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Understanding Seahorse Distribution and Ecology in Malta through Citizen Science

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Abstract

Two seahorse species (*Hippocampus guttulatus* and *Hippocampus hippocampus*) are frequently encountered on the Maltese archipelago. Both species are considered to be endangered and are globally data deficient. They are under-studied in Maltese waters when compared to data published from Mediterranean studies. Seahorses are sporadically encountered by the public (particularly by scuba divers) in Maltese waters and as Malta is considered a centre for recreational Scuba diving in Europe, it was deemed a good candidate for a sub-aquatic citizen science initiative. In this study, citizen science approaches were compared to systematic surveys. The surveys found very few seahorses, whereas, once well publicised, records of seahorses from citizen scientists were relatively frequent in the summer months. Citizen science surveys were used to discover the population composition of those seahorses reported and observed in terms of species, sex and gravidity in relation to environmental factors. Citizen science can confirm the long-term presence of both species in Maltese waters. The nature of the data collected by citizen science allows for some comparative work between species. For example, a significant shift in species composition over time was observed which may indicate the decrease of *H. hippocampus* in Maltese waters. However, opportunistic citizen science data has limits. For example, whilst sea temperatures are correlative to this apparent decline in *H. hippocampus*, the data is less abundant for months where scuba diving and particularly tourist scuba diving is less common and the correlation could therefore be a result of more records occurring when the water is warmer. Nevertheless, it could be concluded both species of seahorse are present in Maltese waters and that pregnant males for *H. guttulatus* were observed with relative frequency indicating that Maltese waters are active breeding sites for at least one of the seahorse species in the Mediterranean.

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Throughout the text, Maltese language words are shown in bold font.

Author's declaration

Author may be contacted for full data access including site name disclosure for genuine research purposes. Site names are not included in the publicly available version to protect against poaching of seahorses and unpermitted dives with the intention to encounter. Historic data from The Seahorse Trust global database may have been used for previous research which may have been published.

Understanding Seahorse Distribution and Ecology in Malta through Citizen Science Foreword

The Maltese archipelago is a unique ecosystem of Southern Mediterranean garrigue on several small land masses between Italy, the African continent and with the Suez Canal to the South-East. The Maltese Islands are home to several unique terrestrial and fresh-water species including **il-Gremxlula**, Maltese wall lizard (*Podarcis filfolensis*), **il-Qabru**, the Maltese freshwater crab (*Potamon fluviatile ssp. lanfrancoi*) and **naħla**, the Maltese honeybee subspecies (*Apis mellifera ruttneri*). Holding a politically strategic position in the Mediterranean Sea, Malta has been subject to a succession of occupations, governances and trade routes which have resulted in well-established populations of alien species such as **Kamaleonti**, the common chameleon (*Chamaeleo chamaeleon recticrista*) and **Bajtra**, the prickly pear (*Opuntia ficus*).

Being the most densely populated country in Europe (1,693 persons per square kilometre in 2022) (EC Eurostat, 2023) by a factor of three, Malta faces environmental pressure through construction, waste management, noise pollution, fresh water supply and energy provision. All these challenges are amplified during the tourist season when the population of Malta increases significantly.

In terms of environmental risk and protection, the coastline to land area ratio for Malta is high. Using the World Resources Institute value for coastline length (World Vector Shoreline, United States Defense Mapping Agency, 1989) and the CIA data for land area (Central Intelligence Agency, 2024), Malta ranks number one in Europe in terms of coastline to land mass ratio at an estimated 800 m/km². By comparison and using the same methods, Norway has a ratio of 274 m/m².

The Marine environment is a vital economic resource for Malta in terms of fishing, aquaculture and tourism and the challenges this environment faces in terms of pollution through eutrophication, marine vessel waste, noise and seasonal rainfall land run-off poses a significant challenge to conservation and restoration efforts of specific species and biodiversity as a whole.

Malta's Marine Natura 2000 network covers more than 35% of Malta's Fisheries Management Zone according to ERA (Environmental & Resources Authority, 2024). Designated for the protection of marine habitats and species under the EU habitats directive (European Union, 2024), special areas of conservation have been established for the protection of seabirds, cetaceans, seagrass and other species. The most recognised marine protected site in Malta is Ċirkewwa Marine Park managed by Nature Trust Malta, arguably as important to tourism as it is to conservation. Indeed, the Malta tourism Authority financially supports this Marine Park.

Conservation in Malta is challenging but vital to preserve a unique ecosystem comprised of many unique species.

Introduction

Seahorses are a flagship species around the world, engaging the general public with the marine environment and marine conservation. Yet are generally data-deficient due to their cryptic nature (CITES, 1983). Seahorses have had an important role in Maltese culture, but anecdotally seahorse observations have declined steeply in line with actual human population increase in Malta. As the islands continue to be developed residentially and touristically, it is important to understand current population dynamics in order to track changes over time. Understanding recognising changes in current populations is critical to structuring successful conservation efforts immediately and dynamically over time.

Flagship species

The word for seahorse in Maltese is a direct translation of “Horse of the Sea”, **Żiemal tal- Baħar**. Seahorses have featured in Maltese architecture, particularly as large door knockers (**ħabbata**) which are an iconic part of Maltese culture. In modern times, the seahorse is often depicted in logos for businesses and the seahorse is commonly found depicted in filagree in traditional jewellers. Iconic through its history in Maltese architecture and culture from at least the time of the Phoenicians, the Seahorse is well-loved by many and provides a good conservational focus.

On the IUCN red list (IUCN, 2021 - 2023), vulnerable to many factors and considered to be “data deficient”, the seahorse is unique in terms of physiology and inherent in terms of mythology. A true fish, and responsible for drawing the chariot of Poseidon, seahorses are also important financially in the SCUBA diving tourist industry, ranking in the top ten best marine animals to see by PADI (PADI, 2023), and featuring fourth after sharks, whales and dolphins in SSI’s marketing campaign for the most fascinating aquatic life (SSI, 2023).

Although declared as an indicator species of biodiversity by Lazic et al. (2020) amongst others, the elusive and data-deficient nature of the seahorse makes this species a problematic candidate for biodiversity indication because they are not easily observed and the study of patterns over time is hindered by a lack of information on the species in general, which conflicts with the requirements Siddig (2016) sets out for a good indicator species.

Seahorse species are both intrinsically susceptible to population decline (Lazic et al., 2020) because of their restricted range / habitat through a lack of mobility (Correia, 2020) and at the same time, apparently comfortable in the most polluted waters an area has to offer (Gristina, 2015). Both *Hippocampus hippocampus* and *H. guttulatus* species are protected under Maltese Law (Minister for Rural Affairs and the Environment, Malta, 2006) and all species of seahorse are protected under the CITES convention (CITES, 1983). Malta has been a contracting party of CITES since 1989.

While potentially not a good ecological indicator species, due to their appeal with scuba divers and tourists, integration in local culture and conservation concern, seahorses can be considered

a flagship species, especially in Maltese waters, capable of galvanising multiple marine conservation aims (Cohen 2017)._

The Mediterranean

The waters of the Maltese archipelago have marine environments similar to other sites in the Mediterranean and so seahorse research from the Mediterranean should provide useful insights to help guide research into the Maltese seahorse. Malta is in the Southern half of the Mediterranean Sea (the Triton Fountain in Valletta has co-ordinates of 35.895 N, 14.508 W placing it below the 'centre of gravity' of the sea) which makes the waters of Malta an interesting research area. Historically Malta has warmer waters than the better studied Northern Mediterranean meaning the effects of climate change may be seen here, before other areas. The more southerly status also means conservative diurnal breadth of sunlight hours throughout the year, lessening the effects of any light induced seasonal changes, potentially important in the ecology and behaviour of more northern seahorses, for example seasonal migration for *H. hippocampus* suggested by Lourie (Lourie, 1999). Malta is also relatively close to the Suez Canal and so surveillance for *H. fuscus*, a seahorse from the Red Sea and not native to Maltese waters, is important as this species has already been observed in Turkish waters (Gokoglu, 2004) and in Israel (Mediterranean coast above Tel Aviv) (Golani & Fine, 2002).

The Maltese Archipelago

The Maltese archipelago sits around 100 km to the South of Sicily, above the Medina trench in what is termed the Ionian Sea. The coastal waters directly surrounding the islands of Malta are shallow (<200m) (Mojetta, 2005). The sun delivers between 670 and 750 Joules/cm² in December, which is significantly more than 500 Joules/cm² in December for the waters surrounding more northerly areas of the Mediterranean such as Venice (Mojetta, 2005). This illustrates the importance of recognising the different environments within the Mediterranean itself and the possibility of localised adaptation of species in different environments, particular in seahorses which demonstrate low mobility and in-cohort lack of genetic diversity (Lazic, 2020). Nevertheless, research of the seahorse in the Mediterranean is still relevant to Maltese seahorses which have not been subject to published research to the best knowledge of this author.

The two species of seahorse prevalent in Maltese waters are *H. guttulatus* and *H. hippocampus* (Lourie, 1999), both of which have previously been reported in Maltese waters (The Seahorse Trust, 2004 - 2021), though seahorses in Europe are elusive (Curtis, et al., 2017). There is a lack of published data on Maltese seahorses, which persists to the current date meaning that the only baseline data currently available is the Global Seahorse Database and extrapolation from other Mediterranean studies.

As a SCUBA diving destination for many and the European Headquarters of DAN (Divers Alert Network) Europe, the Maltese archipelago is surrounded by shallow mixed habitat/terrain inlets

where sightings have previously been recorded and where previous research shows seahorses are likely to be found (Correia, 2020), (Lazic, 2020).

