ECOLOGICAL ZONATION AND ABIOTIC VARIABILITY IN AN ENCLOSED NATURE PARK: THE CASE OF THE SALINI SALTWORKS IN MALTA

Martina Busuttil, Robin Caruana Montaldo, Lena Ehmcke, Elise Bartl, Francesco Giacalone, Alessandro Giuffrida, Sarina Hilke, Giulia Lembo, Andrea Li Vorsi, Shona Murphy, Elia Ponte, Natalia Szklarczyk, Katya Debono, Belinda Gambin, Sandro Lanfranco

Abstract: The Salini saltern complex (SSC), was a natural wetland that now functions as a nature park. The SSC is oriented along a NE to SW axis measuring 740 m by 140 m, with a surface area of 90000 m^2. It comprises two reservoirs at the seaward extremity (each 12000 m^2 in area) and 33 smaller pans in the inland portion, with surface areas ranging from 900 m^2 to 2000 m^2. This study addressed a knowledge gap by recording abiotic and ecological conditions across the SSC and relating them to the external marine conditions. The salina was visited monthly between 25 July 2022 and 5 July 2023. During each visit, the temperature, pH, electrical conductivity, nitrate and phosphate concentration of water were measured in the open sea, in the two reservoirs, and in 15 pans. The diversity of macroalgae, spermatophytes, and non-planktonic fauna was evaluated through direct observation, collection of specimens and photographic monitoring. Water quality changed in spatial and temporal dimensions within the SSC. The principal trend was a sharp gradient of abiotic conditions along the primary axis of the SSC from the seaward side to the landward side, suggesting changes in the characteristics of water during its movement from the open sea into the reservoirs and the pans. The species richness increased from the open sea to the reservoirs and decreased to much lower levels in the pans. The elevated species richness in the reservoirs is attributable to the higher stability relative to the sea and pans.

Keywords: salinas, Malta, electrical conductivity, species richness

Martina Busuttil, University of Malta, Malta, martina.f.busuttil.17@um.edu.mt, 0009-0001-8230-3552
Robin Caruana Montaldo, University of Malta, Malta, robin.caruana-montaldo.20@um.edu.mt
Lena Ehmcke, University of Malta, Malta, lena.ehmcke.20@um.edu.mt
Elise Bartl, University of Malta, Malta, elba.book@freenet.de
Francesco Giacalone, University of Malta, Malta, francesco.giacalone996@gmail.com
Alessandro Giuffrida, University of Malta, Malta, alessandro_01@hotmail.it
Sarina Hilke, University of Malta, Malta, sarina.hilke@freenet.de
Giulia Lembo, University of Malta, Malta, giuliab.lembo@gmail.com
Andrea Li Vorsi, University of Malta, Malta, andrealv.alv@gmail.com
Shona Murphy, University of Malta, Malta, shonamariemurphy@gmail.com
Elia Ponte, University of Malta, Malta, turn@live.it
Natalia Szklarczyk, University of Malta, Malta, nat.szklarczyk@gmail.com
Katya Debono, University of Malta, Malta, katya.debono.22@um.edu.mt, 0009-0007-2389-478X
Belinda Gambin, University of Malta, Malta, belinda.gambin@um.edu.mt, 0000-0001-5233-254X
Sandro Lanfranco, University of Malta, Malta, sandro.lanfranco@um.edu.mt, 0000-0002-0360-7065

Referee List (DOI 10.36253/fup_referee_list)
FUP Best Practice in Scholarly Publishing (DOI 10.36253/fup_best_practice)

Martina Busuttil, Robin Caruana Montaldo, Lena Ehmcke, Elise Bartl, Francesco Giacalone, Alessandro Giuffrida, Sarina Hilke, Giulia Lembo, Andrea Li Vorsi, Shona Murphy, Elia Ponte, Natalia Szklarczyk, Katya Debono, Belinda Gambin, Sandro Lanfranco, *Ecological zonation and abiotic variability in an enclosed nature park: the case of the salini saltworks in Malta*, pp. 101-111,
DOI: 10.36253/979-12-215-0556-6.08

