

Article

Assessing the Motile Fauna of Eastern Mediterranean Marine Caves

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Abstract

Although marine caves are among the most species-diverse habitats in the Mediterranean Sea, most available studies have focused on their sessile fauna. This study provides the first quantitative assessment of motile fauna in 27 marine caves across four geographical subareas of the Aegean and Ionian Seas, using a rapid assessment visual census protocol, applied through 3 min time transects in each ecological cave zone. Multivariate analysis revealed that the motile community structure of the cave entrance was differentiated from that of the semidark and dark zones. Deeper caves were distinct from shallower ones while caves of the east Aegean differed from those around Crete Island. A total of 163 taxa were recorded, 27 of which are reported herein for the first time in marine caves of the eastern Mediterranean Sea, while three species (two native and one introduced) are recorded in Greek waters for the first time, enriching our knowledge on the permanent and occasional cave residents. Seventeen species were introduced, comprising more than half of the total fish abundance in the southeasternmost cave. Our limited knowledge of the motile fauna of Mediterranean marine caves coupled with the continued spread of introduced species highlights the urgent need for monitoring and conservation actions, especially within marine protected areas.

Keywords: motile species; introduced species; visual census; sea caves; ecological zone; Aegean Sea; Ionian Sea; Greece

Key Contribution: A visual census protocol can be applied to rapidly assess the motile community structure of eastern Mediterranean marine caves, a species-diverse habitat differentiated depending on the depth and geographic area.



Academic Editors: Lorenzo Vilizzi and Ying Xue

Received: 11 May 2025

Revised: 14 July 2025

Accepted: 24 July 2025

Published: 5 August 2025

Citation: Digenis, M.; Ragkousis, M.; Dimitriadis, C.; Katsanevakis, S.; Gerovasileiou, V. Assessing the Motile Fauna of Eastern Mediterranean Marine Caves. *Fishes* **2025**, *10*, 383. <https://doi.org/10.3390/fishes10080383>

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1. Introduction

The rocky coasts of the Mediterranean Sea are characterized by the presence of numerous marine caves, which can be either semi-submerged or entirely submerged [1]. Marine caves rank among the richest habitats in terms of biodiversity in the Mediterranean Sea, with approximately 2400 taxa reported from 350 caves (mostly semi-submerged and/or shallow) documented to date [2].

Most studies on marine cave fauna have focused on sessile communities, e.g., [3–8]. Although efforts to study motile fauna in Mediterranean marine caves date back to the 1960s [9,10], most available studies are either qualitative or limited to specific taxonomic groups [11–19]. Recent reviews on marine cave fishes and crustaceans point to a rich diversity of motile species that find refuge in these habitats [20–22]. In addition, the exploration of marine caves in previously under-studied areas has led to several new records of rarely observed species [23–25], suggesting that marine cave biodiversity is greater than previously estimated.

However, most studies on motile cave fauna have been conducted in the western and central Mediterranean [13,26–30], leaving significant knowledge gaps in the eastern and southern sectors of the basin. These areas are experiencing rapid ecological shifts as they have been impacted by mass mortality events and biological invasions over the past decades [31,32]. Marine caves are threatened by multiple global and local pressures [33], such as the effects of climate change, coastal infrastructure development, unregulated tourism, and marine pollution [1,2,34,35]. Recent studies have highlighted the growing numbers of introduced species in marine caves of the eastern and southern Mediterranean [36–38], with the term “introduced species” being used to include non-indigenous, cryptogenic (species with unclear native or introduced origin), crypto-expanding (species with unclear natural or human-mediated expansion), and species with questionable status [37]. Specifically, within only eight years, the number of introduced species inhabiting Mediterranean marine caves has more than doubled [37]. Approximately half of these species (60 species or 47.6%) are motile, with the majority recorded in the eastern basin (50), particularly in the Levantine (45) and Aegean (20) ecoregions. Most of these introduced species originate from the Indo-Pacific and have entered the Mediterranean Sea through the Suez Canal [37]. In light of such abrupt ecological changes, there is an urgent need to obtain quantitative data on the population structure of motile fauna in eastern Mediterranean caves, to better understand the potential responses of native biodiversity to cumulative pressures [20]. Systematic sampling in such environments is particularly challenging due to sharp ecological gradients, limited visibility, and time and space constraints of cave habitats [34,39]. Marine caves are characterized by steep environmental gradients, such as the decrease in light levels and hydrodynamism, accompanied by increased oligotrophy towards the inner zones, caused by their unique topographic and morphological features [40,41]. These gradients structure the cave biota into three distinct ecological zones: the well-lit entrance zone (CE), dominated by macroalgae (mostly rhodophytes); the intermediate semidark zone (SD), dominated by sciaphilic animals; and the inner dark zone (D), where both diversity and biotic cover diminish [2]. While such environmental gradients are primarily known to influence sessile communities, increasing evidence indicates notable spatial variability among motile taxa, particularly fish assemblages. Most fish species tend to occur in the entrance and semidark zones of caves, whereas only a limited number of larger or hyperbenthic species, along with small epibenthic and cryptobenthic fishes, are typically found in the innermost dark sections ([22] and references therein).

Beyond this small-scale heterogeneity, cave assemblages significantly differ among Mediterranean biogeographic regions, supporting the view that caves are highly fragmented habitats, functioning as isolated “islands” that host unique and often isolated populations [42].

In this study, a novel visual census protocol was developed and applied to rapidly and quantitatively assess the motile faunal community in 27 marine caves of the eastern Mediterranean Sea. Specifically, we tested whether motile community structure (i) shows high similarity between equivalent ecological zones across different caves; (ii) differs significantly among geographic subareas and among caves within each subarea; and (iii)

exhibits similar patterns for species with high (e.g., fish) and low mobility (e.g., crustaceans, mollusks). Given the high numbers of introduced fishes observed in the studied caves, we also investigated whether the ratios of introduced to native fishes (in terms of both species richness and abundance) correspond with the known southeast-to-northwest gradient observed in the Mediterranean Sea [31]. In addition, a broader assessment of motile biodiversity in the studied caves is presented, combining visual census data with qualitative sampling, being the first large-scale study of this kind in the most vulnerable sector of the Mediterranean basin.

2. Materials and Methods

2.1. Study Area

Twenty-seven marine caves in the eastern Mediterranean, distributed across ten islands and spanning four geographical subareas of Greece (east Aegean, central Aegean, Ionian Sea and Crete), were surveyed for their motile fauna during different sampling periods between May and December of the years 2020 and 2022 (Figure 1, Table 1) by a single observer during a single dive per cave. Eleven of these caves had fully submerged entrances, whereas sixteen had semi-submerged entrances. The maximum entrance depth ranged from 3.5 to 32 m for submerged caves and from 3.5 to 20 m for semi-submerged ones; cave lengths ranged from 11 to 163 m (Table 1). All studied caves had an entrance and a semidark zone, while eleven also featured a dark zone. Most of the studied caves (20) are located within national parks (National Marine Park of Zakynthos and National Park of Samaria) and/or protected areas of the European Union's Natura 2000 network (Table 1), including nineteen designated as Habitats Directive Sites and two as Birds Directive Sites.

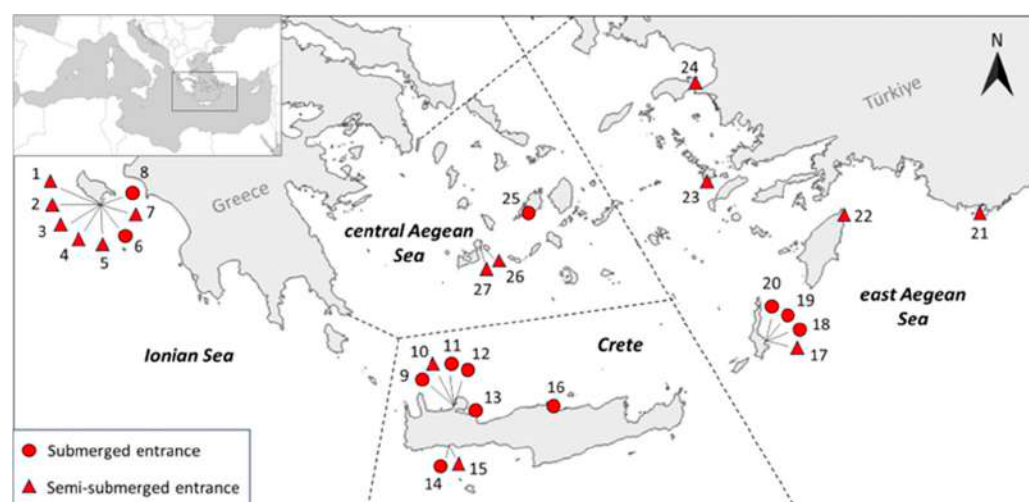


Figure 1. Location of the studied marine caves with fully submerged (circles) and semi-submerged (triangles) entrances and given reference numbers (RN) (see Table 1). Biogeographical subareas (Ionian Sea, central Aegean, east Aegean, and Crete) based on [43] and modified. The inset map (upper left) indicates the location of the surveyed area within the Mediterranean Sea.

2.2. Sampling

A visual census protocol was developed and applied to rapidly and efficiently assess the motile faunal communities in marine caves. According to this protocol, a single scientist/scientific diver recorded both species richness and abundance of all observed motile taxa within a 3 min visual survey transect conducted in each of the three ecological cave zones (i.e., entrance, semidark, and dark) while SCUBA diving. Motile species were recorded on cave walls, crevices, overhangs, the cave bed, and ceiling (when present in submerged caves) within each zone. Basic topographic and morphological features of

each cave (e.g., submersion level, depth) were also recorded after the visual census. This protocol was designed for non-decompression single-dive surveys in marine caves up to a depth of 30 m, considering the logistical constraints of diving time at such depths (16 to 20 min maximum). This approach allowed for a safe exit from inner cave zones under a precautionary approach and standardized research effort across caves with different topographic features (i.e., depth and varying extent of ecological zones). Overall, a maximum time of nine minutes (three minutes per ecological zone) is required for each survey, with less time needed in caves lacking a dark zone. For deeper caves or those with complex morphology, supplemental diving rules need to be considered.

Table 1. Reference number (RN), location, depth, year of sampling, and protection status (Natura 2000 code; * for National Parks) of the 27 studied marine caves. Oct: October; Nov: November; Dec: December; Jun: June; Aug: August.