Habitat

In general terms, seahorse observations derive from the interlittoral zone, typically 0 to 50m in the Mediterranean owing to its clear waters and in Malta specifically, this will be either low energy rock, biogenic reef or sand bottom; MB15, MB25 and MB55 EUNIS classification respectively (European Environment Agency, 2022). It is noted that both citizen science and survey observations *it-situ* are restricted by safe SCUBA diving limits (both ultimate depth and (no-) decompression limits. In previous Mediterranean studies, the habitats occupied by the two species are thought to differ. *H. guttulatus* is considered a shallow inshore water species in seagrass beds, lagoons, bays and inlets (Lourie, 1999), (Riquet, 2019). *H. hippocampus* is considered by Lourie (1999) to be found in shallow, muddy waters; estuaries; inshore among algae, rocky areas.

However, just over half of all seahorses observed during the survey of Stratoni, Greece (Correia, 2020) were observed without anchor (that is not holding on to a fixed point with their prehensile tail) and of those which were anchored, the majority were anchored to either fan worms or artificial anchor points. None of these scenarios are covered in the generally accepted “typical habitats” of Mediterranean seahorses. Interestingly, as Correia (2020) notes, no seahorses were observed in the seagrass, *Posidonia oceanica*. This is in deviation to the findings of studies on these species in other areas, especially in the Northern Atlantic (Lourie, 1999), suggesting Mediterranean seahorses may show differences in habitat preference to those documented elsewhere.

Whilst it would be valuable to qualify sightings according to the EUNIS habitat classification, there are many factors which are only possible observationally (for example anchor type). Future research on images captured for this study will prove useful in categorising non-EUNIS factors for future studies.

Genetic Conservation & Reproduction

Seahorses typically exhibit monogamous mating behaviours, at least seasonally, if not for life, (Garrick-Maidment, 2003), and as such genetic diversity may be low within populations. The reproductive requirement for a true pregnancy (in the males) also restricts the number of reproductive cycles per male per annum. As the gestation period for *H. guttulatus* is 28 days (Garrick-Maidment, 2002), for example, the number of cycles per breeding season (reported to be 7 months – Lourie 1999), is probably in the region of six cycles per season and the number of live young per brood is uncertain and based on data from captive breeding programmes so is unlikely to reflect wild breeding numbers. Given the low mobility of seahorses and the short gestational period, the presence of gravid males does strongly indicate the presence of breeding populations. Whilst this may be true, the sustainability of such breeding populations can only be predicted and may need to be based on factors such as water quality, habitat stability and

changing predation and competition risk from indigenous and alien species as well as anthropological by-catch and poaching profiles. The study of images captured under this citizen science project are being assessed for their potential to correctly identify individuals over time (repeat sightings) with the hope that future studies can begin to track individuals over time which will help further support the hypothesis that there are breeding populations in Maltese waters.

A lack of mobility of both juveniles and adults further enhances reproductive and genetic isolation across even proximal habitats and this inevitably results in interbreeding as Lazic found in Italian *H. guttulatus* cohorts (2020). Dispersal of young is limited (Woodall, 2015), especially within lagoon populations. It has been suggested that lagoon populations should be managed as “distinct genetic units” (Lazic, 2020) Furthermore, Riquet (2019) notes that partial isolation of the lagoon populations from the Atlantic and even Black Sea populations of *H. guttulatus* has existed for a period of time, long enough for genetic variation to become significantly distinguishing to allow divergence of taxonomic lineages. In other words, panmixia, or the ability of individuals in a population to interbreed without restrictions (resulting in uniform random fertilisation), is unlikely (Woodall, 2015). This localised adaptation of populations means that careful consideration of reintroduction policies in the Mediterranean are needed. However, as adaptation can be even more localised, it demonstrates the care with which data should be extrapolated from even proximal Mediterranean sites to Maltese waters, and certainly from other water bodies with far different environments such as the Atlantic. Riquet (2019) touches on the possibility of witnessing the origin of a species and certainly the discussions surrounding seahorse taxonomy are rich when comparing specimens from different cohorts.

Population Density

As Correia (2020) states prior to their own discovery of seahorses in Greek Mediterranean waters, few locations have been studied within the Mediterranean Sea. Certainly, there is an absence of published data regarding seahorses in Maltese waters as there was in Turkey prior to the work of Filiz beginning in 1999 (Filiz & Taskavak, 2012). It is likely that seahorse distribution is relatively widespread, and anecdotal sightings in areas currently data-deficient continue to be reported to The Seahorse Trust to this day.

Whilst population densities can be calculated in terms of individuals per metre squared (e.g. Correia, 2020), the low detection rate of seahorses means the value of this data can only be useful in terms of reference to a baseline for the same site using the same survey techniques. Estimating all individuals in a location is inaccurate because of low survey areas (restriction of dive time), changing environment (storms, landslides, shifting topography) and inaccurate counts (low detectability due to passive and incomplete observation of a camouflaged species). Comparability to other populations of the same species is less reliable when different survey techniques are used (for example dredging of seagrass, compared to active searches of the seagrass, compared to passive observation). In addition, some population estimates are not fully based on surveys. For example, the work of Filiz (2012) and some of the work of Pierri (2021) was based on local surveys and landed catch (fresh and dried) and is difficult to extrapolate to

Malta where laws on the trade in seahorses are enforced. Any illegal trade in Malta is on the black market and thus is likely to reduce the response and the honesty of answers to surveys.

The use and relevance of general fish distribution methodologies needs to be assessed on a case-by-case basis before applying to this rather unique fish. With a species of limited mobility (Lourie, et al., 1999) and a heavy reliance on camouflage for both ambush predation and avoidance of predation itself, predilection cannot be assumed (especially from low sample sizes). Whether niche predilection in terms of physical and biological environment (including food web nuance) can ever override the low mobility limiting factor of this species is arguable and perhaps laboratory behaviour studies would be a good starting point. Though simulative aquaria studies are limited in terms of full ecosystem and topography representation, they can produce valuable data in terms of predilection in the absence of a given variable, for example. Laboratory behavioural research for non-native and invasive species has proved useful for niche modelling of some fish species (Rehage, et al., 2015) and it is likely that the statistical robustness of this approach may be borrowed for behavioural model inputs into niche predilection modelling for data-lacking and/or evasive species such as sea-horses as well. In this case though, the cohort genetic conservatism must be considered (Lazic, et al., 2020); it is unclear whether wild-caught seahorses from southern England, for example, would behave in the same way as aquaria-bred seahorses of the same species but originating from Turkish waters.

Threats

Despite the difficulties of estimating populations, in general, seahorse populations in the Mediterranean are in decline (Pierri, 2021). Although firm evidence to indicate one single cause is lacking, over-fishing has been cited as a key factor in the decline of seahorse populations (Pierri, 2021). A catch of high monetary value, not least for export to Asia as a traditional medicine (Pierri, 2021), the seahorse is less likely to be returned as unwanted catch than less lucrative by-catch species and in fact the IUCN consider the aquarium trade to be a possible factor in seahorse by-catches not being returned to the sea (IUCN, 2016).

Pierri et al. (2021) conclude that several factors contributed to a successful and relative abundant historic population of seahorses in Taranto, Italy, namely, large industry absence driven by tourism, extensive aquaculture (mussels) and high eutrophication. Based on the premise that food availability (smaller crustacea) was likely to be plentiful, and water quality was historically poor, Pierri et al. (2021) conclude that poaching was the reason for the population decline in Taranto starting in 2015.

Citizen Science

The active engagement of the general public in scientific research tasks is referred to as “citizen science” (Vohland, 2021). An emerging method of gathering data, often passively for the lead researcher, and perhaps particularly cost-effective, citizens with a passion for science are self-motivated to contribute to the research project. Not in any way exempt from ethical

considerations such as working without financial remuneration & academic recognition and technical competency (particularly in the natural world where vulnerable ecosystems can easily be damaged), citizen science presents challenges in planning, execution and interpretation quite different to traditional research techniques where paid competent workers can be expected to follow deliberately designed protocols and record data accurately and thoroughly.

Observational research in natural sciences and in particular biodiversity monitoring, benefits from citizen science according to Vohland (2021) as "*it expands the spatial and temporal scales of investigation*". Whilst citizen science does often bring a bias in data which would be mitigated against in a planned experiment, for example, Schmeller et al. did find that less than 15% of participants in species-monitoring programs were professionals: In their 2008 publication (2008) which indicates a likelihood that many researchers, on balance, would use citizen science data, warts and all, rather than take the alternative which may be to have no data at all.

In terms of sub-aquatic citizen science, some drawbacks of using non-professional researchers are intrinsically mitigated. Scuba divers already have training and certified competency in safety and many, if not all, certification schemes have teaching and competency aspects based on environmental protection and on marine species interaction. The modern scuba diver dives with a dive computer which offers several valid data points which can be easily recorded and with a high degree of accuracy such as depth and sea temperature. Furthermore, many scuba divers carry underwater photography / videography equipment with them which would be expensive for the researcher to buy and offers excellent data collection potential which the researcher can analyse at leisure. Whilst it's true that a scuba diver size estimation of a seahorse may not be accurate, the photograph they share can provide valuable information about species, sex, gravidity and surrounding environments/topography.

Groffedo (2004) conducted a successful seahorse citizen science project in Italian waters concluding that "*recreational divers and other resource users can play an active part in monitoring the marine environment*" which demonstrated that success was possible for a Maltese sub-aquatic citizen science project to research the presence of seahorses (2004).

However, citizen science collected data are not without issue. Citizen science collected data, based on opportunistic sightings of a species present a series of challenges for analysis and interpretation of biological trends within the data (e.g. Boakes et al. 2023). For example, unequal sampling effort is likely to occur both spatially (e.g. some dive sites being far more popular than others) and temporally (more dives in the summer months, and project uptake may be higher in some years than others). Furthermore, there is a tendency to only receive positive records (for example, the submission of a record when a seahorse is seen is much more likely than a submission of a record when a seahorse is not seen) to the extent that many projects only ask for positive sightings. This 'presence only' data would cause issues when trying to determine aspects such as habitat preference of a species (see discussions in (Stafford, 2010); (Boakes, 2023).