Introduction

The climatic and oceanographic conditions in the central Mediterranean, characterised by long, dry summers and mild, wet winters on microtidal shores, are ideal for the natural evaporation processes required for salt production. Over centuries, these conditions have been exploited for economic purposes through the construction of 'salinas', coastal complexes focused on the production, harvesting, and storage of sea salt. Although the practice continues to the present-day, modern salinas not only serve economic purposes but also have significant ecological roles [1, 2], with many transformed into nature reserves, providing critical habitats for various species. These habitats, characterised by high salinity and fluctuating water levels, support uncommon communities adapted to these conditions. The relatively undisturbed nature of most salinas, due to their remote locations and regulated human intervention, has allowed these ecosystems to persist over time [3].

The Salini Saltern Complex, henceforth referred to as the 'SSC', is the largest saltworks facility in Malta, and forms part of the Salini Nature Reserve, a managed protected area. The SSC measures approximately 740 m by 140 m, with a surface area of 90000 m^2 and is oriented along a NE to SW axis. It comprises two large reservoirs at the seaward extremity (each 12000 m^2 in area) and 33 smaller enclosures ('pans') in the inland portion, with surface areas ranging from 900 m^2 to 2000 m^2 (Figure 1). The top of the walls of the pans are approximately one metre above mean sea level, although the height difference between the top of the walls and actual sea level changes depending on microtidal variations and marine surges. The SSC receives water from the sea through the two large reservoirs at the seaward end and a series of channels convey the water to the 33 inner pans. The SSC also receives episodic freshwater input from rainfall and surface runoff.

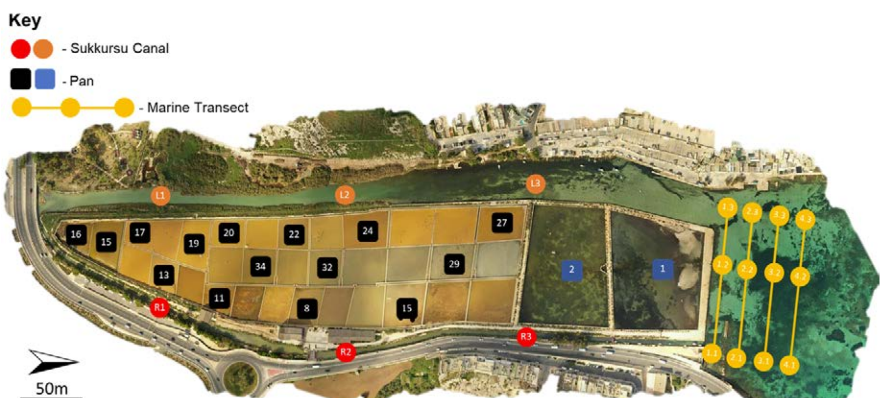

Figure 1 – General view of the SSC and the adjacent marine environment. The arrow at lower left indicates North. The numbered saltpans are the ones that were sampled during this study. Pans 1 and 2 are the large 'reservoirs'. The 'Sukkursu' canal is a seawater channel that encircles the SSC and opens to the sea at both ends.

General Aim

Although the SSC has been the subject of recent studies [4, 5, 6], these have either focused on a specific taxon, usually avifauna, or have assessed the nature reserve in isolation with little regard for its external context. There is little consistent information regarding the variation of physico-chemical conditions in the SSC and their possible linkages with the marine environment, from which almost all their water derives. This study therefore aimed to address this knowledge gap by recording trends in abiotic and ecological conditions across the primary axis of the SSC over a period of one year and relating them to the external marine conditions.

Materials and Methods

The broad aims of the study were achieved by measuring 'water quality', defined as the temperature, pH, electrical conductivity, nitrate concentration, and phosphate concentration of water and the overall 'biological characteristics', described by 'plankton', macroalgae, spermatophytes, and macrofauna in representative parts of the SSC and its environs over the period of study. Subsequent analysis of these data elucidated any relationships between abiotic gradients and biotic characteristics.