RN	Site Name	Location (Island)	Latitude (DD)	Longitude (DD)	Entrance Bottom Depth (m)	Month–Year of Survey	Protection Status
1	Butterfly cave	Zakynthos	37.64525	20.83013	9	Nov-2022	GR2210001 *
2	Mavros Kavos cave	Zakynthos	37.64573	20.83250	20	Nov-2022	GR2210001 *
3	Sulfur 1 cave	Zakynthos	37.64800	20.84108	13	Nov-2022	GR2210001 *
4	Sulfur 2 cave	Zakynthos	37.64798	20.84129	12	Nov-2022	GR2210001 *
5	Altar cave	Zakynthos	37.64717	20.84536	16.5	Nov-2022	GR2210001 *
6	Shrimp cave	Zakynthos	37.64700	20.84600	32	Nov-2022	GR2210001 *
7	Keri 2 cave	Zakynthos	37.66146	20.85858	5.2	Nov-2022	*
8	Marathia cave	Zakynthos	37.66496	20.85869	3.5	Nov-2022	GR2210002 *
9	Seal's 1 cave	Crete	35.55017	24.06942	13	May-2021	-
10	Blue 1 cave	Crete	35.55051	24.07011	7	May-2021	-
11	Skotino cave	Crete	35.55138	24.06999	11	May-2021	-
12	Cathedral cave	Crete	35.55181	24.07052	12	May-2021	-
13	Elephant cave	Crete	35.46921	24.24484	10	Dec-2022	GR4340010
14	Glika Nera cave	Crete	35.20124	24.11633	8	Jun-2021	GR4340008 *
15	Lithistid cave	Crete	35.20134	24.11981	4	Jun-2021	GR4340008 *
16	Alikes cave	Crete	35.41676	24.98657	16	Oct-2021	-
17	Vronti cave	Karpathos	35.53733	27.21255	4	Aug-2020	GR4210002
18	Neptune's cave	Karpathos	35.55571	27.20962	27	Aug-2020	GR4210002
19	Achata 1 cave	Karpathos	35.55906	27.20420	10	Aug-2020	GR4210002
20	Achata 2 cave	Karpathos	35.55919	27.20450	11.5	Aug-2020	GR4210002
21	Blue 2 cave	Kastellorizo	36.12572	29.57881	17	Aug-2020	GR4210004
22	Seal's 2 cave	Rhodes	36.32617	28.21452	4	Aug-2020	-
23	Kalymnos cave	Kalymnos	36.92587	26.97193	4	Aug-2020	GR4210019
24	Seal's 3 cave	Samos	37.77239	27.05983	2	Aug-2020	-
25	Pantieronisi cave	Pantieronisi	36.96853	25.12141	14	Aug-2020	GR4220025
26	Polyaigos cave	Polyaigos	36.78619	24.63650	8	Aug-2020	GR4220006
27	Efstathios cave	Agios Efstathios	36.77380	24.58107	6	Aug-2020	GR4220006

In the present study, the visual census protocol was consistently applied once in each cave, by the same scientific diver (M.D.), who always entered first, during morning to

mid-day hours, to minimize disturbance to the motile fauna and ensure consistency across observations. A second diver accompanied the lead, being responsible for maintaining time intervals between zones and ensuring diving safety. Following the visual census, the team of diving scientists recorded the presence and abundance of motile taxa observed outside the time transects. They also collected qualitative samples for morphological identification (approximately 76 specimens) and close-up photos when in situ identification was not feasible (e.g., for small-sized taxa), to produce a biodiversity inventory for the studied caves. Conspicuous taxa (mostly fishes) were identified during the dives, while small-sized taxa (e.g., mollusks) were mainly identified from collected samples.

2.3. Statistical Analyses

Data on the abundance of all motile taxa recorded within time transects were square-root transformed, and a triangular similarity matrix was generated using the Bray–Curtis similarity index [44]. A two-way non-parametric multivariate analysis of variance (PERMANOVA) [45] was performed, with the factor ‘Cave’ (random, 27 levels—see Table 1 for site names) nested within the factor ‘Subarea’ (fixed, 4 levels—Ionian, Crete, east Aegean, central Aegean). To visualize multivariate patterns, non-metric multidimensional scaling (nMDS) and cluster analysis were applied. The SIMPROF test was used to examine the null hypothesis of no meaningful clustering among samples (significance level: 5%, confidence level: 95%) [44,46]. A Spearman rank order correlation was used to investigate the monotonic relationship of species abundance with the resulting multivariate community patterns. Species that exhibited correlation values greater than 0.6 (and $p < 0.05$) were overlaid onto the nMDS plot as vectors [47,48]. Similarity percentages (SIMPER) analysis was performed to estimate the contribution of individual taxa to dissimilarity patterns [47]. PERMANOVA, nMDS, and SIMPER analyses were also independently conducted for fish and invertebrate taxa, following the above-mentioned design. If there was no significant variation in community structure among caves within the same geographic subarea, a one-way PERMANOVA was applied, based on pooled abundance data from caves within each subarea to examine potential differences in community structure across the three cave ecological zones. Multivariate, non-parametric resemblance analysis was performed using the software PRIMER-6 [48] with the PERMANOVA+ add-on package [49].

3. Results

3.1. Species Richness

Within the time transects, 70 motile taxa belonging to seven phyla were identified: Chordata (37), Arthropoda (14), Echinodermata (11), Mollusca (4), Annelida (2), Platyhelminthes (1), and Cnidaria (1). These were identified to the species (55), genus (9), family (4), and class (2) levels by a single observer during a single dive per cave (Table 2).

Table 2. List of all taxa recorded during the visual census in the studied caves by major taxonomic group (in bold). *: taxa recorded within time transects; †: taxa recorded out of transect. *Gammogobius/Corcyrogobius*: refer to the species *Gammogobius steinitzi* Bath, 1971 and/or *Corcyrogobius liechtensteini* (Kolombatovic, 1891), two small-sized cryptobenthic fish with similar color patterns, often difficult to be distinguished visually by the observer within the time transects.

Phylum Cnidaria	Echinoidea
Scyphozoa	<i>Arbacia lixula</i> (Linnaeus, 1758) *
<i>Pelagia noctiluca</i> (Forsskål, 1775) *	<i>Centrostephanus longispinus</i> (Philippi, 1845) *
Phylum Platyhelminthes	<i>Diadema setosum</i> (Leske, 1778) *
<i>Monobicerus langi</i> Faubel, 1984 *	<i>Paracentrotus lividus</i> (Lamarck, 1816) *
Phylum Mollusca	<i>Sphaerechinus granularis</i> (Lamarck, 1816) *
Cephalopoda	<i>Stylocidaris affinis</i> (Philippi, 1845) *
<i>Octopus vulgaris</i> Cuvier, 1797 †	Holothuroidea
<i>Sepia officinalis</i> Linnaeus, 1758 *	<i>Holothuria</i> spp. *
Gastropoda	Phylum Chordata
<i>Berthella ocellata</i> (Delle Chiaje, 1830) †	Osteichthyes
<i>Charonia seguenzae</i> (Aradas & Benoit, 1871) †	<i>Anthias anthias</i> (Linnaeus, 1758) *
<i>Clanculus</i> spp. †	<i>Apogon imberbis</i> (Linnaeus, 1758) *
<i>Homalopoma sanguineum</i> (Linnaeus, 1758) *	<i>Atherina</i> spp. *
<i>Luria lurida</i> (Linnaeus, 1758) †	<i>Blenniidae</i> spp. †
<i>Naria spurca</i> (Linnaeus, 1758) *	<i>Boops boops</i> (Linnaeus, 1758) *
<i>Peltodoris atromaculata</i> Bergh, 1880 *	<i>Chromis chromis</i> (Linnaeus, 1758) *
<i>Plocamopherus ocellatus</i> Rüppell & Leuckart, 1828 †	<i>Conger conger</i> (Linnaeus, 1758) †
<i>Umbraculum umbraculum</i> ([Lightfoot], 1786) †	<i>Coris julis</i> (Linnaeus, 1758) *
Phylum Annelida	<i>Diplodus annularis</i> (Linnaeus, 1758) *
Polychaeta	<i>Diplodus puntazzo</i> (Walbaum, 1792) *
<i>Bonellia viridis</i> Rolando, 1822 *	<i>Diplodus sargus</i> (Linnaeus, 1758) *
<i>Hermodice carunculata</i> (Pallas, 1766) *	<i>Diplodus vulgaris</i> (Geoffroy Saint-Hilaire, 1817) *
Phylum Arthropoda	<i>Enchelycore anatina</i> (Lowe, 1838) †
Malacostraca	<i>Epinephelus costae</i> (Steindachner, 1878) *
<i>Brachycarpus biunguiculatus</i> (Lucas, 1846) *	<i>Epinephelus marginatus</i> (Lowe, 1834) *
<i>Carupa tenuipes</i> Dana, 1852 †	<i>Gammogobius/Corcyrogobius</i> spp. *
<i>Dardanus calidus</i> (Risso, 1827) *	<i>Gobiidae</i> sp. †
<i>Dromia personata</i> (Linnaeus, 1758) *	<i>Gobius vittatus</i> Vinciguerra, 1883 *
<i>Galathea strigosa</i> (Linnaeus, 1761) †	<i>Grammonus ater</i> (Risso, 1810) †
<i>Herbstia</i> sp. *	<i>Marcelogobius splechnai</i> (Ahnelt & Patzner, 1995) *
<i>Lysmata</i> spp. *	<i>Microilipophrys nigriceps</i> (Vinciguerra, 1883) *
<i>Maja</i> sp. *	<i>Mugilidae</i> spp. *
<i>Paguroidea</i> spp. *	<i>Mullus surmuletus</i> Linnaeus, 1758 *
<i>Palaemon serratus</i> (Pennant, 1777) *	<i>Muraena helena</i> Linnaeus, 1758 †
<i>Palinurus elephas</i> (Fabricius, 1787) *	<i>Oblada melanurus</i> (Linnaeus, 1758) *
<i>Paragalene longicrura</i> (Nardo, 1869) *	<i>Parupeneus forsskali</i> (Fourmanoir & Guézé, 1976) *
<i>Plesionika narval</i> (Fabricius, 1787) *	<i>Pempheris rhomboidea</i> Kossman & Räuber, 1877 *
<i>Scyllarides latus</i> (Latreille, 1803) *	<i>Phycis phycis</i> (Linnaeus, 1766) *
<i>Stenopus spinosus</i> Risso, 1827 *	<i>Pterois miles</i> (Bennett, 1828) *
<i>Urocaridella pulchella</i> Yokes & Galil, 2006	<i>Sargocentron rubrum</i> (Forsskål, 1775) *
<i>Xanthidae</i> spp. †	<i>Sciaena umbra</i> Linnaeus, 1758 *
Phylum Echinodermata	<i>Scorpaena scrofa</i> Linnaeus, 1758 †
Crinoidea	<i>Scorpaena</i> spp. *
<i>Antedon mediterranea</i> (Lamarck, 1816) †	<i>Scorpaenodes arenai</i> Torchio, 1962 *
Asteroidea	<i>Serranus cabrilla</i> (Linnaeus, 1758) *
<i>Coscinasterias tenuispina</i> (Lamarck, 1816) †	<i>Serranus scriba</i> (Linnaeus, 1758) *
<i>Hacelia attenuata</i> Gray, 1840 *	<i>Siganus luridus</i> (Rüppell, 1829) *
<i>Marthasterias glacialis</i> (Linnaeus, 1758) *	<i>Siganus rivulatus</i> Forsskål & Niebuhr, 1775 †
<i>Ophidiaster ophidianus</i> (Lamarck, 1816) *	<i>Sparisoma cretense</i> (Linnaeus, 1758) *
Ophiuroidea	<i>Symphodus</i> sp. *
<i>Ophiuroidea</i> spp. *	<i>Synodus saurus</i> (Linnaeus, 1758) *