A global database for seahorse sightings by citizens is already in place and is curated and managed by The Seahorse Trust (The Seahorse Trust, 2004 - 2021) and has been active in its current form since 1994. This database is open access for research purposes although the data set is not in the public domain to protect seahorses from potential disturbance and poaching. Sightings for the Maltese archipelago go back to 2004 when initial surveys were undertaken by The Trust in Maltese waters. Between 2012 and 2014 some surveys were undertaken by the Trust in Maltese waters and sightings from these are included in the historic data base. Database records up to the 31 December 2019 are considered to be historical records because they were undertaken according to protocols undefined by this study and reproducibility of those methods is poor owing to a lack of recorded methodology.

Hypotheses

This study examines the distribution and ecology of seahorses in Malta. It uses data from a combination of literature searches, citizen science records and scuba surveys to address the following hypotheses:

- 1) There are established breeding populations of seahorses in the Maltese archipelago.
- 2) There will be differences in distribution between the two species, with *H. guttulatus* being found in seagrass on sand beds and *H. hippocampus* being found amongst algae on rocks.
- 3) Despite limitations, citizen science provides a cost-effective way of understanding the ecology, population trends and distribution of seahorses.
- 4) There are differences in the biology, ecology or behaviour of Maltese populations of seahorses when compared to those found in the rest of the Mediterranean.

Methods

This thesis utilises data from surveys and citizen science data. The discussions with authorities and databases used to store data were established prior to commencement of this thesis and were conducted for the purposes of The Seahorse Trust. The details of the surveys are provided here to enable understanding and repeatability of the techniques and allow an evaluation of the approaches, and the data collection itself, and storage of the data, are separate to this research, although performed by myself, with data collected for the Seahorse Trust, and subsequently analysed in this thesis. This research has been based on the provision of the survey data and anonymised citizen science data from The Seahorse Trust.

Surveys

Structured and planned surveys were undertaken according to the Standard Operating Procedure (SOP) agreed upon with the Environmental Resource Authority, Malta (ERA) as part of the permit granted to undertake scientific surveys looking for seahorses in the Maltese archipelago. The SOP is included in Appendix 1 and provided a standard technique to follow ensuring consistency, reproducibility and protection of the marine world, particularly for seahorses. Basic scuba survey techniques of transects were used most commonly, Buddy pairs of volunteer divers with at least Advance Open Water qualifications and demonstrated good buoyancy control swam parallel at less than 1m apart and less than 1m from the base substrate. A slow swim speed was used in order to maximise the time allowed for searching. Care was taken not to disturb the sediment and sand whilst scanning from side to side. Nothing was moved or touched by hand. Survey sites were chosen according to various criteria identified in the literature search (shallow sheltered bay with some boat use), accessibility and previously sighting history. Sites are not named here due to the protected nature of seahorses and the risks of illegal diving and disturbance, illegal fishing and poaching. Full data can be obtained from The Seahorse Trust for genuine research purposes. Adjacent land use was determined by the author from existing knowledge and was based on the most common anthropological use (e.g. dive site, boat use etc.)

Site 1 Numerous sightings in this location, a sheltered leisure boat-use shallow bay. This is a fairly large site popular with larger “booze cruise” vessels which provide subaquatic noise pollution frequently. The site is easily accessible.

Site 2 No previous sightings in this location. A sheltered leisure-boat use shallow bay of fairly large size. The site is easily accessible.

Site 3 Numerous sightings in this location, a busy commercial and large vessel use harbour. A large site with frequent subaquatic noise pollution. The site is easily accessible.

Site 4 Numerous sightings in this location, a busy leisure boat temporary moor (usually day visit). A small site with frequent subaquatic noise pollution. The site is easily accessible.

Site 5 No previous sightings and in a no-boat zone protected area with prohibited fishing and scuba diving without a permit. The natural sheltered bay is infrequently disturbed and is only accessible by boat.

Site 6 Some sightings in this location, a busy small leisure and commercial boat moor (usually short tourist excursions). A small site with frequent subaquatic noise pollution. The site is easily accessible.

Some surveys were undertaken in 2013 by The Seahorse Trust, which are combined with the citizen science data set. The methods for these surveys is unknown and the temporary increase in data for 2013 is reflective of these surveys. The techniques for these surveys in 2013 are unknown by the author.

Table 1 Structured survey sites, their sighting history, current use and number of surveys

	Island	Site use	Previous reports of seahorses?	Site type	Boat use	Number of surveys
Site 1	Malta	Recognised dive site	Yes	Protected shallow bay	Leisure boat use	7
Site 2	Malta	Recognised beach area	No	Protected shallow bay	Leisure boat use	1
Site 3	Malta	Recognised dive site	Yes	Marina	Commercial and leisure boat use	0*
Site 4	Gozo	Recognised dive site	Yes	Protected shallow bay	Leisure boat use	2
Site 5	Malta	Protected site	No	Offshore island, protected bay	Restricted boat use	1
Site 6	Malta	Recognised dive site	Yes	Marina	Small boat use	1

*planned survey dive was cancelled due to inclement conditions

Table 2 Surveys undertaken

Site	Date	GPS?	Method
1	2 June 2022	No	Linear transect
1	18 June 2022	No	Linear transect
1	19 July 2023	No	Linear transect
1	23 June 2022	No	Durrant transect
1	26 May 2022	No	Linear transect
1	30 September 2022	No	Linear transect
1	30 October 2023	No	Linear transect
2	16 July 2022	No	Linear transect
4	24 July 2022	No	Linear transect

4	29 July 2023	No	Durrant transect
5	6 August 2022	No	Linear transect
6	22 July 2023	No	Linear transect (modified for vertical plane)

Statistical Analysis

No statistical analysis was undertaken on survey data since only one positive record was observed (see results).

Citizen Science

In 2020, The Seahorse Trust renewed efforts to begin survey dives and to encourage citizen science reports. Records from 1 January 2020 are considered to be current records in the dataset presented here as those records were obtained using the revised methodologies described here.

A Google form was developed by The Seahorse Trust to capture records of sightings by the public. The Google form method was chosen due to its ability to capture image files as part of the reporting process and the automatic adaption to completion using a smart phone. It was anticipated that more reports would be received if the method of reporting was widely available. Google forms allow reporting via PC, tablet, smart phone and on Apple and Android operating systems. Google supports encryption of all files uploaded or created to the google drive at transit and at rest and is compliant with regulatory requirements (security and privacy). The form was established on an EU account and adhered to EU legislation; a representation is given in Appendix 2.

A QR code for the form URL was obtained and shared on social media, via a poster campaign and also through stickers distributed in dive schools & shops. Anonymous data was extracted from the results of The Seahorse Trust citizen science survey and used for this project. All responses were from scuba divers except one which was from an individual marina clean-up operation.

A Facebook page was launched by The Seahorse Trust in June 2022 to support the citizen science initiative and to raise awareness locally. The most successful post was on 21 July 2023 which advertised the reporting stickers availability (A post reach of 12.5K). The facebook page currently has 548 followers and 513 likes.

All positive sightings of seahorses, from citizen science and current and historic surveys were combined for the analysis:

- Date
- Site / GPS
- Depth
- Visibility
- Water temperature
- Size*

- Colour**
- Position**
- Photographs
- Time
- Island
- Topography**

And some additional parameters were included;

*The size of the seahorse was prescribed in the Google form to be “from the top of the head to the bottom of the uncurled tail”

**Recorded from a drop-down menu including “other”

Statistical Analysis

Data was synchronised with a Microsoft Excel .csv file, which had been manipulated for consistency to enable more meaningful statistical analysis; All topographical information was collated into words which corresponded to the google form drop-down menu and empty cells were filled with “.”.

Statistical analysis was performed using JASP and R. JASP (University of Amsterdam, 2018) is a front-end interface for an extensive list of R routines (The R Foundation, 2024). The R repository was cloud-based (<https://cloud.r-project.org>) and the JASP software was installed on a stand-alone desktop PC with internet access via ethernet cable and wi-fi.

JASP was used for Fisher’s exact tests, correlations and comparisons of average values across categories (e.g. t-tests, ANOVA and non-parametric equivalents). R (version 4.3.2) was used for ANCOVA, Fisher’s exact tests and multivariate analysis described below.

A multiple correspondence analysis (MCA) was performed on the data, using the categories of year, site use, recorded water temperature when the seahorse was sited, depth, sex, gravidity, size and habitat type to distinguish between the two species of seahorse. To minimise the number of categories, data were regrouped as follows. Historic data was up to and including 2019. 2020, 2021, 2022 onwards classed as recent, due to the start of the citizen science campaign. Site temperature was placed into groupings of 15-18, 19-22, 23-27 and > 27 °C. Depth into categories of <10, 10-15, and >15 m, and size into categories of <10, 10-15, and >15 cm. Only the primary habitat type was used, where primary was defined by the presence of structural complexity using the hierarchy of seagrass, algae, rock, sand. Therefore, if seagrass was present in low abundance, the category was still defined as seagrass. . The use of primary habitat only was necessary to ensure sufficient replication between habitat types to meaningfully perform the analysis and the hierarchy used is based on known habitat preferences by seahorses (i.e. their close association with seagrass and algae) (Lourie, et al., 1999). The analysis was performed in R using the FactoMineR package (Le, 2008).

The sample was voluntary, positive (presence only) citizen scientist reports, predominantly using Facebook via a Google form. No negative (absence of seahorses) reports were received or were intended to be received. Historical sightings were reported through the global reporting form. Though the non-response and voluntary response bias is unknown because the survey had no established invite list, the survey was established for voluntary reported sightings and so negative bias is considered to be unlikely (i.e. many sightings were unreported).