Data were collected from several sample sites within the SSC and its immediate environs (Figure 1) as follows:

(1) The open sea outside the salterns. This was sampled along three virtual transects, with six sample points on each transect, giving a total of eighteen sample points.

(2) The two large reservoirs at the seaward part of the SSC, each of which represented one sample point.

(3) The 'inner pans': a sample of 15 from the 33 smaller saltpans were selected for study. The 15 pans were selected systematically, ensuring a spread of sample sites from the seaward part to the landward part. The individual pans were numbered sequentially for ease of identification.

Collection of data

The SSC was visited at approximately monthly intervals for one calendar year, between 25 July 2022 and 5 July 2023. Visits were generally carried out during the late morning and early afternoon, to maximise comparability of measurements recorded during different sessions. During each visit, the water parameters of interest were measured in all sample points (18 in the open sea, the two large reservoirs, and 15 smaller pans). The diversity of plankton, macroalgae, spermatophytes, and non-planktonic fauna (excluding interstitial fauna) was evaluated through the methods described later in this section

Water quality

Electrical conductivity (EC), pH, water temperature, nitrate concentration, and phosphate concentration were measured monthly at each sample point during every field visit using Hanna Instruments HI98194 and HI9829-13042 multiparameter meters that were calibrated before use. In each case five replicate readings were taken and averaged. In parallel with this, pH and water temperature were also measured at 90-minute intervals throughout the duration of the study, in the two large reservoirs using two HOBO Onset MX2501 data loggers. These were initially installed on July 25, 2022, and remained submerged in the water until April 3, 2023. The loggers were only taken out of the water briefly during the monthly visits to download the data and reinitialise them.

Measurement of nitrate and phosphate concentration was carried out in the laboratory after each field visit. Water samples were collected from each sample point, transported to the laboratory in a refrigerated container, and subsequently stored at a temperature of -20C until analysis. Nitrates were measured using a Hanna Instruments HI96786C Nitrate ISM spectrophotometer. Phosphates were measured using a Hanna Instruments HI83399 for 'ultra-low range' phosphates, and a Hanna Instruments HI97713 for 'low range' phosphates in cases where the phosphate reading exceeded the range of the HI83399 device. Device protocol was followed for all nitrate and phosphate measurements.

Plankton

'Plankton', here defined as microscopic organisms passively transported by water currents, were considered as a single functional group to give an indication of the comparative productivity across sample sites. A volume of 0.5 L of water from the surface layer (0-50cm depth) was collected from each sample point during every visit and refrigerated at 4C. These were subsequently transported to the laboratory and analysed as soon as possible. Initial microscopic surveys were carried out on unmodified samples whilst enumeration of plankton was carried out following fixation with Lugol's Iodine. To ensure an even distribution of any plankton present, the water samples were gently shaken prior to microscopic examination. Plankton densities were determined through direct enumeration of plankton cells or aggregates in water. Subsequently, one drop from each sample was taken, transferred into a glass cavity slide, and examined using a Bresser ADL 601 microscope, equipped with a Nikon D5300 Digital Camera. Five micrographs were captured at a magnification of x100. This process was repeated ten times for every sample. All micrographs were subsequently processed using ImageJ 1.53c [7] to facilitate supervised counting of plankton. Planktonic microorganisms were identified to the lowest taxonomic level possible considering the instrumentation available.

Macroscopic fauna

Macroscopic fauna in the two large reservoirs were monitored through analysis of video recordings. Two GoPro Hero 10 cameras were installed in these sample sites and were programmed to record video continuously for approximately 45 minutes during each visit.