Osteichthyes (cont.)*Thalassoma pavo* (Linnaeus, 1758) **Thorogobius ephippiatus* (Lowe, 1839) **Torquigener flavimaculosus* Hardy & Randall, 1983 **Tripterygion* spp. *

The taxa recorded within transects comprised 77.8% of the total motile fauna recorded in situ; an additional 20 taxa were observed out of time transects (Table 2). Osteichthyes represented more than half (52.8%) of the taxa which were recorded within transects. At a cave level, the number of motile taxa recorded in transects ranged from 6 to 18, representing 33.3% to 91.7% of the total in situ observed fauna (Figure S1), with a mean of $61.2\% \pm 2.8$ (SE).

The highest number of taxa was recorded in the semidark zone (45), followed by the cave entrance (42), and the dark zone (30). Osteichthyes were the most frequently observed group in all three zones, representing 73.8% of the motile fauna in the cave entrance, 55.5% in the semidark, and 36.6% in the dark zone. The most common taxa were the cardinal fish *Apogon imberbis* (present in all caves), followed by the polychaete *Hermodice carunculata* (in 22 caves), the sea urchin *Arbacia lixula* and holothurians (each recorded in 21 caves).

Species richness per zone (recorded within transects) ranged from 2 to 12 taxa, comprising 16.7% to 100% of the total taxa observed in situ in each respective zone (mean $65.1\% \pm 3.3$ SE, Figure S2). The lowest percentage values (16.7%) were observed in the semidark zones of Blue 2 and Polyaigos caves, while the highest values (100%) were observed in the entrance zones of six caves (Sulfur 1, Sulfur 2, Marathia, Achata 2, Seal's 2 and Polyaigos), the semidark zone of one cave (Alikes), and the dark zone of two caves (Sulfur 2 and Cathedral) (Table 3). On average, the proportion of motile taxa recorded within transects to the total recorded taxa was highest in the entrance zone (75.1%), followed by the dark (64.4%) and the semidark zone (49.7%) (Table 3).

Table 3. Species richness and abundance per ecological zone recorded in transect in the studied caves. The percentage of species richness and abundance recorded in transects in relation to the total (in and out of transects) is presented in brackets. CE: cave entrance; SD: semidark zone; D: dark zone; SE: standard error.

Cave	Species Richness			Abundance		
	CE (%)	SD (%)	D (%)	CE (%)	SD (%)	D (%)
Butterfly	10 (71.4)	9 (42.9)	-	55 (80.9)	1054 (91.2)	-
Mavros Kavos	9 (90)	8 (44.4)	-	20 (90.9)	198 (76.5)	-
Sulfur 1	4 (100)	2 (25)	5 (62.5)	14 (100)	51 (85)	38 (90.5)
Sulfur 2	5 (100)	7 (58.3)	5 (100)	8 (100)	185 (96.4)	42 (100)
Altar	9 (75)	10 (66.7)	-	35 (61.4)	65 (65)	-
Shrimp	6 (60)	6 (85.7)	10 (90.9)	324 (97.9)	776 (99.6)	1311 (99.5)
Keri 2	4 (75)	5 (62.5)	-	110 (97.4)	1010 (99.6)	-
Marathia	7 (100)	6 (85.7)	-	18 (100)	28 (87.5)	-
Seal's 1	4 (80)	4 (30.8)	-	6 (60)	8 (38.1)	-
Blue 1	6 (75)	6 (40)	3 (37.5)	15 (57.7)	11 (21.2)	7 (46.7)
Skotino	5 (50)	5 (55.6)	-	6 (50)	25 (78.1)	-
Cathedral	6 (46.2)	4 (44.4)	3 (100)	160 (94.7)	54 (90)	3 (100)
Alikes	7 (77.8)	3 (100)	-	27 (65.9)	83 (100)	-
Glika Nera	6 (66.7)	6 (54.6)	-	11 (34.4)	31 (47)	-
Lithistid	10 (90.9)	4 (33.3)	5 (38.5)	75 (98.7)	110 (88.7)	39 (61.9)
Elephant	8 (61.5)	8 (57.1)	4 (57.1)	132 (95.7)	74 (87.1)	21 (70)
Vronti	3 (75)	4 (57.1)	-	6 (85.7)	32 (91.4)	-
Neptune's	7 (87.5)	2 (33.3)	-	110 (99.1)	1105 (99.6)	-

Cave	Species Richness			Abundance		
	CE (%)	SD (%)	D (%)	CE (%)	SD (%)	D (%)
Achata 1	7 (87.5)	3 (75)	-	65 (98.5)	13 (92.9)	-
Achata 2	3 (100)	7 (53.9)	-	12 (100)	206 (96.7)	-
Blue 2	5 (45.5)	2 (16.7)	5 (71.4)	160 (42.6)	5 (20.8)	266 (98.5)
Seal's 2	10 (100)	5 (55.6)	5 (45.5)	131 (98.5)	33 (86.8)	18 (69.2)
Kalymnos	7 (46.7)	6 (42.9)	-	91 (57.6)	28 (45.2)	-
Seal's 3	8 (38.1)	5 (22.7)	-	104 (57.5)	33 (27.7)	-
Pantieronisi	8 (88.9)	9 (45)	4 (36.4)	264 (45.5)	36 (55.4)	61 (83.6)
Polyaigos	12 (100)	2 (16.7)	4 (57.1)	27 (100)	26 (59.1)	10 (71.4)
Efstratios	8 (47.1)	5 (35.7)	5 (83.3)	21 (31.8)	15 (33.3)	28 (87.5)
Mean $\pm$ SE	6.8 $\pm$ 0.4	5.3 $\pm$ 0.4	4.8 $\pm$ 0.6	74.3 $\pm$ 15.7	196.1 $\pm$ 66.2	153.7 $\pm$ 107.2
(Mean % $\pm$ SE)	(75.1 $\pm$ 3.9)	(49.7 $\pm$ 4.2)	(64.4 $\pm$ 6.9)	(77.9 $\pm$ 4.6)	(72.6 $\pm$ 5.2)	(81.6 $\pm$ 5.1)

3.2. Abundance

The abundance of motile taxa recorded within transects per cave ranged from 14 to 2411 individuals, comprising between 35.5% and 99.5% of all individuals observed in situ (mean 76.6% $\pm$ 4.1 SE, Figure S3). Osteichthyes were the most abundant group comprising 64.2% of all individuals recorded, followed by Arthropoda (32.3%), Echinodermata (2.1%), and Annelida (1.2%). The remaining groups (Mollusca, Cnidaria, and Platyhelminthes) comprised 0.2% of the total abundance. *Apogon imberbis* was the most abundant fish in most caves (19), followed by atherinids (6) and *Anthias anthias*, which dominated in the two deepest caves (Shrimp and Neptune's cave). The semidark zone often presented the highest transect abundance (in 15 caves). In total, the highest transect abundance in a single cave was observed in the dark zone (up to 1311 individuals of mostly *Plesionika narval*), followed by the semidark zone (1054 individuals of mostly *Atherina* spp.) and the cave entrance (324 individuals of mostly *Anthias anthias*). The proportion of transect-recorded abundance relative to the total abundance was highest in the dark zone (81.6%), followed by the entrance (77.9%) and the semidark zone (72.6%) (Table 3, Figure S4).

3.3. Resemblance Patterns

Two-way PERMANOVA analysis results indicated that motile community structure significantly differed among the geographic subareas (pseudo-F = 1.622; df = 3; p = 0.021); no significant differences were evident among individual caves within subareas (pseudo-F = 1.154; df = 23; p = 0.083). Pairwise comparisons showed significant differentiation between the east Aegean and Crete (t = 1.428, p = 0.032), with *Apogon imberbis*, *Atherina* spp., *Chromis chromis*, *Plesionika narval*, *Hermodice carunculata*, and *Arbacia lixula* contributing 52.2% to this differentiation (Tables S1–S3). The average similarity within each area ranged from 20.3% for the east Aegean to 27.3% for the central Aegean. Within all geographic areas, significant differences in community structure were detected between the cave entrance and the inner semidark and dark zones (see Tables S4–S7) (Ionian: pseudo-F = 2.682, df = 2, p = 0.001; Crete: pseudo-F = 2.690, df = 2, p = 0.002; east Aegean: pseudo-F = 1.699, df = 2, p = 0.041; central Aegean: pseudo-F = 2.192, df = 2, p = 0.038). *Apogon imberbis* and *Plesionika narval* showed the strongest positive associations with the semidark and dark cave zones, while *Coris julis*, *Chromis chromis*, *Thalassoma pavo*, and *Arbacia lixula* were more closely associated with the cave entrance (Figure 2).

Cluster analysis indicated that the species composition of the deepest caves with fully submerged entrance (Neptune's and Shrimp cave) was significantly different from all other caves (Figure 3), with an average dissimilarity of 80.3% (SIMPER and SIMPROF analyses). This differentiation was primarily driven by the shrimp *Plesionika narval* (45.4%) and the fish *Anthias anthias* (10.5%).