Due to lack of normality of most data analysed, non-parametric comparison of medians (Mann-Whitney tests) or Spearman correlations were conducted on data where there was a single categorical or continuous independent variable, respectively. Since no viable non-parametric tests exist to determine differences in relationships (e.g. species differences in changes in numbers of records over time) ANCOVA was used with a Poisson distribution link function to minimise departures from non-normality or heteroskedasticity and account for the 'count' data being analysed.

Literature Review

Literature was collected to examine Hypothesis 4: There are differences in the biology, ecology or behaviour of Maltese populations of seahorses when compared to those found in the rest of the Mediterranean. A search for books revealed that all relevant books had already been identified and acquired in research preparation. Literature was identified using a progressively focussed set of search terms and criteria and was used for searches The University of Malta Library Platform (using HyDi search engine) (University of Malta, 2025). Search terms and platforms used are shown in Table 3. Titles were reviewed for relevance and irrelevant articles were not read further. Of those considered relevant, the abstract was reviewed with irrelevant articles being discarded. If clarity was required for those relevant articles left, conclusions and methods were scanned to establish relevance. Table 4 shows the phases of review and record refinement figures.

Table 3 Literature search terms and platforms

Data base	Search term	Refinement
University of Malta	Seahorse mediterranean	
University of Malta	Seahorse mediterranean	Search term in title only Last five years All items In English
University of Malta	Seahorse mediterranean	Search term in title only Last 10 years All items In English
University of Malta	Hippocampus guttulatus	
University of Malta	Hippocampus hippocampus	
University of Malta	Hippocampus mediterranean	
University of Malta	Hippocampus mediterranean	Last 5 years In English
University of Malta	Hippocampus mediterranean	Search term in title only
University of Malta	Seahorse ecology	
University of Malta	Seahorse ecology	Search term in title only Title does not contain "florida" In English Last 5 years Articles only

Table 4 Phases of literature review and record refinement

Identification	Records identified through data base searches according to Table 3.	=	Σ	462 219
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Screen	Records screened for*	Search in title	=	Σ	1 673
		Exclusion of term (i.e. “florida”)			
		Articles only			
		English language			
		Last five years			

Included	Abstract review	=	Σ	25**
	Conclusion & methods scan			

*Terms screened for depended on number of initial hits

**After reviewing the papers, on 12 were of use

A Google Scholar alert was also established to capture articles released whilst research was ongoing.

The broad search term was "Seahorse mediterranean" and provided alerts for eight articles whilst the alert was live:

- (Castejón-Silvo et. al, 2023)
- (Vivas, 2023)
- (Ergüden, 2023)
- (Vivas, 2023)
- (Planas, 2023)
- (Bosso, 2024)
- (Rida Salam, 2024)
- (Meyer, 2023)

One alert has not been included here as it was an article relating to familial Mediterranean fever and “Seahorse metabolic flux analysis”. Alerts after 30 March 2024 were not taken into account for this project.

Literature sources identified from the standard literature review above (excluding new literature) were plotted on a map of the Mediterranean. Pinmaps.net (Tobon Web Tools L.L.C., 2024) was used to plot 23 sites mentioned in 12 papers. Papers specified as being in countries with a Mediterranean coast had sites plotted for completeness, despite some of the sites being Atlantic. Sites in the Adriatic are considered to be Mediterranean for the purposes of this study. Those papers without any Mediterranean sites were not plotted.

Results

Hypothesis 1. There are established breeding populations of seahorses in the Maltese archipelago

The data support this hypothesis. The presence of gravid male seahorses in 2013 (historic data), 2020 and 2023 confirms that there are breeding seahorses in Maltese waters and there have been for at least ten years. This suggests that established populations are present in the Maltese archipelago. Interestingly, those gravid males from 2013 were all *H. hippocampus* and those since 2020 were *H. guttulatus* (see Table 5). It can therefore be further qualified that only *H. guttulatus* can be confirmed to be currently breeding in Maltese waters. There is a lack of positive data which would confirm currently breeding *H. hippocampus* in Maltese waters after 2013. Whilst this study could not confirm the presence of breeding *H. hippocampus*, it could not exclude the possibility since the citizen science data is comprised only positive sightings.

Table 5 Positive citizen science reports of gravid male *H. hippocampus* & *H. guttulatus*

Year	Date	Species	Sex	Gravidity
2013	01/05/2013	<i>H. hippocampus</i>	Male	Gravid
2013	03/05/2013	<i>H. hippocampus</i>	Male	Gravid
2013	07/05/2013	<i>H. hippocampus</i>	Male	Gravid
2013	09/06/2013	<i>H. hippocampus</i>	Male	Gravid
2013	12/06/2013	<i>H. hippocampus</i>	Male	Gravid
2013	24/06/2013	<i>H. hippocampus</i>	Male	Gravid
2013	25/06/2013	<i>H. hippocampus</i>	Male	Gravid
2020	24/05/2020	<i>H. guttulatus</i>	Male	Gravid
2023	29/10/2023	<i>H. guttulatus</i>	Male	Gravid
2023	12/06/2023	<i>H. guttulatus</i>	Male	Gravid
2023	29/05/2023	<i>H. guttulatus</i>	Male	Gravid
2023	02/06/2023	<i>H. guttulatus</i>	Male	Gravid
2023	28/07/2023	<i>H. guttulatus</i>	Male	Gravid
2023	27/07/2023	<i>H. guttulatus</i>	Male	Gravid
2023	23/04/2023	<i>H. guttulatus</i>	Male	Gravid

The breadth of size reported for both species indicates a diversity of growth stage (4-16 cm for *H. guttulatus* and 7-16cm for *H. hippocampus*) (see

Table 6) which supports the existence of established breeding populations which are present in Maltese waters at maturity and younger stages of development.

Table 6 Mean seahorse size (cm)

	Size cm	
	H.guttulatus	H.hippocampus
Valid	34	17
Missing	14	2
Mean	10.029	11.059
Std. Deviation	2.990	2.609
Minimum	4.000	7.000
Maximum	16.000	16.000

Note. Excluded 16 rows from the analysis that correspond to the missing values of the split-by variable Species

The presence of both males and females of both species further supports the possibility of breeding populations being present in the Maltese archipelago. There was also a significant difference in the composition of sightings by species in terms of sex, (Fisher's Exact Test, $p = 0.050$; Figure 1), potentially indicating different reproductive strategies between the Maltese populations of these species.

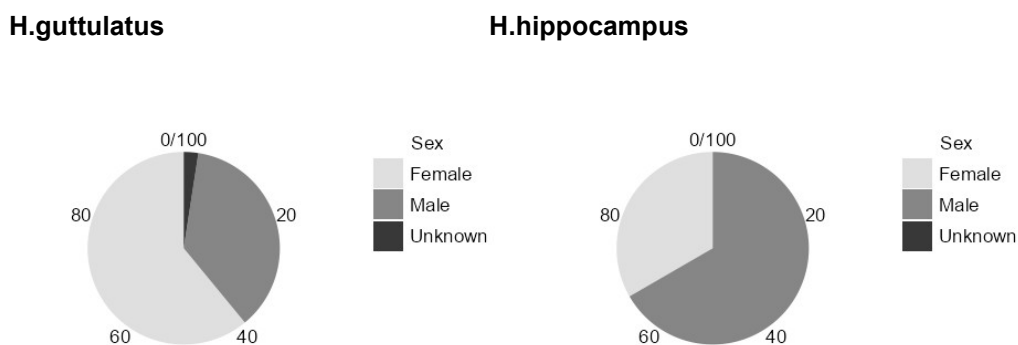


Figure 1 Percentage of each sex of the two species of seahorse. Note, 'unknown' was removed for statistical analysis

Hypothesis 2. There will be differences in distribution between the two species, with *H. guttulatus* being found in seagrass on sand beds and *H. hippocampus* being found amongst algae on rocks.

The data do not fully support this hypothesis, but this may be due to limited records. Topography data for *H. hippocampus* is predominantly from historical reports which must be taken into consideration. Nevertheless, it is clear that *H. Hippocampus* was reported as being on seagrass, seagrass and sand or just sand in over 60% of sightings (see Table 7). Algae and rock topography made up the remaining sightings. *H. guttulatus* was found most commonly in Algae alone but observations in seagrass, seagrass and sand or just sand were also relatively common at 36%.

Fisher's exact tests comparing the counts of the two species found either on sand or any habitat containing seagrass, or any remaining habitat containing rocks and algae (but no seagrass) showed borderline significant difference ($p = 0.051$), likely due to a limited sample size. As such, there may be habitat preferences between species, but it is also clear that both species use a variety of habitats in Maltese waters.

Table 7 Frequencies of seahorse sightings by topography

Species	Topography	Frequency	Percent	Valid Percent	Cumulative Percent
H.guttulatus	Algae	15	31.250	34.091	34.091
	Sand	7	14.583	15.909	50.000
	Rock	3	6.250	6.818	56.818
	Seagrass	8	16.667	18.182	75.000
	Seagrass, Sand	3	6.250	6.818	81.818
	Algae, Rock	3	6.250	6.818	88.636
	Algae, seagrass	1	2.083	2.273	90.909
	Seagrass, Algae, Sand	2	4.167	4.545	95.455
	Algae, Sand	2	4.167	4.545	100.000
	Missing	4	8.333		
	Total	48	100.000		
H.hippocampus	Algae	2	10.526	11.111	11.111
	Sand	3	15.789	16.667	27.778
	Rock	1	5.263	5.556	33.333
	Seagrass	9	47.368	50.000	83.333
	Seagrass, Sand	1	5.263	5.556	88.889
	Algae, Rock	2	10.526	11.111	100.000
	Algae, seagrass	0	0.000	0.000	100.000
	Seagrass, Algae, Sand	0	0.000	0.000	100.000
	Algae, Sand	0	0.000	0.000	100.000
	Missing	1	5.263		
	Total	19	100.000		

Valid percent is the percent adjusted for missing values

Hypothesis 3. Despite limitations, citizen science provides a cost-effective way of understanding the ecology, population trends and distribution of seahorses.