Data from the marine sample points was collected by conducting underwater visual surveys (UVSs). These surveys involved establishing a transect measuring 150 m in length and 2 m in width. To ascertain this distance accurately, the cycle kicks during the 150 m swim were tallied. The combination of cycle kick counts, navigation proficiency, and the utilisation of GPS data confirmed both the length and direction of the transect [8]. Data acquisition employed a non-invasive approach through the utilisation of video capture using a GoPro Hero 10 camera to mitigate potential biases and induced behaviours during the research [9, 10, 11]. Video footage of the transect was systematically recorded at regular intervals to provide a comprehensive record of species presence and abundance that was subsequently subjected to detailed analysis [9]. Transect surveys were rigorously conducted in accordance with a standardised fish visual census protocol [12]. Adherence to this protocol necessitated maintaining a consistent swimming speed of approximately ten metres per minute for each 50 metre transect, resulting in an average survey duration of around five minutes. While executing the survey, a two-metre distance on either side of the transect midline was diligently observed while swimming at the water's surface. Ensuring a minimum spatial separation of 20 metres between consecutive transects was imperative. Upon the completion of each transect, reviewing the virtual transect allowed for the analysis of photographic data portraying the habitat landscape and any observed instances of fish sightings. To address the challenge of counting fish within large groups, the frame-by-frame mode, in conjunction with the geometric measurements tool, was employed. This approach facilitated precise and systematic fish counting to differentiate individual fish within shoals. The combined use of ethograms, species-specific coding, and geometric measurements offered a comprehensive method for accurate and detailed counting of fauna.

Spermatophytes and macroalgae

Spermatophytes and macroscopic algae were systematically sampled through direct observation by traversing the sample sites in a grid pattern. Each specimen encountered was collected, stored in a refrigerated plastic container, and transported to the laboratory for analysis. Each specimen collected was initially examined using an AmScope stereo microscope at magnifications up to x45. Where necessary, specimens were further examined using a Bresser ADL 601 microscope at a magnification of x100. Specimens were identified to the lowest possible taxonomic level using various guides [13, 14].

Management and analysis of data

All data collected was coded in 'tidy' format [15] and analysed using R [16] through the RStudio interface [17].

Results

Water quality: spatial variation

The physico-chemical characteristics of the water in the marine samples and in the samples taken from the SSC are summarised in Table 1 and in Figures 2 to 4.

Table 1 – Water quality parameters in the sea and in the SSC (large reservoirs and small pans considered together). The pH value marked with an asterisk may represent instrumental error. Mean values are accompanied by the standard deviation.

Parameter	**Range (Marine)**	**Mean (Marine)**	**Range (SSC)**	**Mean (SSC)**
EC (µS/cm)	4320 ÷ 59740	52351 ± 9320	1425 ÷ 200000	109389 ± 40423
pH	6.28 ÷ 8.44	7.90 ± 0.37	6.30 ÷ 14.00*	9.03 ± 1.89
Nitrate (mg/L)	0.10 ÷ 68.10	7.96 ± 11.33	0.4 ÷ 99.3	11.20 ± 16.84
Phosphate (mg/L)	4.00 ÷ 2500.00	121.60 ± 259.28	1.0 ÷ 2500.0	122.02 ± 339.55

The results indicated that the principal trend in water quality was a gradient of electrical conductivity (EC), pH, and nitrate concentration along the primary axis of the SSC from the seaward side to the landward side, suggesting clear changes in the characteristics of water during its movement from the open sea into the reservoirs and the pans. The spatial trend was statistically significant for EC and pH and marginally non-significant for nitrate concentration (Table 2). Variations in the phosphate content of the water showed no discernible spatial pattern between the open sea and SSC (Figure 2).