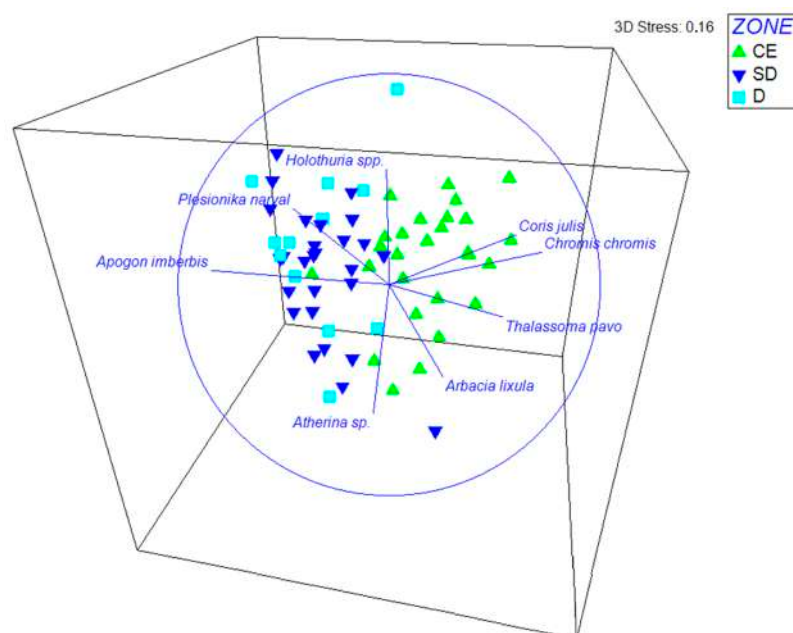


Figure 2. Non-metric multidimensional scaling (nMDS) three-dimensional plot showing the dissimilarities among motile taxa recorded in transects at different ecological zones and the species contributing the most to this differentiation. CE: cave entrance; SD: semidark zone; D: dark zone.

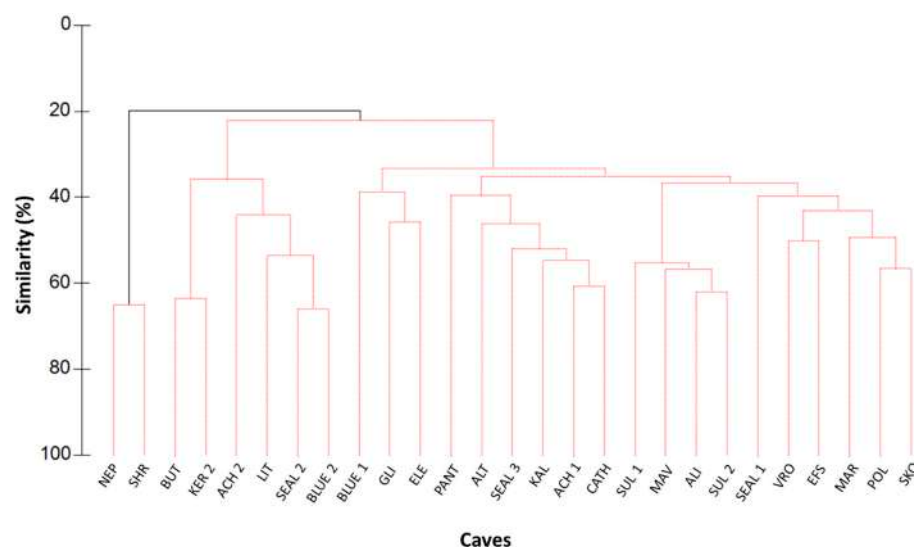


Figure 3. Cluster dendrogram with the percent similarity among all studied caves (NEP: Neptune's; SHR: Shrimp; BUT: Butterfly; KER 2: Keri 2; ACH 2: Achata 2; LIT: Lithistid; SEAL 2: Seal's 2; BLUE 2: Blue 2; BLUE 1: Blue 1; GLI: Gliko Nera; ELE: Elephant; PANT: Pantieronisi; ALT: Altar; SEAL 3: Seal's 3; KAL: Kalymnos; ACH 1: Achata 1; CATH: Cathedral; SUL 1: Sulfur 1; MAV: Mavros Kavos; ALI: Alikes; SUL 2: Sulfur 2; SEAL 1: Seal's 1; VRO: Vronti; EFS: Efstathios; MAR: Marathia; POL: Polyagios; SKO: Skotino). Red lines indicate non-significant differentiation; Black lines indicate significant differentiation.

SIMPER analysis also showed that Neptune's and Shrimp caves had an average similarity of 64.8%, mainly due to *P. narval* (72.1% contribution), while all other caves clustered with an average similarity of 31.9%, primarily due to *A. imberbis* (52.3% contribution). When fish and motile invertebrate taxa were analyzed separately, two-way PERMANOVA indicated no differentiation among the four subareas or between the caves within the same geographic subarea. However, for fish species alone, PERMANOVA detected significant differences in community structure between the entrance and inner zones across all subar-

eas: Ionian: pseudo-F = 3.139, df = 2, p = 0.001; Crete: pseudo-F = 3.314, df = 2, p = 0.004; central Aegean: pseudo-F = 2.941, df = 2, p = 0.034; east Aegean: pseudo-F = 2.101, df = 2, p = 0.030 (see Tables S8–S13). *Apogon imberbis* was predominantly associated with inner zones, whereas *Coris julis*, *Chromis chromis*, and *Thalassoma pavo* were mostly related to the cave entrance (Figure 4).

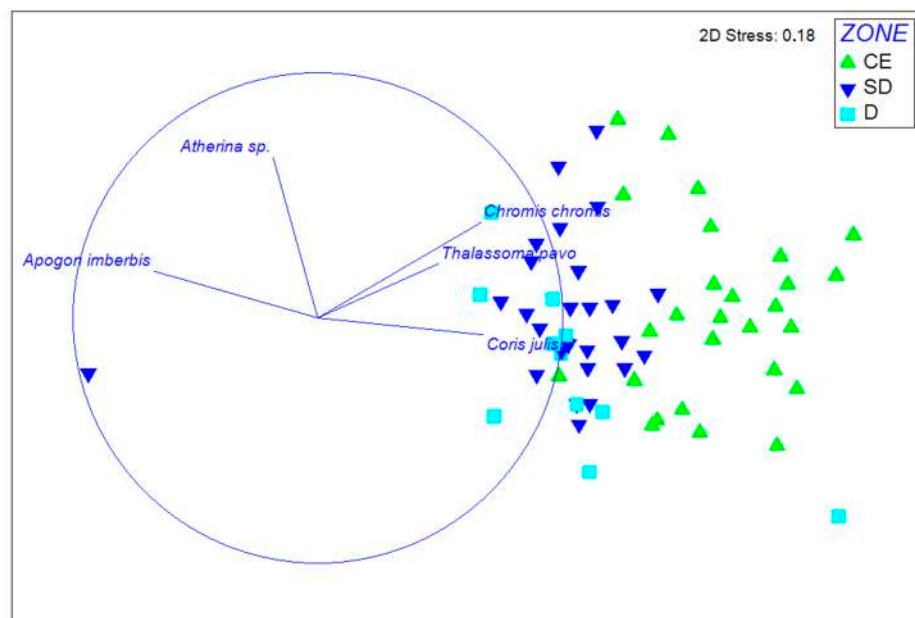


Figure 4. Non-metric multidimensional scaling (nMDS) two-dimensional plot showing the dissimilarities among the fish taxa recorded in transects at different ecological zones and the fish species contributing the most to this differentiation. CE: cave entrance; SD: semidark zone; D: dark zone.

3.4. Biodiversity Inventory

A total of 163 motile taxa belonging to seven phyla were identified: Chordata (57), Mollusca (51), Arthropoda (30), Echinodermata (18), Platyhelminthes (4), Annelida (2), and Cnidaria (1). These were identified to the species (142), genus (9), family (9), superfamily (1), class (1), and order (1) levels, and included 12 protected and 17 introduced species (Table 4). These taxa were identified visually during the time transects and through out-of-transect in situ observations (1–3 dives per cave and 1–3 observers per dive), and by examining the supplementary collected material (photos and samples).

The total number of motile species recorded per cave ranged from 9 to 54 taxa. The Lithistid cave in southern Crete and Seal's 3 cave on Samos Island (eastern Aegean) were the most species-rich sites, with 54 and 49 taxa recorded, respectively.

Table 4. Complete list of all motile taxa recorded in the studied caves by major taxonomic group (in bold). *: Introduced species; †: Protected species; °: species morphologically identified with collected specimens; f: species recorded for the first time in eastern Mediterranean marine caves (black colored) and in Mediterranean marine caves (orange colored). Colored circles indicate the presence of taxa in different ecological zones (white: entrance zone; gray: semidark zone; black: dark zone); numbers in brackets indicate the number of caves where each taxon was recorded; blue triangles in squares indicate the geographical subarea each taxon was recorded (left: Ionian; bottom: Crete; right: east Aegean; top: central Aegean).