The data support this hypothesis. Of 12 survey dives undertaken, only one seahorse was found, whereas, once well publicised, records of seahorses from citizen science were relatively frequent in the summer months. Dive surveys sites were chosen based on historical citizen science reports and were selected based on their use (dive site, protected site etc.). In terms of EUNIS habitats, all accessible dive sites in Malta can be described as low energy rock, biogenic reef or sand bottom; MB15, MB25 and MB55 respectively (European Environment Agency, 2022). It is noted that both citizen science and survey observations *it-situ* are restricted by safe SCUBA diving limits. Future studies at greater depth may need to consider additional EUNIS classifications. Citizen science surveys were used to discover the population composition of those seahorses reported and observed in terms of species, sex and gravidity in relation to environmental factors.

Total reports of seahorses in the Maltese archipelago, as facilitated by The Seahorse Trust, have shown a general increase over time. Report numbers have been historically low, aside from a spike of reports in 2013 which was due to a number of planned surveys undertaken during that year (results of which were included in the database). Increased publicity through Facebook, QR code stickers and posters at popular dives sites has increased the number of sightings reported since 2020 significantly (see Figure 2).

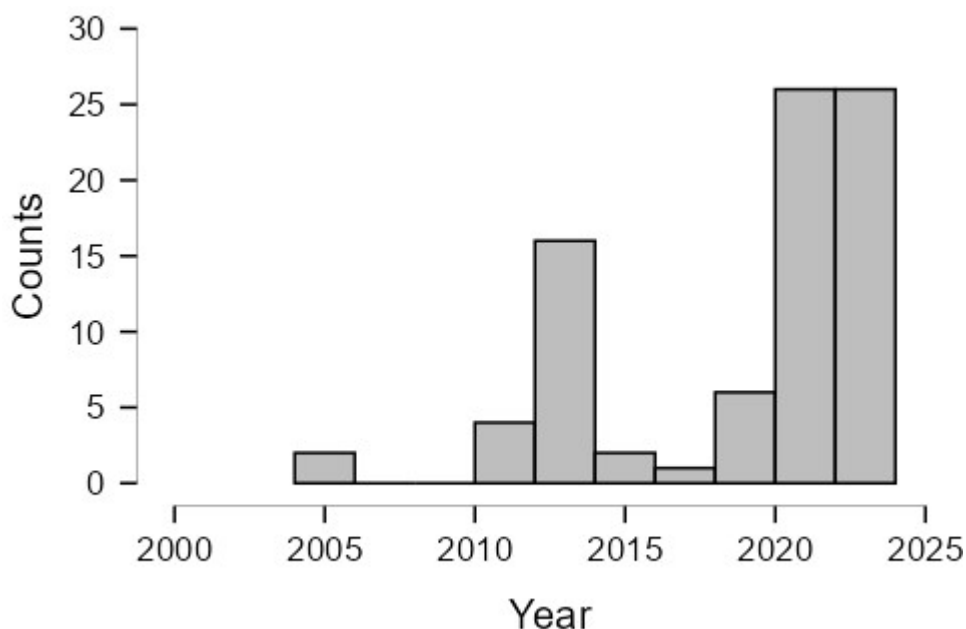


Figure 2 Citizen science seahorses reports in Malta over time

Multiple correspondence analysis showed that whilst there were more sightings of *H. guttulatus* (red points) than *H. hippocampus* (blue points) over the full data collection period, there were still enough data points to allow comparison between species (Figure 3). Proportionally more *H. hippocampus* were found on the left-hand side of the figure. However, the right-hand side of

the figure aligned more to missing data, where factors had not been recorded (e.g. depth, temperature etc) and to sightings in marinas, rather than the main dive sites. Warmer waters (associated with the middle top section) showed a roughly even split of species, but given the overall higher number of *H. guttulatus*, this may suggest *H. hippocampus* are favouring warmer waters, or may be more visible in the mid-summer season. Some of these general trends in the data are explored in more detail below.

Red dots denote sightings of *H. guttulatus*; blue dots denote sightings of *H. hippocampus*. Top 1 (purple text) denotes the topography in words.

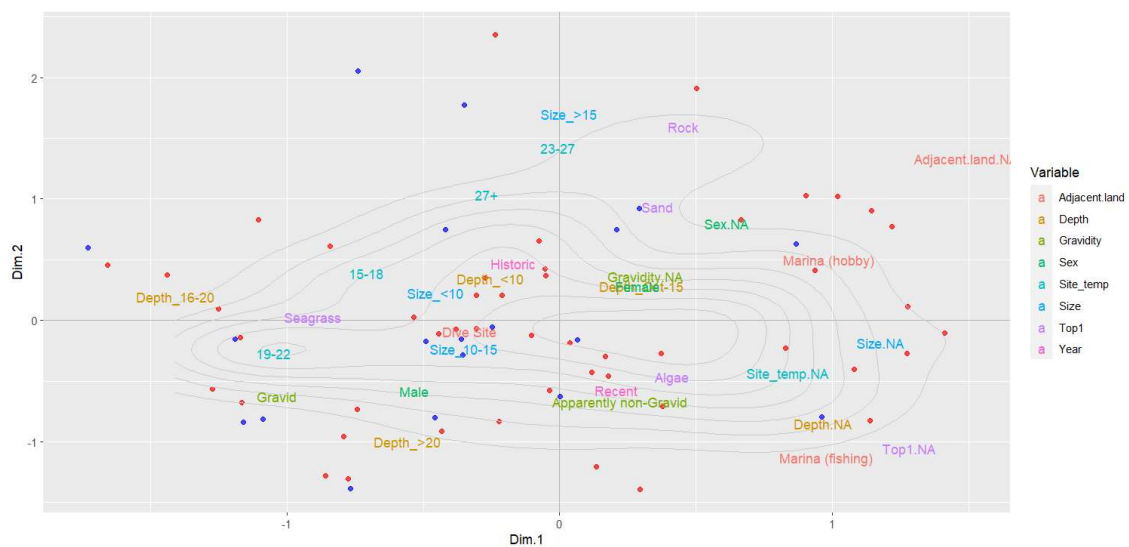


Figure 3 Dimensional map of multiple correspondence from citizen science report of seahorses in the Maltese archipelago

Comparison of the Two Species Versus Variables

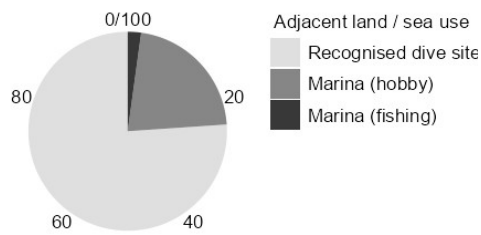
There was no significant difference between species based on the depth of sighting (t-test, $t = 0.200$, d.f. = 51, $P = 0.842$). However, a sighting at 66m (without photograph to confirm) highlights the potential importance that surveys at depth (within technical diving limits or by remotely operated vehicles, ROVs) may hold.

Adjacent land use was predominantly a recognised dive site regardless of species observed (see Figure 4), but with a slightly higher proportion of *H. guttulatus* found in marinas, as also suggested by the MCA analysis

Temperature over time

The temperature reported by citizen scientists in the sea at the point of observing the seahorse was statistically significant when compared with the year of the sighting (linear regression, $F_{1,45} = 3.99$, $P = 0.011$, $r^2 = 0.136$).

H.guttulatus



H.hippocampus

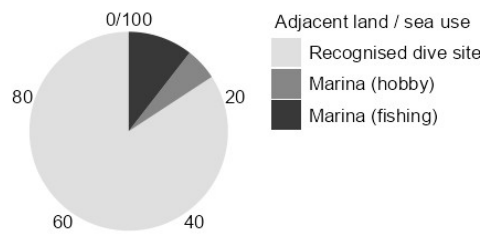
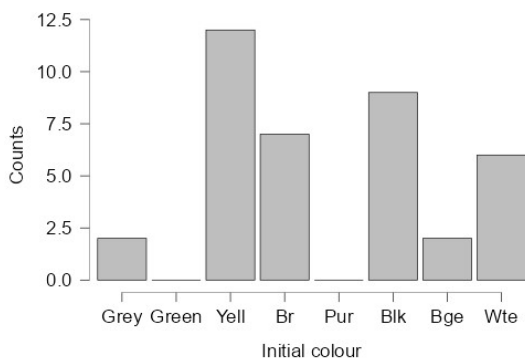


Figure 4 Adjacent land use by species (pie chart)

Where yellow was frequently observed as being the initial colour of *H. guttulatus*, it was never noted for *H. hippocampus*. Conversely, purple was noted with some frequency for *H. hippocampus* and not for *H. guttulatus*. No other colours showed a clear difference between species (see Figure 5).

H.guttulatus



H.hippocampus

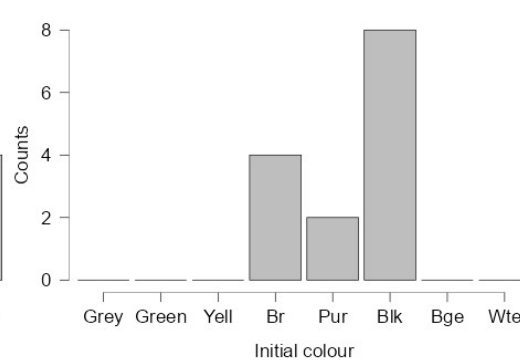


Figure 5 Initial colours of seahorses at sighting

There was a significant difference in the number of records in each year by species (ANCOVA Species*Year interaction term: Deviance = 10.2, d.f. = 1, 20, $p = 0.0014$), with the numbers of *H. guttulatus* increasing much more in recent years than *H. hippocampus*, which appears to be decreasing. This could potentially indicate a change in species assemblage.