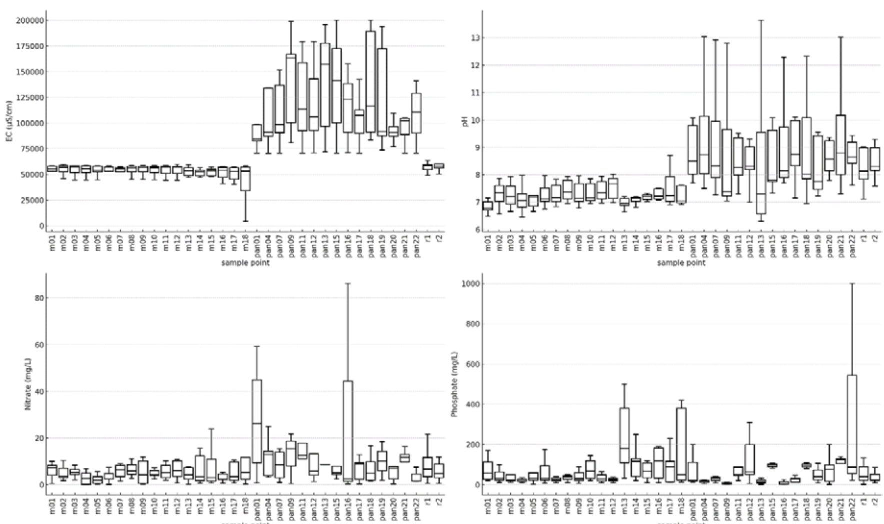

Figure 2 – Variation of four physico-chemical parameters (EC, pH, nitrate, phosphate) in the marine sample points (m01 to m18), the large reservoirs (r1 and r2) and the inner saltpans (pan01 to pan15).

Table 2 – Comparison of the median value of four physico-chemical parameters in the marine sample points and the SSC. The p-value represents the probability of a statistically significant difference between the marine samples and those taken from the SSC.

Parameter	Median (Marine)	Median (SSC)	t	p
EC (µS/cm)	55550	96700	-25.33	< 0.0001
pH	7.16	8.34	-14.9	< 0.0001
Nitrate (mg/L)	3.8	7.6	-1.80	0.075
Phosphate (mg/L)	38.0	37.5	0.43	0.670

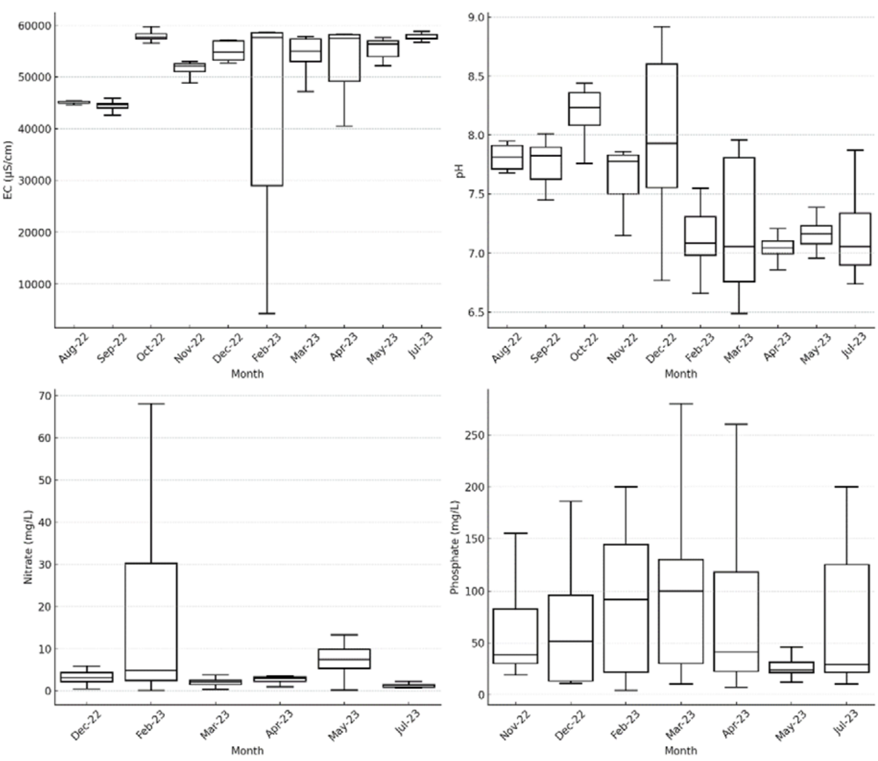

Figure 3 – Variation of four physico-chemical parameters (EC, pH, nitrate, phosphate) across all sample points in different months for the marine samples.