Phylum Cnidaria**Scyphozoa**

Pelagia noctiluca (Forsskål, 1775) f  (7) 

Phylum Platyhelminthes

Planocera sp. °  (1) 

Prostheceraeus moseleyi Lang, 1884 f  (2) 

Monoboceros langi Faubel, 1984 ° f  (5) 

Thysanozoon brocchii (Risso, 1818)  (1) 

Phylum Mollusca**Cephalopoda**

Octopus vulgaris Cuvier, 1797 f  (2) 

Sepia officinalis Linnaeus, 1758 f  (3) 

Gastropoda

Aplus gaillardoti (Puton, 1856) ° f  (2) 

Aplus scacchianus (R. A. Philippi, 1844) °  (1) 

Berthella aurantiaca (Risso, 1818) ° f  (1) 

Berthella ocellata (Delle Chiaje, 1830) f  (2) 

Berthellina edwardsii (Vayssièrre, 1897) ° f  (1) 

Bittium sp.  (5) 

Bittium latreillii (Payraudeau, 1826) °  (2) 

Calliostoma laugierii (Payraudeau, 1826) °  (2) 

Caloria elegans (Alder & Hancock, 1845) f  (3) 

Cerithium scabridum R. A. Philippi, 1848 * °  (2) 

Cerithiidae sp.  (5) 

Charonia sequezenae (Aradas & Benoit, 1871) †  (2) 

Clanculus corallinus (Gmelin, 1791) °  (4) 

Clanculus cruciatus (Linnaeus, 1758) °  (2) 

Conomurex persicus (Swainson, 1821) *  (1) 

Cratena peregrina (Gmelin, 1791) f  (1) 

Dendrodoris grandiflora (Rapp, 1827) f  (1) 

Diaphorodoris papillata Portmann & Sandmeier, 1960 f  (1) 

Episcomitra cornicula (Linnaeus, 1758) °  (2) 

Ergalatax junoniae Houart, 2008 * °  (1) 

Euthria cornea (Linnaeus, 1758) °  (2) 

Facelina annulicornis (Chamisso & Eysenhardt, 1821) ° f  (1) 

Facelina rubrovittata (A. Costa, 1866) f  (1) 

Felimare gasconi (Ortea, 1996) ° f  (1) 

Felimida krolni (Vérany, 1846) f  (1) 

Fissurellidae sp.  (1) 

Flabellina affinis (Gmelin, 1791)  (2) 

Flabellinidae sp.  (1) 

Fusinus sp. °  (2) 

Hexaplex trunculus (Linnaeus, 1758)  (1) 

Homalopoma sanguineum (Linnaeus, 1758) °  (5) 

Luria lurida (Linnaeus, 1758) †  (8) 

Muricopsis cristata (Brocchi, 1814) °  (11) 

Naria spurca (Linnaeus, 1758) †  (3) 

Peltodoris atromaculata Bergh, 1880  (5) 

Phyllidia flava Aradas, 1847 ° f  (2) 

Pisania striata (Gmelin, 1791)  (1) 

Plocamopherus ocellatus Rüppell & Leuckart, 1828 *  (1) 

Gastropoda (cont.)

Polycera quadrilineata (O. F. Müller, 1776) f  (1) 

Pseudofusus rolandi (Buzzurro & Ovalis, 2005) ° f  (1) 

Similiphora similior (Bouchet & Guillemot, 1978)  (1) 

Spurilla neapolitana Bergh, 1889 f  (1) 

Steromphala rarilineata (Michaud, 1829) ° f  (1) 

Tarantinaea lignaria (Linnaeus, 1758) f  (2) 

Trapania lineata Haefelfinger, 1960 °  (1) 

Tritia incrassata (Strom, 1768) f  (1) 

Tyloclina perversa (Gmelin, 1791) °  (1) 

Umbraculum umbraculum ([Lightfoot], 1786)  (7) 

Bivalvia

Pectinidae sp.  (1) 

Phylum Annelida**Polychaeta**

Bonellia viridis Rolando, 1822  (10) 

Hermodice carunculata (Pallas, 1766)  (22) 

Phylum Arthropoda**Malacostraca**

Brachycarpus biunguiculatus (Lucas, 1846)  (15) 

Calcinus tubularis (Linnaeus, 1767)  (1) 

Carupa tenuipes Dana, 1852 * °  (5) 

Dardanus calidus (Risso, 1827)  (5) 

Dromia personata (Linnaeus, 1758) °  (11) 

Galathea strigosa (Linnaeus, 1761)  (1) 

Gonioinfradens giardi (Nobili, 1905) * f  (2) 

Herbstia condyliata (Fabricius, 1787) °  (1) 

Herbstia spp.  (7) 

Hemimysis spp. °  (8) 

Lysmata nilita Dohrn & Holthuis, 1950  (2) 

Lysmata seticaudata (Risso, 1816)  (3) 

Maja sp.  (1) 

Mysida spp.  (21) 

Pagurus anachoretus Risso, 1827  (5) 

Pagurus chevreuxi (Bouvier, 1896) °  (1) 

Paguroidea spp.  (10) 

Palaemon serratus (Pennant, 1777)  (9) 

Palinurus elephas (Fabricius, 1787) †  (1) 

Paractaea monodi Guinot, 1969 ° f  (2) 

Paragalene longicrura (Nardo, 1869)  (1) 

Percnon gibbesi (H. Milne Edwards, 1853) *  (1) 

Plesionika narval (Fabricius, 1787)  (8) 

Scyllarides latus (Latreille, 1803) †  (6) 

Scyllarus pygmaeus (Spence Bate, 1888) †  (2) 

Siriella gracilipes Nouvel, 1942 °  (5) 

Stenopus spinosus Risso, 1827  (17) 

Urocaridella pulchella Yokes & Galil, 2006 * °  (5) 

Xantho pilipes A. Milne-Edwards, 1867 °  (1) 

Pycnogonida

Pantopoda sp. °  (1) 

Phylum Echinodermata

Crinoidea

Antedon mediterranea (Lamarck, 1816) ° (4)

Asteroidea

Coscinasterias tenuispina (Lamarck, 1816) (5)

Hacelia attenuata Gray, 1840 (1)

Marthasterias glacialis (Linnaeus, 1758) (4)

Ophiaster ophidianus (Lamarck, 1816) † (9)

Ophiuroidea

Ophiactis sp. ° (1)

Ophioderma longicaudum (Bruzellus, 1805) f (3)

Ophiopsila aranea Forbes, 1843 ° f (1)

Ophiothrix fragilis (Abildgaard in O.F. Müller, 1789) (10)

Ophiuroidea sp. (13)

Echinoidea

Arbacia lixula (Linnaeus, 1758) ° (21)

Centrostephanus longispinus (Philippi, 1845) † (2)

Diadema setosum (Leske, 1778) * (4)

Paracentrotus lividus (Lamarck, 1816) † (2)

Sphaerechinus granularis (Lamarck, 1816) (7)

Stylocidaris affinis (Philippi, 1845) (2)

Holothuroidea

Holothuria (Platyperona) sanctori Delle Chiaje, 1823 (13)

Holothuria spp. (21)

Phylum Chordata

Osteichthyes

Anthias anthias (Linnaeus, 1758) (2)

Apogon imberbis (Linnaeus, 1758) (27)

Atherina spp. (8)

Blenniidae spp. (3)

Boops boops (Linnaeus, 1758) (3)

Chromis chromis (Linnaeus, 1758) (20)

Conger conger (Linnaeus, 1758) (3)

Corcyrogobius liechtensteini (Kolombatovic, 1891) (5)

Coris julis (Linnaeus, 1758) (19)

Diplodus annularis (Linnaeus, 1758) (5)

Diplodus puntazzo (Walbaum, 1792) (1)

Diplodus sargus (Linnaeus, 1758) (7)

Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817) (7)

Enchelycore anatina (Lowe, 1838) * (4)

Epinephelus costae (Steindachner, 1878) (4)

Epinephelus marginatus (Lowe, 1834) † (6)

Osteichthyes (cont.)

Gammogobius steinitzi Bath, 1971 (10)

Gobiidae sp. (2)

Gobius bucchichi Steindachner, 1870 (1)

Gobius vittatus Vinciguerra, 1883 (1)

Grammonus ater (Risso, 1810) (5)

Lepadogaster candolii Risso, 1810 (1)

Lepadogaster lepadogaster (Bonnaterre, 1788) (2)

Marcelogobius splechnai (Ahnelt & Patzner, 1995) (6)

Microlophrys nigriceps (Vinciguerra, 1883) (11)

Mugilidae spp. (3)

Mullus surmuletus Linnaeus, 1758 (3)

Muraena helena Linnaeus, 1758 (2)

Oblada melanurus (Linnaeus, 1758) (7)

Parablennius gattorugine (Linnaeus, 1758) (1)

Parablennius rouxi (Cocco, 1833) (1)

Parupeneus forsskali (Fourmanoir & Guézé, 1976) * (3)

Pempheris rhomboidea Kossmann & Räuber, 1877 * (4)

Phycis phycis (Linnaeus, 1766) (5)

Pterois miles (Bennett, 1828) * (10)

Sargocentron rubrum (Forsskål, 1775) * (6)

Sciaena umbra Linnaeus, 1758 † (1)

Scorpaena maderensis Valenciennes, 1833 (19)

Scorpaena notata Rafinesque, 1810 (1)

Scorpaena porcus Linnaeus, 1758 (1)

Scorpaena scrofa Linnaeus, 1758 (2)

Scorpaenodes arenai Torchio, 1962 (1)

Serranus cabrilla (Linnaeus, 1758) (8)

Serranus scriba (Linnaeus, 1758) (9)

Siganus luridus (Rüppell, 1829) * (5)

Siganus rivulatus Forsskål & Niebuhr, 1775 * (1)

Sparisoma cretense (Linnaeus, 1758) (8)

Spicara smaris (Linnaeus, 1758) (1)

Symphodus mediterraneus (Linnaeus, 1758) (3)

Symphodus sp. (1)

Symodus saurus (Linnaeus, 1758) f (2)

Thalassoma pavo (Linnaeus, 1758) (16)

Thorogobius ephippiatus (Lowe, 1839) (6)

Torquigener flavimaculosus Hardy & Randall, 1983 * (3)

Tripterygion delaisi Cadenat & Blache, 1970 (1)

Tripterygion melanurus Guichenot, 1850 (18)

Tripterygion tripteronotum (Risso, 1810) (3)

3.5. Introduced Species

Seventeen introduced species were recorded, belonging to four phyla: Chordata (8), Arthropoda (4), Mollusca (4), and Echinodermata (1). The lionfish *Pterois miles* was the most frequently encountered introduced species, recorded in ten caves across the east Aegean, Crete, and the Ionian Sea, followed by *Sargocentron rubrum* in six caves of the east Aegean, and *Pempheris rhomboidea* in four caves of the east Aegean and Crete (Figure 5, Table 4). The

highest number of introduced species was recorded in the easternmost caves of the east Aegean and Crete, with Blue 2 cave (Kastellorizo Island) hosting the most (ten species).