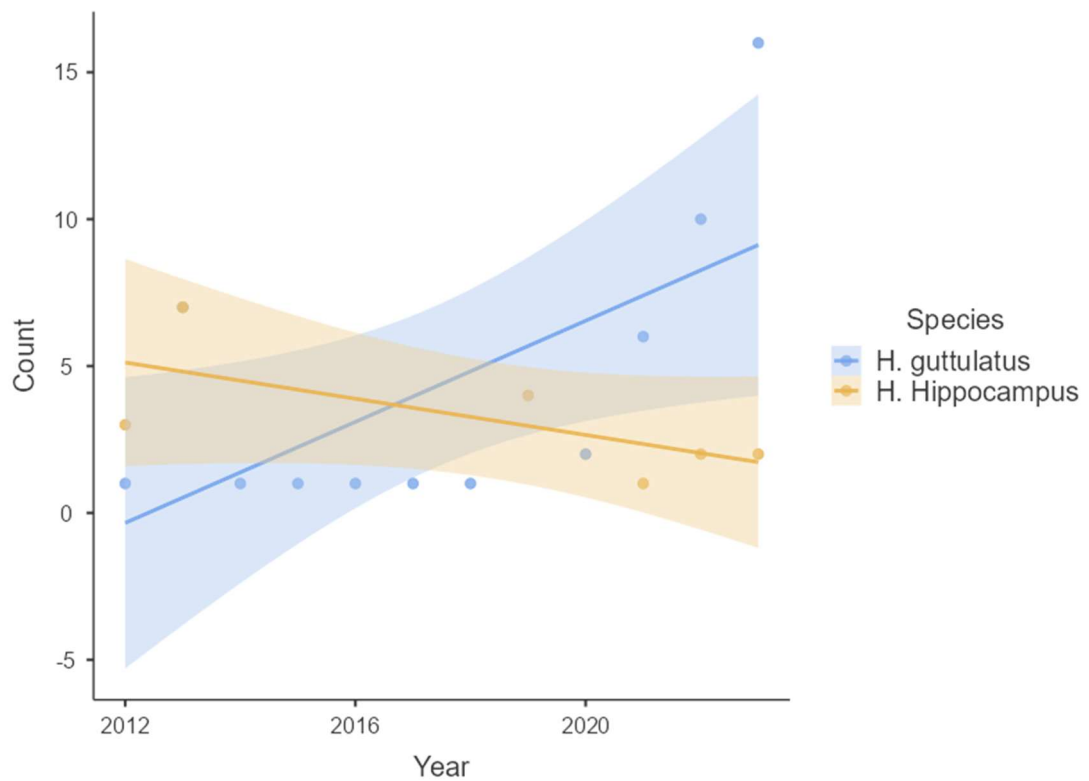


Figure 6 Changes in counts of the two seahorse species over time. Lines are linear lines of best fit, with shading indicating 95% confidence intervals of the lines. Data prior to 2012 and unknown species sightings have been removed for clarity.

Height data was normally distributed. There was no statistically significant difference in size between the two species recorded from citizen science reports ($P=0.0.233$, $df=49$, $t=-1.207$). Despite this, Figure 7 shows overlap of distribution that agrees with established data on the two species; Lourie (1999) sets the adult height of *H. guttulatus* at 8.5-18.0 cm and *H. hippocampus* at 7.0-13.0 cm, measuring the height of the seahorse from the coronet to the tip of the tail (as this study also defined), see

Table 6.

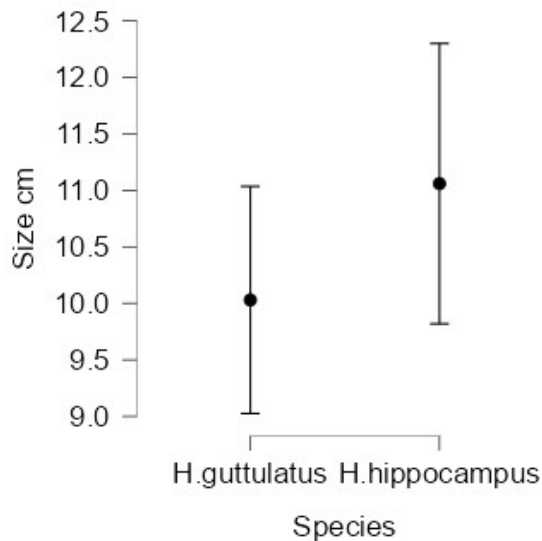


Figure 7 Mean (+/- S.D.) size of the two species of seahorse

Hypotheses 4. There are differences in the biology, ecology or behaviour of Maltese populations of seahorses when compared to those found in the rest of the Mediterranean.

The data obtained do not allow for a meaningful test of this hypothesis. Figure 8 shows the specific sites of study identified through the literature search

The literature review does show presence in these sites as indicated here. Sites tend to be chosen for study because seahorses have been seen there - but these studies do confirm presence. We have also found out that there is a lack of consistency between how data are collected - and as such, trying to compare the data further than presence in a site may be misleading.

The majority of sites do appear to be in close proximity to artificial marinas (15/20 sites presented) though it is unclear whether the presence of an artificial construct enables easier access to the sites for humans or whether the presence of seahorses is because of the artificial construct; possibly owing to increased shelter, for example.



Figure 8 Pinmaps.net graphic of seahorse sites mentioned in literature (Mediterranean), excluding Atlantic sites (Credit Google Maps)

Comparisons of species distribution, individual size and population densities are hampered by the variation in methodologies between studies (for example passive citizen science reporting versus dredge sampling). Of 11 papers published and used for this study, only 5 employed passive observational sampling which could be considered similar to this study (survey dives). The remaining studies involved capture and release or destructive sampling and two studies cited beach seine / dredging techniques which are ethically divisive, especially in an area of such low population (see Table 8).

Table 8 Methods of seahorse observation and study in the Mediterranean (from literature)

Article	Species	Purpose	Method & Notes	Sample fate
(Aurelio, 2013)	<i>H. guttulatus</i>	Environmental adaptation	Beach seine	Transfer to aquaria
(Correia, 2018)	<i>H. guttulatus</i> <i>H. hippocampus</i>	Population monitoring 1760 individuals over 6 sites	Linear transects, (2 x 30m)	No samples taken
(Costa, 2023)	<i>H. guttulatus</i>	Behavioral monitoring 11 males and 11 females	Scuba collection	Transfer to aquaria
(Curtiz & Vincent, 2006)	<i>H. guttulatus</i>	Mark and recapture/ territory Frozen sample collection (2735 individuals)	No collection method provided Beams trawls / Beach seine	Release but disabled Frozen, destructive
(Filiz & Taskavak, 2012)	<i>H. guttulatus</i> <i>H. hippocampus</i>	Interviews (394) 32 locations	-	Some dried samples were collected
(Gristina, 2013)	<i>H. guttulatus</i> <i>H. hippocampus</i>	Population monitoring 242 individuals over 11 transect seadivesrches	Linear transects, (2 x 150mtransects per site)	No samples taken
(Lazic, 2020)	<i>H. guttulatus</i>	DNA analysis of 8 populations 119 individuals	No collection method provided Tissue samples (dorsal fin clip)	Return to site
(Pierri, 2021)	<i>H. guttulatus</i>	Population monitoring	Linear transects, (2 x 150m) over 2 sites 2 divers, morning	No samples taken
(Riquet, 2019)	<i>H. guttulatus</i>	DNA analysis, one site 172 individuals collected over 3 years	No collection method provided Tissue samples (dorsal fin clip)	Return to site
(Spinelli, 2020)	<i>H. hippocampus</i>	Study of one male and one female	Incidental	No samples taken
(Tiralongo & Baldacconi, 2014)	<i>H. guttulatus</i>	23 separate days 196 individuals over 3 years	Scuba and snorkelling	No samples taken

Discussion

Following publicity on social media, by posters and freely distributed QR code stickers, citizen science report numbers increased significantly and successfully confirmed the presence of two species of seahorse in the Maltese archipelago where structured surveys only succeeded in recording one individual over twelve dives. Citizen science data proved that breeding populations of seahorses have existed in Maltese waters for at least ten years and that they can be observed in topography commonly believed to be atypical of their species with *H. hippocampus* being found in sites with hard substrata and algae present, rather than just on seagrass, and *H. guttulatus* being found over both hard and soft substrates. Furthermore, an absence of *H. hippocampus* sightings over a number of recent years indicates a potential shift in species composition with the possible reality being that Malta is losing an iconic marine species from its waters. Though biased to the months of warmer sea temperatures, citizen science has provided data which invites both discussion and further research as to how the Maltese seahorse fits into the wider Mediterranean and global populations of both *H. guttulatus* and *H. hippocampus* and it has done so economically:

Population Health

Only *H. guttulatus* can be confirmed to be currently breeding in Maltese waters due to a lack of positive data which would confirm currently breeding *H. hippocampus*. The size of the seahorses reported appears to show agreement with established data on the two species in general; Lourie (1999) set the adult height of *H. guttulatus* at 8.5-18.0 cm and *H. hippocampus* at 7.0-13.0 cm, measuring the height of the seahorse from the coronet to the tip of the tail (as this study also defined). A good distribution of size supports the presence of breeding communities over time though seahorse size data reported by citizen scientists cannot be relied upon to the degree the data indicates at first glance. Size estimates made by divers with differing mask composition and possible prescription lenses along with human population variation in size-estimates means the data should really be considered approximate, rather than exact.