Water quality: temporal variation

Temporal variations in pH on daily and seasonal scales were unremarkable and reflected predictable patterns driven by photosynthetic activity, whilst EC, nitrate, and phosphate concentrations showed no discernible trend over one calendar year (Figure 2). Conditions in the innermost saltpans, those furthest from the sea, were extreme during the dry season with supersaturated hypersaline water, underwater precipitation of evaporites, and water temperatures sometimes exceeding 50 °C. It should be remarked that some very high pH values (approximately pH 14) recorded from the inner saltpans were initially assumed to be the results of instrumental error. These measurements were however repeated with separate instruments, giving very similar results.

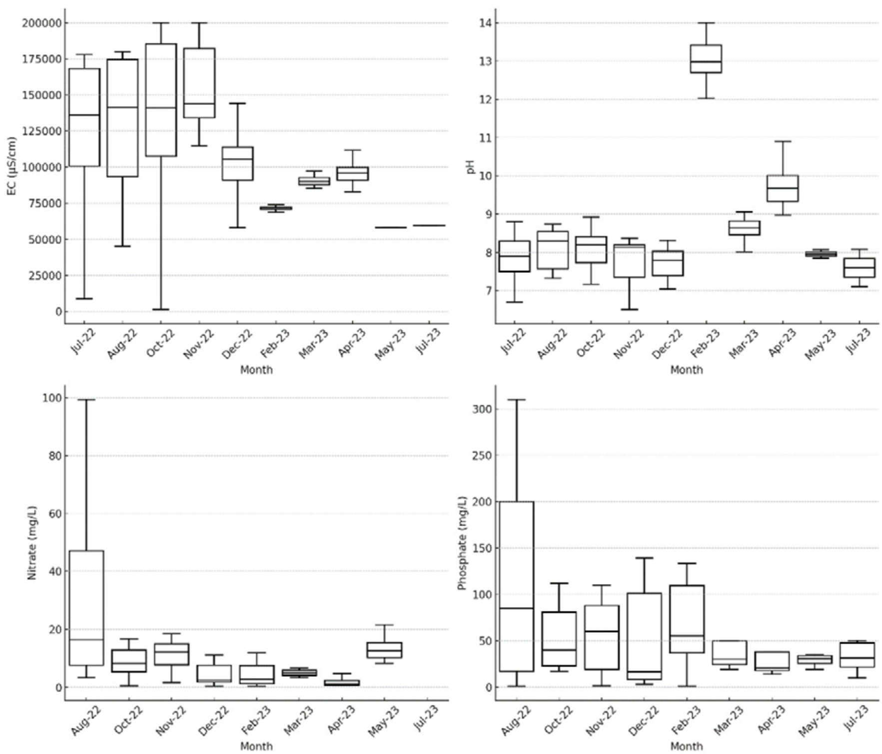

Figure 4 – Variation of four physico-chemical parameters (EC, pH, nitrate, phosphate) across all sample points in different months for the saltpan samples.

Biota

The results of the ‘biodiversity’ surveys are here summarised qualitatively. This assessment highlighted a clear decrease in species richness from the large reservoirs to the inner saltpans. The reservoirs, characterised by more stable conditions, supported a diverse array of macroalgae, spermatophytes, and macroscopic fauna.

Overall plankton density was higher in the saltpans relative to the open sea. These counts were comparatively high between August to October 2022, declining sharply from November 2022 onward. The frequency of macrofaunal sightings was much higher in the two large reservoirs relative to the open sea and the inner saltpans, with sightings increasing throughout 2023 compared to 2022. The macrofauna within the reservoirs included 27 species of fish, a much higher species density compared to the open sea. The reservoirs were also colonised by various species including the Blue Swimmer Crab (*Portunus armatus*), Mediterranean Stone Crab (*Eriphia verrucosa*), and Upside-Down jellyfish (*Cassiopea andromeda*), in much higher densities than the open sea. The inner saltpans were attractive to avifauna, with 20 species of birds recorded using the saltpans as feeding grounds during the study. The most abundant were gulls (*Larus* spp.) whilst occasional visitors included flamingos (*Phoenicopterus ruber*).