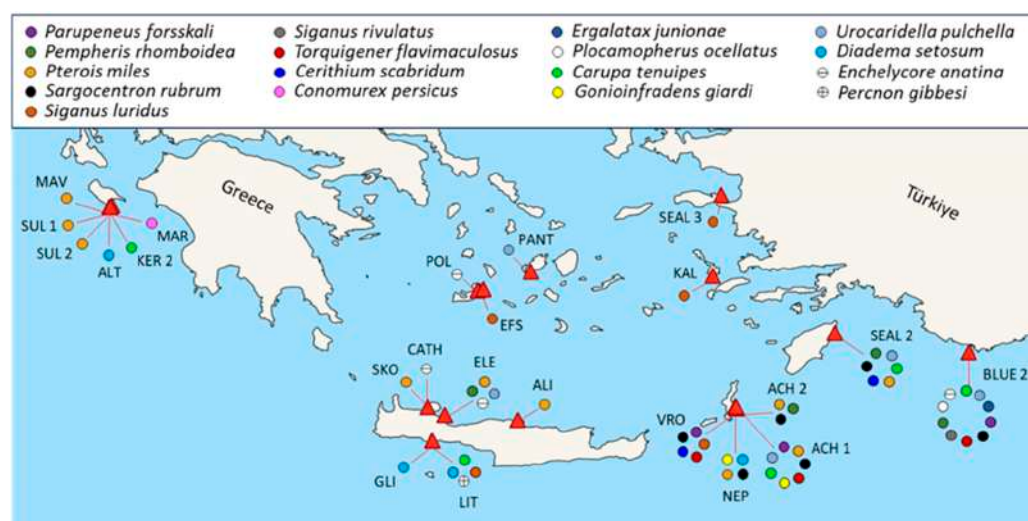


Figure 5. Introduced species recorded in the studied caves in and out of transects labelled with different colors (MAV: Mavros Kavos; SUL 1: Sulfur 1; SUL 2: Sulfur 2; ALT: Altar; KER 2: Keri 2; MAR: Marathia; SKO: Skotino; CATH: Cathedral; ALI: Alikes; GLI: Gлика Nera; LIT: Lithistid; VRO: Vronti; NEP: Neptune's; ACH 1: Achata 1; ACH 2: Achata 2; BLUE 2: Blue 2; SEAL 2: Seal's 2; KAL: Kalymnos; SEAL 3: Seal's 3; PANT: Pantieronisi; POL: Polyaigos; EFS: Efstathios). Caves with no introduced species are not presented.

Fishes constituted the most abundant introduced group in most caves. Blue 2, the southeasternmost studied cave, presented the highest overall abundance of introduced species, with more than 200 individuals of *P. rhomboidea* in its semidark zone and over 200 juveniles in its entrance zone, comprising approximately 53% of the cave's total fish abundance (Figure 6).

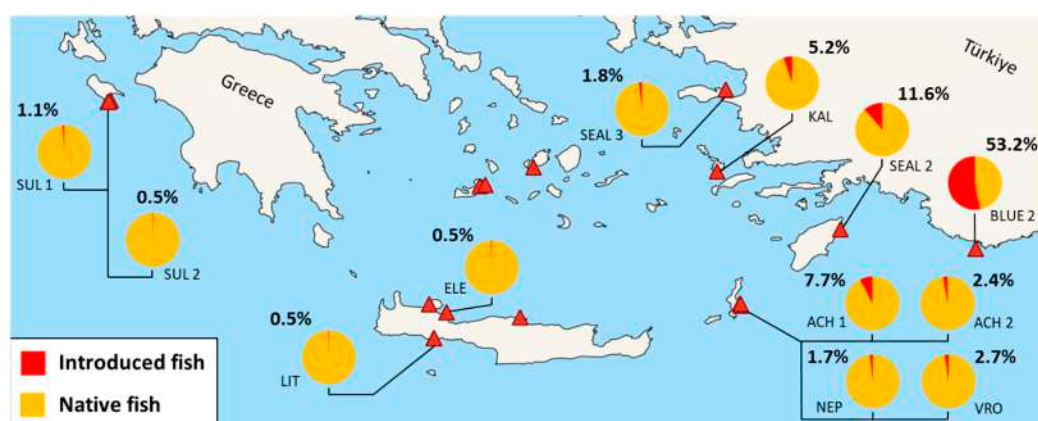


Figure 6. Percent ratio of introduced to native fish species recorded in transect for the studied marine caves (SUL 1: Sulfur 1; SUL 2: Sulfur 2; ELE: Elephant; LIT: Lithistid; VRO: Vronti; NEP: Neptune's; ACH 1: Achata 1; ACH 2: Achata 2; BLUE 2: Blue 2; SEAL 2: Seal's 2; KAL: Kalymnos; SEAL 3: Seal's 3). Caves with no introduced species are not presented.

4. Discussion

This study provides the first large-scale biodiversity assessment of motile fauna in 27 marine caves across the eastern Mediterranean, using a standardized quantitative methodology based on a rapid visual census protocol, complemented by qualitative material (photos and specimens). These caves were surveyed for the first time for their motile

fauna, revealing rich biodiversity, with 163 taxa identified. Motile community structure exhibited considerable variation at a large spatial scale (i.e., among the four surveyed geographic subareas). In contrast, the lack of significant differentiation among caves within the same subareas suggests a more homogenized pattern in motile communities, likely due to the high mobility of most taxa, possible differences at local species pools and environmental filters. This finding contrasts with the widely accepted concept of cave “individuality” for sessile communities [3,4,6,50]. Species with distinct ecological niches and traits had a significant effect on the community structure across ecological zones. Reef-associated species dominated the cave entrance (e.g., *Coris julis*, *Chromis chromis*, *Thalassoma pavo*, and *Arbacia lixula*), while nocturnal (*Apogon imberbis*) and deeper-water species (*Anthias anthias* and *Plesionika narval*) prevailed in darker cave zones and deeper caves.

Consistent with previous faunistic zonation schemes for sessile communities in Mediterranean marine caves [40,51,52], it was observed that species typical of darker cave zones tend to shift towards the cave entrance with increasing depth. Examples include *Thorogobius ephippiatus*, *Stenopus spinosus*, and *Plesionika narval*, which were encountered closer to the entrance in deeper caves.

Apogon imberbis was the most widespread and abundant fish in most caves, echoing findings from Italy and France [11,53], where it comprised around 75% of the total fish density in caves [54]. However, in deeper caves, *A. imberbis* was usually replaced by *Anthias anthias*, while atherinids dominated the entrance and semidark zones of some semi-submerged caves forming large schools close to the surface. Among decapods, *Stenopus spinosus* was the most common (in 17 caves), followed by *Brachycarpus biunguiculatus* (in 15 caves) and *Dromia personata* (in 11 caves), in contrast with earlier studies where *Palaemon serratus* and *Herbstia condyliata* were more prominent [13,20].

Based on our findings and prior faunal categorization schemes, e.g., [2,19,20,22,53–55], motile taxa in the studied marine caves of the eastern Mediterranean can be assigned into the following ecological groups in relation to their degree of association with the cave environment: (1) accidental visitors (e.g., *Pelagia noctiluca*), often carried by currents and trapped inside the cave; (2) diurnal reef-associated species (e.g., *Diplodus sargus*, *Coris julis*, and *Chromis chromis*), which can occasionally be found in caves, usually close to the entrance; (3) trophic associates dependent on prey (typically but not exclusively) found within caves (e.g., *Peltodoris atromaculata* feeding on sponges); (4) cryptic habitat dwellers or “cave within cave” species, i.e., species associated with cryptic cave-like “mesolithial habitats”, such as crevices, empty holes of endolithic bivalves, overhangs, and fissures (e.g., *Gammogobius steinitzi*, *Scyllarus pygmaeus*, and the introduced *Urocaridella pulchella*); (5) nocturnal shelter-seekers, i.e., species finding shelter inside caves during daytime (e.g., *Apogon imberbis*, mysids, and the introduced *Pempheris rhomboidea*, and *Sargocentron rubrum*); (6) deeper-water species (e.g., *Grammonus ater*, *Scorpaenodes arenai*, and *Stylocidaris affinis*); (7) speleophilic species (typically but not exclusively) inhabiting and reproducing in marine caves (e.g., *Stenopus spinosus* and *Palaemon serratus*); and (8) cave-exclusive species (stygobionts), not observed in other environments than caves. Species belonging to the latter category were not observed in the studied marine caves, but they are known from other studies, e.g., [56,57]. It should be noted that some taxa could be assigned to more than one category (e.g., *Muraena helena* is both cryptic habitat dweller and nocturnal shelter-seeker). Categories 4–7 include species considered to exhibit secondary stygobiosis, since they originate from external marine environments and only secondarily inhabit marine caves [2,58].

In this study, the biodiversity inventory from the 27 studied marine caves revealed several rarely reported, protected, and introduced species, including new records for the

study area and the marine cave habitat, thereby filling biogeographical and ecological knowledge gaps.

4.1. Rarely Reported Species

Overall, 17.8% of the identified taxa (29 species) were recorded for the first time in marine caves of the eastern Mediterranean Sea (Table 2), while 9.8% (16 species) were recorded for the first time in Mediterranean marine caves [15,19,20,22,25,59]. Some notable examples are presented below along with photographic evidence (Figure 7).

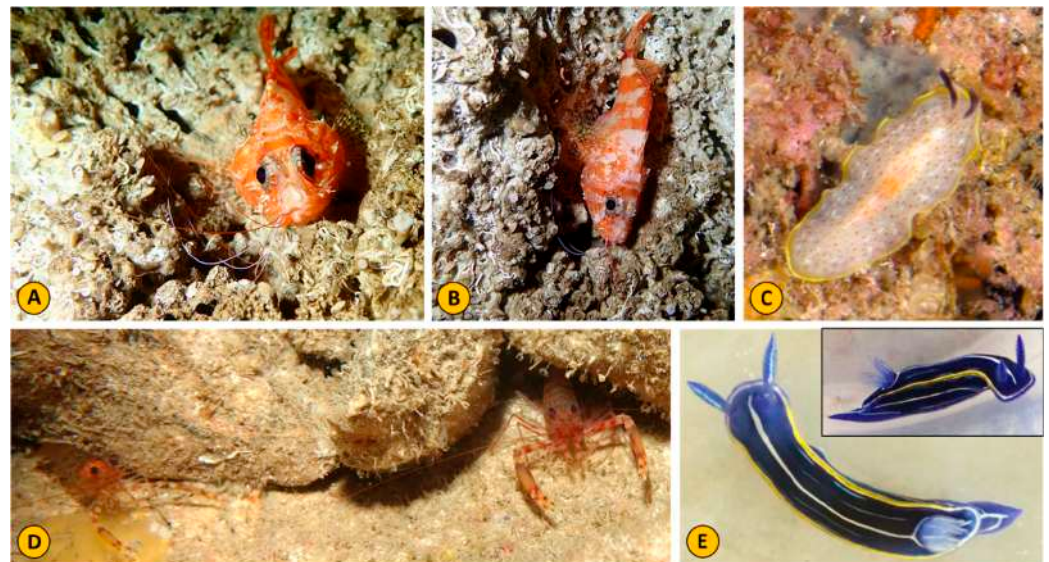


Figure 7. The Messina rockfish *Scorpaenodes arenai* eating the shrimp *Plesionika narval* in a deep marine cave of Zakynthos Island ((A): front view; (B): side view); the flatworm *Prostheceraeus moseleyi* photographed at the entrance of a cave in Saria Island (C); two individuals of the decapod *Brachycarpus biunguiculatus* (D) and the collected nudibranch *Felimare gasconi* from a shallow marine cave of Crete (E). Photos by M. Digenis (A,B,E), T. Dailianis (C), and M. Ragkousis (D).