Conversely, the sex identification of individuals was undertaken by scientists using images captured by citizen scientists and is reliable. Assuming monogamous behaviour, this data indicates that sexual reproduction is possible since both males and females have been observed. The difference in sex ratio between the two species invites a hypothesis that reproductive mechanisms (in terms of monogamy which is often claimed for seahorses, for example) may differ between species. The female:male ratio for *H. guttulatus* may indicate monogamous mating behaviour as reproductive productivity is limited by the lower number of males (remembering males experience true pregnancy. Conversely, *H. hippocampus* data would allow one female impregnating more than one male during the same cycle which in turn could indicate lower genetic variability. However, these behaviours have not been observed, and can not be fully confirmed by sex ratio data alone.

Seahorse species assemblages appear to be changing over time, and with temperature. Sightings of *H. hippocampus* are declining both over time and with reported temperature while the opposite is seen with *H. guttulatus*. As discussed above, the demographic of citizen scientists here are recreational divers reporting observations during conditions which are safe to dive and with a seasonal bias. Nevertheless, the data may demonstrate the effect of climate change on seahorse assemblages and future viability of species in Malta. If there was any topography preference for *H. hippocampus* in Malta, this study appears to find that it was seagrass, which deviates from the habitat Lourie describes (1999). No such clear preference could be suggested for *H. guttulatus* which was commonly found in Algae alone and in seagrass, seagrass and sand or just sand just as likely. There was borderline statistical significance implying that there could be a species preference for topography showing seahorse adaptation to habitat though the data may not yet be sufficient to conclude on complexity preferences as Vivas did (2022).

Fisher's exact tests comparing the counts of the two species found either on sand or any habitat containing seagrass, or any remaining habitat containing rocks and algae (but no seagrass) showed borderline significant difference ($p = 0.051$), likely due to a limited sample size. As such, there may be habitat preferences between species, but it is also clear that both species use a variety of habitats in Maltese waters.

The Value of Citizens

Bosso et al (2024) looked at citizen science reports over a period of less than a year in 2022 from the Italian coastline through an appeal to dive centres and concluded that citizen science data has an importance worth highlighting in respect of marine species classed as data deficient, which this study agrees with.

The research budget for the project analysed in this thesis would not have covered the number of professional surveys required to gather the same amount of data submitted by citizen scientists which was submitted *pro-bono*, particularly with regards to image capture. Provided the bias and short-comings of citizen science data are considered and understood, citizen science data can contribute to areas of research currently data deficient, especially for those areas where research is lacking due to restricted funding and at a lower cost than traditional research may need.

Current technology cannot track seahorses through eDNA (anecdotally due to a lack of shedding tissue), have exceptional camouflage in terms of shape, position and the ability to change colour and profile (*H. guttulatus* predominantly). Seahorses may inhabit areas where surveys have not yet been undertaken due to location, cost and time. Thus, the data gathered by citizen scientists does have a high comparative importance, especially for a species on the CITES list and furthermore a species where data is limited from a specific geographical region.

True for both research divers and citizen scientists alike, the limits of recreational diving do restrict the completeness of this data, in some ways to a further extent because the wildlife corridor cannot be followed deeper than 40 m by most divers.

Whilst it would be useful to predict the sample size required to successfully collect statistically valid data from the survey data (one observation in 12 sightings), it is unfortunately in itself not a large enough sample size to allow this at this stage. Rare or elusive species require a larger sample size or insight to improve the number of positive sightings per survey. For example, if it was understood that seahorses could be viewed at night using UV light, a night dive survey would be likely to have more statistical power than a survey during daylight hours. The MCA data in this study did not indicate any factors which might improve the success of surveys for this species, but this author believes improving survey techniques/strategies would offer more scientific value than increasing the number of surveys. Survey dives take resource and rely on unpaid volunteers; they are difficult to organise and require funding so optimising their success would be preferable to increasing their frequency.

Adjacent land use is a subjective qualifier for what activity is predominant in the surrounding land area / sea surface. Whilst a site like Ċirkewwa Marine Park can be categorised as a recognised dive site and a marina can be totally excluded because of the ban on boat mooring there, other sites do have a mixed function and the predominant use has been selected here. The data, as discussed previously, is heavily biased towards the demographic of the sample (recreational divers in the majority) and unsurprisingly, the majority of reports came from a recognised dive site. As the data is subjective and assigned by the author it is anticipated that as the citizen science project expands to boat owners, for example, that sites may be reclassified in future to more clearly define human activity on proximal land and in the water. For example, a dive site may also take tourists on boat trips during the day or a site may be a night fishing port for larger vessels. Though the robustness of the data set is compromised by being made up of only positive sightings and from citizen scientists mainly reporting from a limited number of popular dives sites (as adjacent land use data shows) with restricted depths (40m for most recreational divers) and mainly in the most popular season(s) for diving, it is also true that the data may be more robust comparatively because of the species of study which is elusive and data deficient, and as demonstrated, can be hard to identify in standard non-destructive surveys.

The data for species sighted over time by year is perhaps the most significant finding of this study. Though it would be a big leap to say that *H. hippocampus* has all but disappeared from Maltese waters, the data does raise a conservation flag for *H. hippocampus* in Maltese waters and invites action.

Other European StudiesA lack of consensus on basic methodology, like individual length and sampling technique prohibits analysis of data across the Mediterranean and there is an urgent need for methodological framework regarding seahorses in the Mediterranean. This study could

not be directly compared with any of the published studies reviewed but some interesting reasons for this did emerge.

Recent European publications from citizen science projects and targeted surveys reviewed in this study reflect the lower incidence of *H. hippocampus* when compared with *H. guttulatus* but the change over time is more difficult to compare since research into seahorses is often species-specific and sometimes sampling is destructive and therefore not repeated. Cross-study comparison is perhaps possible on a large scale but the difference in survey techniques, length of surveys, seasonal differences in survey etc. make comparison challenging as Pierri et al. (2022) also noted

When Bosso (2024) looked at citizen science reports over a period of less than a year in 2022 from the Italian coastline there did not appear to be a significant difference in species distribution overall between *H. guttulatus* and *H. hippocampus*. Indeed, *H. hippocampus* records were submitted from Sicily to the direct North of Malta and Pantelleria to the direct North-West. Compare this with the 2020 work of Spinelli (Spinelli, 2020) where the return of *H. hippocampus* was being studied in North-East Sicily and it is evident that of the two species, *H. hippocampus* is the least studied (4 of 11 studies encountered *H. hippocampus*; either intentionally or otherwise) and is perhaps divergent from typically understood *H. hippocampus* behaviour in other ecosystems. Certainly, it should be considered that different approaches may be required to study the different species in future research.

Turkey faces a similar challenge to Malta since published historical data is not available. Of note is the discovery of *H. fuscus*, native to the Red Sea, in Turkish waters (Ergüden & Ayas, 2023) and the importance this has for Malta's alien species monitoring in the near future.

Mediterranean collaborations and procedural alignment are needed and in addition, the applicability of emerging technologies (such as eDNA) must be assessed and harmonised to ensure that future data collection is comparable within the Mediterranean. Maltese studies must include a more comprehensive data collection in terms of water qualities and temperature as well as biologically relevant data.

Conclusion

Once well publicised, records of seahorses from citizen science were relatively frequent in the summer months whereas surveys were far less successful in the provision of data.

The long-term presence of both seahorse species in Maltese waters and the composition of these populations in terms of species, sex and gravidity were established from citizen science reports. The observation of pregnant males confirmed the presence of breeding populations though the sustainability of these populations cannot be confirmed and further research is needed to understand the ecological profile and stability of seahorses in Malta. Comparative work between species in terms of environmental factors was possible and significant findings were made with respect to the decline of sightings of *H. hippocampus* over time, and possibly with temperature.. For example, a significant shift in species composition over time was observed which may indicate the decrease of *H. hippocampus* in Maltese waters. However, opportunistic citizen science data has limits. For example, whilst sea temperatures are correlative to this apparent decline in *H. hippocampus*, the data is less abundant for months where scuba diving and particularly tourist scuba diving is less common and the correlation could therefore be a result of more records occurring in when the water is warmer.

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Standard Operating Procedure- Seahorse Surveys in the Waters of Malta, Gozo & Comino

Purpose

The purpose of this SOP is to provide a standard technique and framework for a consistent approach to dive surveys in Malta, Gozo and Comino undertaken to identify the location of two species of seahorse. Other parameters will be recorded. The SOP is provided in English as a common language of divers though translations may be produced where required.

The aim is to carefully record each seahorse with as little disturbance as possible. This will allow seahorse behaviour to be observed and limit stress. Diver safety is as important as that of the seahorses and each volunteer must be fully insured, possess suitable qualifications, have a minimum of 50 dives and be qualified to PADI advanced level or equivalent. This SOP forms part of the Method Statement under the ERA permit application.

Scope

The SOP applies to all divers undertaking seahorse surveys in Maltese waters. Every survey will be covered by the applicable ERA permit.

The two marine seahorse (Żiemal tal-Baħar) species are found in Maltese waters:

- Spiny Seahorse (*Hippocampus guttulatus*)
- Short Snouted Seahorse (*Hippocampus hippocampus*)

Responsibility

All seahorse surveys are conducted under the supervision of the permit holder. Each diver is responsible for their own transport, kit, training, experience, entry and exit and dive techniques according to the instruction of their dive qualification awarding body. All divers are responsible for undertaking the Seahorse Ecology Survey course after a commitment has been made. All divers are responsible for the surveys being undertaken according to the ERA permit and to this SOP. Deviations from the permit and this SOP will be reported to the permit holder.