In the case of algae and spermatophytes, the flora of the two large reservoirs was predominantly of marine origin, including *Posidonia oceanica*, *Cymodocea nodosa*, *Ulva* sp., *Halophila stipulacea*, *Cladophora* sp. and *Acetabularia* sp. However, species associated with brackish water, including *Ruppia* sp. were also present in high densities. The innermost saltpans, subjected to extreme conditions such as hypersalinity and high temperatures, supported only a few highly adapted species, including the halophilic algae *Dunaliella salina*.

Discussion

In general, salinas are relatively simplified habitats with definite boundaries and with predictable physico-chemical and ecological gradients. Electrical conductivity generally increases with distance away from the sea and biological diversity decreases along the same spatial axis [3], although not necessarily proportionately [18]. The electrical conductivity gradient in the SSC largely followed this pattern, with increasing concentration of salt along the inland axis. The pH trend was similar, with an abrupt increase in the saltpans relative to the open sea. This trend is less straightforward to explain, as it depends on the chemical composition of the brine [19]. Nitrate and phosphate content showed no clear spatial trend and the relatively high coefficient of variation for each parameter (nitrate: 142.9, phosphate: 289.7) suggests that conditions in individual pans were the most likely cause of any variations. Much of the organic content in individual saltpans was associated with the presence of avifauna, accounting for higher nitrate concentrations in the saltpans used as feeding and resting grounds by birds. The saltpans with higher nitrate content were usually characterised by dinoflagellate blooms during the warmer parts of the year.

In contrast to the physico-chemical characteristics, the general species diversity observed in the SSC may be best represented by a unimodal pattern, with relatively low diversity in the open sea and in the inner saltpans and with much higher species richness in the intervening reservoirs. This pattern is a consequence of the interaction of the chemical conditions and habitat stability in each of the three habitat segments considered (open sea, reservoirs, inner saltpans). The physico-chemical stability is highest in the open sea and reservoirs and lowest in the smallest saltpans and is related to buffering capacity. The small size of the inner saltpans gives them very little buffering capacity against large-scale environmental changes such as storm surges. Two storm surges were observed during the period of study, during which the associated rise in sea level flooded the SSC completely reverting the chemical conditions in the inner saltpans to marine conditions. The larger size of the reservoirs buffered much of this change. Following subsidence of the surges, conditions gradually reverted to those observed previously. These surges were also accompanied by widespread mortality of macrofauna and replacement of the planktonic assemblages with that characteristic of the open sea. The higher species diversity in the two large reservoirs may be explained in terms of their lower physico-chemical variability compared to the open sea and their different EC, providing habitat space for non-marine species adapted to those conditions, and to their function as a 'species trap'. In this case, species present in low densities in the open sea and therefore potentially undetected by occasional surveys, may translocate into the pans and establish a population there. The unbalanced fauna and abundance of food resources would then promote larger populations than those recorded from the specific marine area that was sampled, leading to higher alpha diversities.

Conclusion

The results of this study provide a preliminary foundation on which to base conservation and management strategies for the SSC, taking the adjacent marine environment into account. The primary function of the SSC is that of an ornithological reserve and the prevalent conditions provide the resources required to sustain this function. The elevated species richness in the two reservoirs is attributable to the greater stability of this portion of the habitat relative to the marine environment (in terms of mechanical energy) and the inner pans (in terms of physico-chemical extremes). In conclusion, the findings underscore the importance of considering both internal conditions and the external marine influence to inform effective habitat management strategies. These should focus on maintaining the conditions that support the assemblage of species, in the more stable reservoirs that serve as biodiversity hotspots within the complex.

References

[1] Petanidou, T. (2000) - *The postmodern saline landscape in Greece and the European Mediterranean: salinas for salt or what*. In Korovessis N. and Lekkas TD eds., *Saltworks: Preserving saline coastal ecosystems*., Global NEST–Hellenic Saltworks SA, Athens.