One of the most striking examples of rarely reported species was the finding of the Messina rockfish *Scorpaenodes arenai* in the same cave where it was first recorded in 2015 (Shrimp cave, Zakynthos Island), representing the shallowest Mediterranean records of this deep-water species and the only site where it can be observed through SCUBA diving [60]. Two individuals were sighted in an upside-down position in the semidark and dark zones of the cave (Figure 7A,B). The rockfish was recorded for the first time as feeding on the shrimp *Plesionika narval*, shedding light on its trophic habits inside marine caves.

The shrimp *Brachycarpus biunguiculatus* was observed almost exclusively in the semi-dark and dark zones of 15 caves, with up to 14 individuals in a single shallow cave in southern Crete (Figure 7D). Due to its highly cryptic habits and its nocturnal behavior, this species seeks shelter in dark environments such as caves, where it is mostly recorded in the Mediterranean Sea [20,61]. The frequent presence of *B. biunguiculatus* in 55% of the herein studied caves indicates that the species is a lot more common than previously thought.

Two of the recorded species, the flatworm *Prostheceraeus moseleyi* and the nudibranch *Felimare gasconi*, are herein reported for the first time in Greek waters as well as in the cave environment increasing the known fauna of Mediterranean marine caves. The flatworm *P. moseleyi* was observed in the entrance and semidark zones of two caves on Karpathos Island (Achata 2 and Vronti cave), and more recently at the entrance of a cave on the nearby Saria Island (35.854402° N, 27.193404° E), during another expedition (authors' personal observation). This flatworm is easily distinguished from other congeneric Mediterranean species by its unique color patterns [62] (Figure 7C). The nudibranch *F. gasconi* was collected

from the dark zone of Lithistid cave in Crete (Table 2, Figure 7E). This dark blue doriid is characterized by a notum edged with a yellow line, white markings in front of the rhinophores and behind the gill tuft, and a prominent thick white longitudinal line running dorsally [63].

Among the identified motile taxa, twelve are protected under the Bern and Barcelona Conventions and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Table 4). Protected taxa were recorded in most studied caves (25) across all geographical areas, with 1–3 protected species found per cave, highlighting the high conservation value of Mediterranean marine caves [2,64].

4.2. Introduced Species in Marine Caves

Approximately 10% of all motile species identified in the studied caves were introduced, with the highest richness and abundance in southeastern areas. This pattern is consistent with their known distribution in other marine habitats [31,65–67]. Several of these species were recorded for the first time in marine caves or in specific areas. The introduced portunid crab *Carupa tenuipes*, which can be distinguished by its pale orange coloration and fingers dark brown distally and along their inner margins [68] (Figure 8A), was recorded for the first time in the Ionian Sea, within the semidark zone of Keri 2 cave on Zakynthos Island. The decapod *Gonioinfradens giardi* and the gastropod *Conomurex persicus* are herein firstly reported from the marine cave habitat, raising the total number of introduced species in Mediterranean marine caves to 128 [37]. Two individuals of the portunid crab *G. giardi*, were recorded in the semidark zone of Neptune's cave on Karpathos Island, as far as 70 m from its entrance (Figure 8B). This species can be distinguished by the shape of its frontal lobes with two spines medially on the upper surface of its palms set close apart, its distinctive body color pattern, the small ancillary teeth between four well-developed teeth at the anterolateral border of the carapace, and light-banded dark-tipped anterolateral spines [69–71]. Another interesting finding was the presence of the shrimp *Urocaridella pulchella* in five caves, with up to 40 individuals in caves on Crete and Karpathos Islands (Figure 8E), mostly on soft substrate. This decapod was recently reported from Aegean and Ionian marine caves [72,73].

Several of the introduced fish species had considerably high abundances in the studied caves. Large aggregations of the well-established sweeper fish *Pempheris rhomboidea* were observed in the Blue 2 cave (Kastellorizo Island), with up to 400 individuals, half of which were juveniles (Figure 8C). Large schools of *Siganus* spp. were observed near the entrance of a cave in southern Crete (Figure 8D). *Pempheris rhomboidea* as well as some other introduced fishes (*Sargocentron rubrum*), undertake diurnal migrations to feed in outer environments, possibly enriching the oligotrophic cave interior with organic matter, in a manner similar to that of native species such as *Apogon imberbis* [53,54,74,75]. In contrast, other introduced species, such as *Pterois miles*, may negatively affect the cave ecosystem through predation and competition with native predators, as observed in [76,77] and through personal observations.

4.3. Marine Caves as Nursery Grounds

During the surveys, some species were found to be more common than expected, displaying interesting and little-known behavioral traits related to the marine cave habitat. For instance, the sea urchin *Arbacia lixula* was recorded in 78% of the surveyed caves (21 caves), constituting the most frequently reported echinoderm (Table 4). Although commonly regarded as a diurnal herbivore feeding primarily on crustose algae, it is actually an omnivore with a strong tendency toward carnivory, occasionally reaching high densities in barren states such as dark caves, where it avoids competition with the more dominant

sea urchin *Paracentrotus lividus* [78,79]. In addition, small and medium sized individuals of *A. lixula*, being more vulnerable to predation, compensate for their less-efficient morpho-functional defenses against predators by adopting cryptic behavior and seeking refuge in concealed microhabitats [78,80–82]. This behavior likely explains the presence of *A. lixula* in all ecological cave zones, with juveniles observed as far as 160 m away from the entrance in the Elephant cave (Crete).

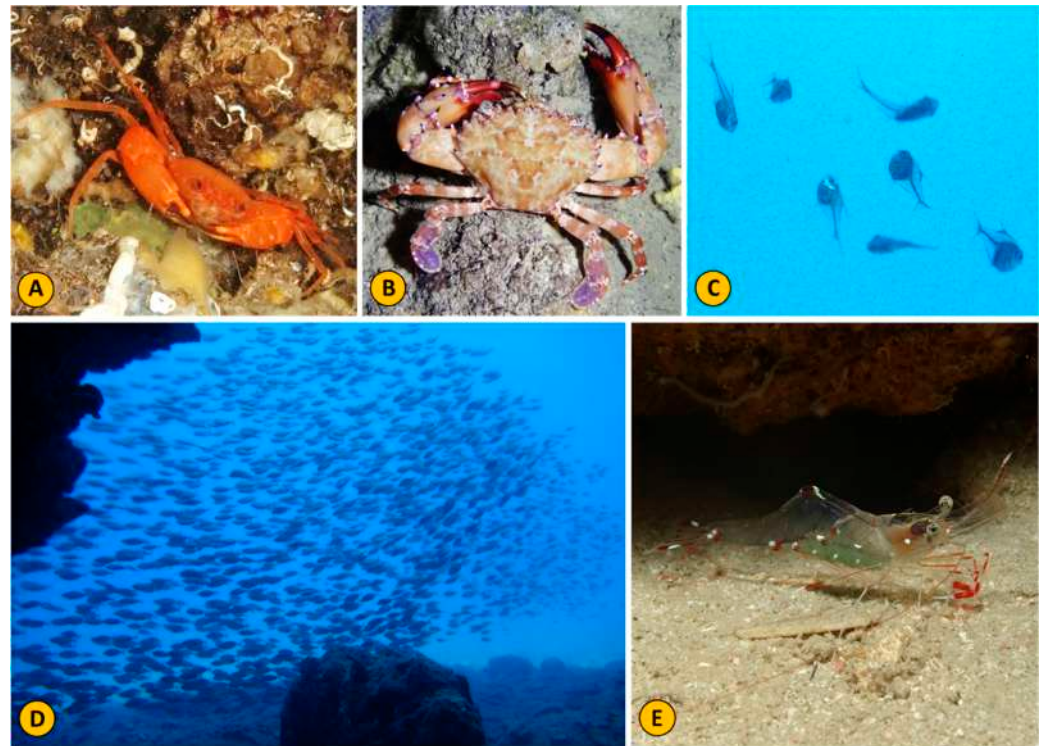


Figure 8. The introduced crabs *Carupa tenuipes* (A) and *Gonioinfradens giardi* (B) from the dark zone in a cave of Rhodes (Seal 2) and Karpathos Islands (Neptune’s cave), respectively; juveniles of the sweeper fish *Pempheris rhomboidea* (C) from the cave entrance of Blue 2 cave in Kastellorizo Island; school of *Siganus* spp. passing in front of Gliko Nera cave entrance in south Crete (D); the shrimp *Urocaridella pulchella* (E) from the semidark zone of a cave in south Crete. Photos by M. Ragkousis (A,E), M. Digenis (B,C), and V. Gerovasileiou (D).

Mediterranean marine caves seem to serve as nursery grounds for certain species, such as *Antedon mediterranea*, a crinoid only sporadically reported from caves [2,83]. In this study, although *A. mediterranea* was observed in low numbers (in only four caves), we recorded a remarkably high abundance with numerous juveniles in the semidark zone of a submerged cave with freshwater inflow in southern Crete (personal observation). This observation probably represents the first documented case of caves functioning as a breeding ground for this echinoderm species.

Additionally, the cave interior may serve as an egg-laying site for cuttlefish, as eggs of *Sepia* spp. were recorded in the semidark zone of three caves distributed from the east to the central Aegean and Crete. Although other cephalopods, such as octopuses, have previously been reported to lay eggs in caves [84], this appears to be the first documented observation of cuttlefish eggs in Mediterranean marine caves.