Materials

- SCUBA kit
- Dive camera, battery, SD card etc
- GPS (if used)
- Buoy (if used)
- Dive slates
- Measuring stick (e.g. ruler) with clear, contrasting length markers

Procedure

Dive specific pre-checks

According to the consensus, and to cover each dive qualification awarding body, a pre-dive risk assessment to supplement the general risk assessment will be undertaken. All standard pre-dive safety checks specific to awarding bodies, clubs, schools and kit type as well as local laws should be adhered to.

A dive plan including contingency exits should be agreed in groups, buddy pairs or individuals suitable to qualification, conditions and equipment.

Pre-dive procedures

At the surface the following information must be recorded:

Date
Land visibility
Weather*
Moon phase*
Wind*
Wave state*

*where reliable sources exist, this data can be gathered retrospectively

Dive Surveys

To support research into territorial behaviour and understand areas preferred by species, it is critical to mark the start point and keep a compass bearing.

Surveys with GPS

Surveys with GPS are not currently possible in Malta, Comino and Gozo. This SOP will be updated when GPS equipment becomes available.

Surveys without GPS

Survey methodology

Searching for seahorses requires the diver to move slowly and with good buoyancy and movement control to concentrate on the seabed and habitat. The Seahorse Trust have used four survey methods and recommend that the dive leader selects which is most suitable for the site e.g. Durant Transect for seagrass meadows and Linear Transect for habitat edges. Survey type diagrams are included in the Annex.

Once a seahorse is located these guidelines should be followed. However, the surveillance and proximity to the seahorse should end immediately if it is suspected that the activity is impacting negatively on the seahorse:

Surveillance procedure

- Maintain a distance and observe the seahorse to check for any signs of stress.
- If the seahorse appears relaxed with the diver's presence, the diver should take a photo of the seahorse within its habitat.
- Check for any signs of stress.
- Slowly approach directly towards the seahorse.
- Take another, closer, photograph with a ruler or measure stick to allow size estimation

- Record as many of the following as time and conditions allow (photographing the dive computer may suffice providing the image is of sufficient quality):
 - Co-ordinates
 - Depth
 - Visibility
 - Temperature
 - Seabed habitat
 - Sex
 - Gravidity
 - Behaviour
 - Associated species
- The maximum time limit for an encounter is 5 minutes to avoid stress.
- Once the observations are complete, the diver(s) should back away slowly from the individuals and continue on their survey trajectory.

A seahorse under stress will	A seahorse not under stress will
• Try to look very small • Turn their back on the perceived threat • Lay flat on the seabed • Turn a very dark colour • Try to swim away (if they do, do not chase them, let them go)	• Raise its head up and look around, it will open up and visibly relax • Turn towards you to see what you are and look directly at you • Lighten up in colour • Settle in front of you and not swim away • Be curious about you and what you are doing

Photography

The seahorse will be photographed without flash but depending on the environment artificial lighting may be required (seahorses are known to be found in caves around the islands) to record key data. Ideally both sides of the seahorse face will be photographed.

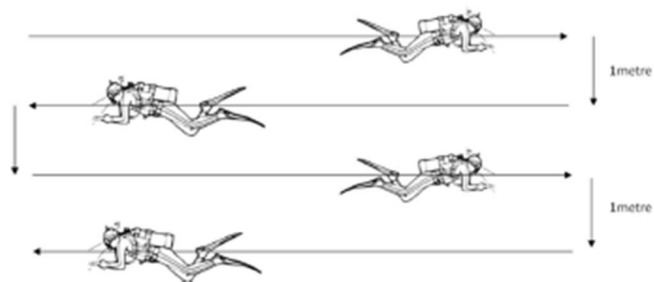
References

Version history

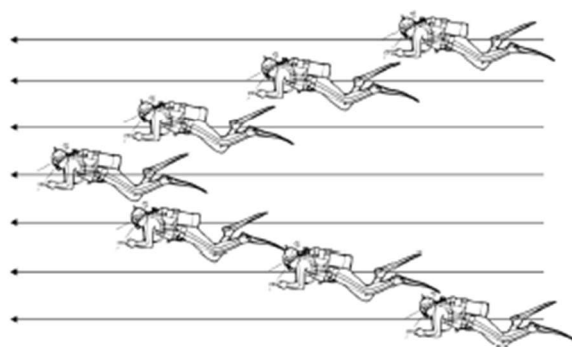
Version 1 23 November 2021

Annex

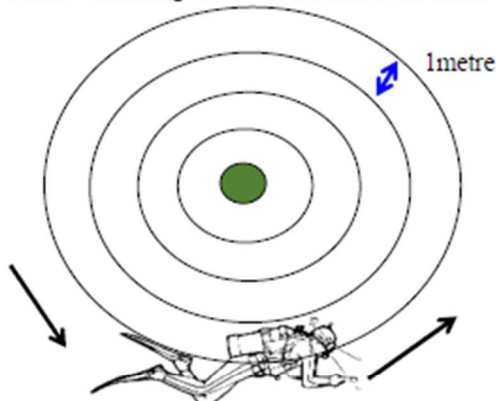
LINEAR TRANSECT – small areas or habitat edges. Transects are 1 m apart for 30-50m.



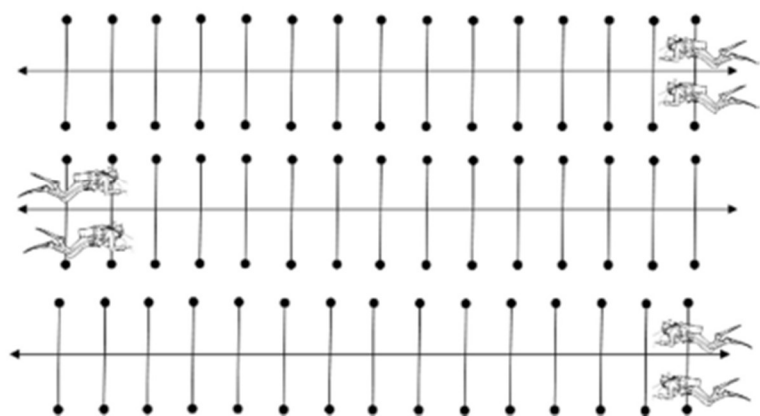
DURRANT TRANSECT – multiple divers covering a large area.



CIRCULAR TRANSECT – searching for individual seahorses within a known territory.



POINTS TRANSECT – covering large areas with small groups. Stop every 2m.



Seahorse Sighting in Malta

You have been lucky enough to have seen a seahorse in the waters of Malta, Gozo or Comino and kind enough to report it to us, Thank-you!

Please use one form per sighting and if you are in a group, only one person needs to file a report.

Please complete the survey honestly and to the best of your ability. Contact us via our facebook page or by email maltaseahorseproject@gmail.com , if you have any questions or complaints. Any data you share with us will be held according to The Seahorse Trust policy found on their website.

Thank-you!

** Indicates required question*

1. What date did you see the seahorse? *

Example: 7 January 2019

2. Was this sighting part of an official seahorse survey under the permit of ERA? Only tick "yes" if you are directed to by the permit holder. If you were not on a planned seahorse survey dive, your answer will be "No"

Mark only one oval.

☐ Yes

☐ No

3. And what time, roughly?

Example: 8.30 a.m.

4. Which Island was the seahorse seen closest to?

Mark only one oval.

- ☐ Malta
☐ Gozo
☐ Comino

5. And what locality or site? For example, Anchor Bay, Mgarr Ix-xini etc.

6. If you know the GPS coordinates (you can get these from google maps), please enter them here. Otherwise, you may use this space to describe where the seahorse was in more detail (for example, on the left side of the bay near the second cave entrance).

7. What was the depth of the sighting in meters?

8. If you were diving, what was the visibility like, in meters?

9. And the water temperature at the sighting depth, in celsius / centigrade?

10. What size do you estimate the seahorse to be, in cm from the top of the head to the bottom of the uncurled tail?

Mark only one oval.

- ☐ 1 cm
☐ 2 cm
☐ 3 cm
☐ 4 cm
☐ 5 cm
☐ 6 cm
☐ 7 cm
☐ 8 cm
☐ 9 cm
☐ 10 cm
☐ 11 cm
☐ 12 cm
☐ 13 cm
☐ 14 cm
☐ 15 cm
☐ 16+ cm

11. What colour was the seahorse when you first saw it? Choose all that apply

Tick all that apply.

- ☐ Yellow
☐ Green
☐ White
☐ Grey
☐ Black
☐ Brown
☐ Sand / Biege
☐ Other: _____

12. Where was the seahorse?

Mark only one oval.

- ☐ Anchored on a horizontal plane (like the sea bed)
- ☐ Anchored on a vertical plane (like the reef wall)
- ☐ Free swimming
- ☐ First sighted out of the water
- ☐ On an artificial reef, like a wreck
- ☐ Other: _____

13. What was the immediately surrounding area like? Choose all that apply.

Tick all that apply.

- ☐ Seagrass
- ☐ Algae/seaweed
- ☐ Rock
- ☐ Sand
- ☐ Cave

14. Do you have any photographs or video of the sighting?

Images are really crucial to our research. We can identify the species, the sex, even each individual and we can tell if a male is pregnant. We'd be really grateful if you could share your images. Please add your best ones here and send as many as you can to our facebook page, whatsapp or email address.

By sending us these images you agree that we can use them for our research and this may include publication in a scientific domain. We will never use your images in any other way without your express permission (please leave contact details if you are happy for us to contact you to seek permission).

Files submitted:

15. If you are happy for us to contact you to further discuss your sighting, please leave your email address or whatsapp number below:

!!! Thank-you !!!

Thank-you so much for sharing your sighting with us. If you have any general questions, any questions about image sharing or would like to send us some images, please do get in touch with us by email: maltaseahorseproject@gmail.com .

The data you have shared will be reported to ERA and ultimately be used to support marine environmental protection in Malta.

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