[2] Petanidou, T. (2004) - *Salinas of the Mediterranean-past and futures*. ALAS all about salt: Salt and Salinas in the Mediterranean. Figueira da Foz: Tipografia Cruz e Cardoso, Lda, 11-15.

[3] Sadoul, N., Walmsley, J. G., & Charpentier, B. (1998) - *Salinas and nature conservation*. Medwet.

[4] Schembri, F. (2006) - *An Ecological Survey of Salini Saline Marshland*. Unpublished BSc Dissertation, Faculty of Science, University of Malta, Malta.

[5] Borg, J. A., Debono, S., Lanfranco, S., & Schembri, P. J. (2017) - *Report of a phytoplankton and water quality survey made in the 'Sukkursu canal, Salina, in connection with a 'red water' episode in June 2017*. Unpublished technical report. University of Malta, Malta.

[6] Caruana Montaldo, R. (2023) - *The Relationship Between Abiotic Factors and Biodiversity at Salina Nature Reserve*. Unpublished Bachelor of Science dissertation. Institute of Earth Systems, University of Malta, Malta.
[7] Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012) - *NIH Image to ImageJ: 25 years of image analysis.* Nature Methods, 9, 671-675. DOI: 10.1038/nmeth.2089.
[8] Thanopoulou, Z., Sini, M., Vatikiotis, K., Katsoupis, C., Dimitrakopoulos, P., & Katsanevakis, S. (2018) - *How many fish? Comparison of two underwater visual sampling methods for monitoring fish communities*. PeerJ, 6, e5066. DOI: 10.7717/peerj.5066.
[9] McCauley, D., McLean, K., Bauer, J., Young, H., & Micheli, F. (2012) - *Evaluating the performance of methods for estimating the abundance of rapidly declining coastal shark populations*. Ecological DOI: 10.1890/11-1059.1
[10] Caldwell, Z., Zgliczynski, B., Williams, G., & Sandin, S. (2016) - *Reef Fish Survey Techniques: Assessing the Potential for Standardizing Methodologies*. PLOS ONE, 11(4), e0153066. DOI: 10.1371/journal.pone.0153066
[11] Baker, D., Eddy, T., McIver, R., Schmidt, A., Thériault, M., & Boudreau, M. et al. (2016) - *Comparative analysis of different survey methods for monitoring fish assemblages in coastal habitats*. PeerJ, 4, e1832. DOI: 10.7717/peerj.1832
[12] Garrabou J., Bensoussan N. & Azzurro E. (2018) - *Monitoring Climate-related responses. Mediterranean Marine Protected Areas and beyond: Five Standard Protocols*. 36 In Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Passeig Marítim de la Barceloneta pp. 37-49, Barcelona, Spain.
[13] Rodríguez-Prieto, C., Afonso-Carrillo, J., Ballesteros, E., & Boisset, F. (2013) - *Guía de las macroalgas y fanerógamas marinas del Mediterráneo Occidental*. Barcelona: Omega.
[14] Cabioc'h, J., Floc'h, J.-Y., Le Toquin, A., Boudouresque, C.-F., Meinesz, A., & Verlaque, M. (2006) - *Guide des algues des mers d'Europe*. 2nd ed., Delachaux et Niestlé.
[15] Wickham, H. (2014) - *Tidy Data*. Journal of Statistical Software, 59(10), 1-23. DOI: 10.18637/jss.v059.i10
[16] R Core Team (2023) - *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
[17] RStudio Team (2023) - *RStudio: Integrated Development Environment for R. RStudio*, PBC, Boston, MA. URL http://www.rstudio.com/.
[18] Britton, R. H., Johnson, A. R. (1987) - *An ecological account of a Mediterranean salina: the Salin de Giraud, Camargue (S. France)*, Biological Conservation, 42(3), 185-230.
[19] Zhiming, W., Xiangmei, C., & Mianping, Z. (2012) - *pH value change trends in salt brine evaporation.* Chinese Journal of Inorganic Chemistry, 28(2), 297-301.