4.4. Limitations of the Applied Methodology

The applied methodology enabled the identification of a high number of motile species through a standardized quantitative protocol requiring only one observer and non-decompression single-dive surveys. Nevertheless, since the proposed rapid assessment

protocol is time limited—due to the logistic constraints of diving in space-limited and dark environments—it does not allow for a complete assessment of motile cave biota. The in-transect/total species richness percentage ratio differed among zones likely due to the varying light level and spatial extent of each zone. Specifically, the highest percentage at the cave entrance was probably due to the better visibility and its smaller size while the lower percentages in the dark zone are attributed to the poorest visibility and low species richness. The semidark zone presented the lowest proportion of in-transect to total species richness and abundance due to its largest size and higher heterogeneity in terms of resources and micro-habitats [3,4,40,85]. Thus, lower ratios are expected to be recorded in caves with extensive semidark zones, lower visibility (e.g., caves with sulfur springs and microbial mats) or increased surface complexity with several micro-habitats (e.g., submerged ceilings, fissures, and overhangs). The high small-scale heterogeneity of the cave habitat affects species diversity and distribution, with different species occupying specific sections and micro-habitats within caves [22]. To better capture this variability, the use of modified transect methods and quadrat sampling across distinct ‘sub-habitats’ (e.g., walls, ceilings, and floor) has been suggested, although these approaches typically require multiple dives or extended dive times [22,53,55].

In this study, taxa that could not be reliably identified in situ at species level were recorded at higher taxonomic ranks (e.g., *Atherina* spp., *Clanculus* spp.). Cryptic and small sized species such as cryptic habitat dwellers (e.g., *Microlipophrys nigriceps*, *Corcyrogobius liechtensteini*, *Marcelogobius splechnai*, *Gammogobius steinitzi*) are likely underrepresented, especially in less-studied caves, such as deeper caves or caves with complex morphology. The combined approach adopted in this work, which included photographic documentation and qualitative sampling, seems to mitigate the restrictions of the time-limited visual census, as evidenced by the high total number of species recorded. However, to accurately assess the distribution and abundance of cryptobenthic fishes in marine caves, more targeted methodologies should be considered, such as the use of anesthetic agents and quadrat sampling [55].

Annual, seasonal, and diurnal variations are also expected to influence the species richness and abundance of motile taxa, especially for occasional visitors and/or highly mobile species (e.g., *Atherina* spp. and mysids). Although our visual census protocol was applied across different years and periods (Table 1), the majority of surveys (63%, corresponding to 17 caves) were carried out during the warm season (typically April to September in the Mediterranean Sea) and consistently during morning to midday hours.

Although having a single observer entering first in the cave is expected to reduce disturbance, some highly mobile species (e.g., fishes) may still relocate toward outer or inner cave sections or hide in micro-habitats before being noticed. Finally, the involvement of multiple observers can introduce variability in species detection and data collection [86]. Thus, to minimize such bias, the same diver consistently applied the visual census protocol in all surveyed caves.

5. Conclusions

The assessment of motile cave fauna in a large number of marine caves in the eastern Mediterranean Sea enabled—for the first time—the quantitative characterization of their motile assemblages, employing a standardized rapid visual census protocol. Through the suggested methodology, more than 60% on average of the motile cave fauna observed in situ can be recorded, providing information on species richness and abundance. Such data are vital for assessing the ecological quality of marine caves [33,87] and for informing long-term monitoring initiatives, especially in marine protected areas.

The high number of new species records for marine caves in the eastern Mediterranean, and for cave habitats overall, demonstrates that our knowledge regarding cave biota is far from complete. In order to understand the distribution pathways of current and future cave inhabitants, it is important to examine the association between motile species assemblages in caves and nearby habitats such as rocky reefs. Given the unique biodiversity of marine caves, their low ecological resilience, and the increasing occurrence of several introduced species, this key habitat emerges as particularly vulnerable. Notably, six of the identified introduced species are listed among the worst Mediterranean invasives [88], and recent studies have highlighted the urgent need to investigate their potential ecological impacts on cave biota [38]. The population explosion of certain introduced species and their increasing abundance in caves of southeastern subareas, as seen in [36,37,72,89] and the present study, indicate a rapid shift in the cave ecosystem. Therefore, the protection of certain caves should be prioritized. Among these caves is the Shrimp cave of Zakynthos Island, a currently uninvaded cave within an otherwise invaded area, which represents the shallowest known Mediterranean habitat of the deep-water Messina rockfish *S. arenai*. Additionally, the Lithistid cave in southern Crete and Seal's 3 cave on Samos Island stand out as the two most species-rich caves studied. In this context, the long-term monitoring of the motile cave communities through seasonal surveys is recommended, particularly within newly established high-priority protected areas. However, the absence of historical baseline restricts our understanding of change in cave communities. This study provides a baseline for future comparisons of cave communities between invaded and uninvaded caves [90], and for future monitoring areas that are still unaffected or only minimally impacted in view of the continuous expansion of thermophilic introduced species due to climate change.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes10080383/s1>. Figure S1: Number of motile taxa recorded within time transects compared with all taxa recorded in situ during a single dive survey in each studied cave. Figure S2: Number of motile taxa recorded within time transects in the cave entrance (CE), the semidark (SD), and the dark zone (D) compared with all taxa recorded in situ during a single dive survey in each studied cave. Figure S3: Abundance recorded within time transects compared with all taxa recorded in situ during a single dive survey in each studied cave. Figure S4: Abundance recorded within time transects in the cave entrance (CE), the semidark (SD), and the dark zone (D) compared with the total abundance recorded in situ during a single dive survey in each studied cave. Table S1: Dissimilarity percentage analysis (SIMPER) showing the contribution of motile taxa to the average dissimilarity (%) between the subareas east Aegean and Crete. Table S2: Pair-wise tests for the two-way PERMANOVA analysis conducted with the factor 'Cave' nested as random within the fixed factor 'Subarea' for the total of motile taxa recorded in transect. Table S3: Construction of pseudo-F ratio from mean squares from the two-way PERMANOVA analysis conducted with the factor 'Cave' nested as random within the fixed factor 'Subarea' for the total of motile taxa recorded in transect. Table S4: Construction of pseudo-F ratios from mean squares from the one-way PERMANOVA analysis conducted with the factor 'Zone' within each subarea for the total of motile taxa recorded in transect. Table S5: Dissimilarity percentage analysis (SIMPER) showing the contribution of motile taxa to the average dissimilarity (%) between the cave entrance (CE) and the semidark zone (SD). Table S6: Dissimilarity percentage analysis (SIMPER) showing the contribution of motile taxa to the average dissimilarity (%) between the cave entrance (CE) and the dark zone (D). Table S7: Dissimilarity percentage analysis (SIMPER) showing the contribution of motile taxa to the average dissimilarity (%) between the semidark zone (SD) and the dark zone (D). Table S8: Pair-wise tests for the two-way PERMANOVA analysis conducted with the factor 'Cave' nested as random within the fixed factor 'Subarea' for fishes recorded in transect. Table S9: Construction of pseudo-F ratio from mean squares from the two-way PERMANOVA analysis conducted with the factor 'Cave' nested as random within the fixed factor 'Subarea' for fishes recorded in transect. Table S10: Construction of pseudo-F ratios from mean squares from the one-way PERMANOVA analysis conducted with the factor 'Zone' within

each subarea for fishes recorded in transect. Table S11: Dissimilarity percentage analysis (SIMPER) showing the contribution of fish species to the average dissimilarity (%) between the cave entrance (CE) and the semidark zone (SD). Table S12: Dissimilarity percentage analysis (SIMPER) showing the contribution of fish species to the average dissimilarity (%) between the cave entrance (CE) and the dark zone (D). Table S13: Dissimilarity percentage analysis (SIMPER) showing the contribution of fish species to the average dissimilarity (%) between the cave entrance (CE) and the dark zone (D).

Author Contributions: Conceptualization and methodology, M.D. and V.G.; sampling: M.D., M.R. and V.G.; identified samples, M.D. and V.G.; formal analysis, M.D., C.D. and V.G.; writing—original draft preparation, M.D.; writing—review and editing, V.G., M.R., C.D. and S.K.; funding acquisition, M.D., S.K. and V.G. All authors have read and agreed to the published version of the manuscript.

Funding: The research was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 10597). Part of the sampling activities was funded through the projects “First Call for HFRI Research Projects to support faculty members and researchers and the procurement of high-cost research equipment grant” (Project ALAS—‘ALiens in the Aegean—a Sea under siege’, project number: HFRI-FM17-1597) (3/2020-3/2023), and the “STUDIOTOPIA—Art meets Science in the Anthropocene (2019–2022)” residency program, hosted by Onassis Stegi and co-funded by the Creative Europe programme of the European Union. Additional support for the 2021 sampling campaign was provided to V.G. by the project “Centre for the study and sustainable exploitation of Marine Biological Resources (CMBR)” (MIS 5002670), implemented under the Action “Reinforcement of the Research and Innovation Infrastructure”, funded by the Operational Programme “Competitiveness, Entrepreneurship and Innovation” (NSRF 2014–2020) and co-financed by Greece and the EU (European Regional Development Fund).

Institutional Review Board Statement: The research has been accepted for implementation by the Research Ethics and Deontology Committee of the Ionian University (Approval code: 1036, Approval date: 2023-04-06).

Informed Consent Statement: Not applicable.

Data Availability Statement: All relevant data are contained within the article and its Supplementary Materials.

Acknowledgments: The authors are grateful to Dimitris Podaras and Maria Naletaki for their assistance with the identification of echinoderm and decapod samples, respectively; Fabio Crocetta for his help with mollusk identification; and Pierre Chevaldonné for verifying the identification of mysid specimens. We also thank Thanos Dailianis for providing one of the photos presented (Figure 7C) and for his contribution to some of the sampling campaigns. We are grateful to the Management Units of the Southeastern Aegean Protected Areas, Samaria National Park, and the Protected Areas of Western Crete, and Zakynthos and Ainos National Parks and Protected Areas of the Ionian islands of the Natural Environment and Climate Change Agency (NECCA) for granting sampling permissions in marine caves within these protected areas. We also wish to thank Dennis Mohr and the staff of Nero Sport Diving Center (Limni Keriou, Zakynthos), Nikos Giannoulakis from the Chania Diving Center (Chania, Crete), and Dinos Protopapas from the Karpathos Diving Center (Pigadia, Karpathos) for their valuable expertise and support during fieldwork in Zakynthos, Crete and in Karpathos Islands, respectively. Finally, we warmly thank Carlos Navarro-Barranco, Juan Sempere-Valverde, Sahar Chebaane, Alfredo Marchiò, and Ioannis Rallis for their assistance during fieldwork.

Conflicts of Interest: The authors declare no conflicts of interest.

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