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Justin Camilleri, Louis F. Cassar & Patrick J. Schembri

DISTRIBUTION AND ABUNDANCE OF THE ALIEN HELICID *OTALA PUNCTATA* (MÜLLER, 1774) (*Mollusca Helicidae*) AT BAĦRIJA, MALTA, A SITE OF AGRICULTURAL AND CONSERVATION IMPORTANCE

SUMMARY

The alien helicid *Otala punctata* has to date been recorded from two localities on the island of Malta. Based on field surveys, this study assesses the distribution and abundance of the species in one of these localities, Baħrija valley, an agriculturally important area and a site of conservation interest. *O. punctata* was found to have colonised three areas along the valley system, with the highest densities where there was low, virescent vegetation. Juveniles had a high abundance at the peripheries of the area of distribution, suggesting active dispersal outwards from the centres of the population, possibly in response to intraspecific competition for resources. Spread of the snail appeared to be slowed or halted by physical barriers, but anthropogenic transport through carriage of agricultural produce from Baħrija to markets elsewhere, and discarded agricultural material, has overcome natural barriers. The occurrence of *O. punctata* in the study area does not appear to have affected the population density of the closely-related indigenous helicid *Eobania vermiculata* thus far. Given the alien species' potential to become an agricultural pest, and the conservation importance of Baħrija valley, close monitoring is recommended.

Keywords: Invasive species, Helicidae, land snail, Maltese Islands, population, agricultural pest.

RIASSUNTO

Distribuzione e abbondanza dell'elicide alieno Otala punctata *(Müller, 1774) a Baħrija, Malta, un sito di importanza agricola e conservazionistica*. L'elicide alieno *Otala punctata* è stato segnalato finora in due località maltesi. Questo studio, basato su attività di campo, mette in luce la distribuzione e la frequenza di questa specie in una delle due località, la valle Baħrija, un'area importante dal punto di vista agricolo ma anche un sito di interesse conservazionistico. *O. punctata* ha colonizzato tre aree lungo la valle, con le maggiori densità dove c'è una bassa e virescente vegetazione. Le forme giovanili mostrano un'elevata abbondanza alla periferia dell'area, suggerendo una dispersione attiva al di fuori del centro della popolazione, possibilmente in risposta alla competizione intraspecifica per le risorse. La diffusione della chiocciola appare diminuire o fermarsi davanti a barriere fisiche, ma il trasporto da parte dell'uomo attraverso la movimentazione dei prodotti agricoli da Baħrija al mercato, e lo scarto del materiale agricolo, le ha consentito di superare le barriere naturali. La frequenza di *O. punctata* nell'area di studio non sembra abbia interferito finora con la densità della popolazione dell'elicide indigeno strettamente imparentato *Eobania vermiculata*. Considerato il potenziale della specie aliena come 'peste' in campo agricolo e l'importanza della conservazione della valle di Baħrija, si raccomanda un suo continuo e rigido monitoraggio.

INTRODUCTION

The helicid *Otala punctata* (Müller, 1774) is a western Mediterranean thermophilic species, with a range extending from the north-western tip of Algeria to France (FALKNER, 1990; CLANZIG & BERTRAND, 2001; FALKNER *et al.*, 2002; MAUREL, 2006; BANK, 2013; HOLYOAK & HOLYOAK, 2017). SACCHI (1965) suggests it is of Ibero-Maghrebian origin, while MARTINEZ-ORTÌ & ROBLES (2001) consider the species native to the Iberian Peninsula. The species is known to have dispersed via anthropogenic agency to other geographical areas, some very distant from its original range (Fig. 1) and to have successfully established breeding populations in areas with similar climatic regimes to its native range, as for example in South Africa (HERBERT & SIRGEL, 2001). Its introduction in non-native environments has the potential to alter local ecosystems (POINTIER & AUGUSTIN, 1999).

In some cases, *O. punctata* has become invasive and has proliferated to the point of becoming a horticultural and agricultural pest (BORAY AND

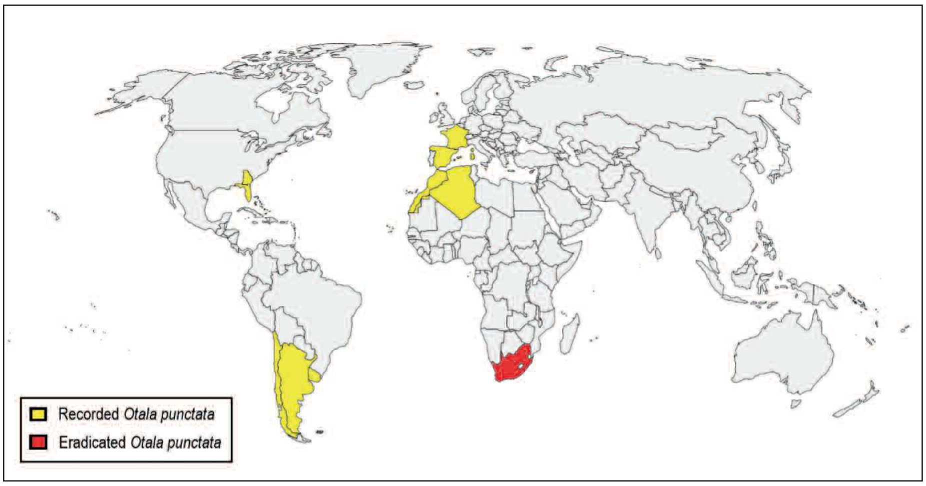

Fig. 1 — World map showing the countries where *Otala punctata* has been recorded (in yellow). South Africa (in red) is the only country where alien *Otala punctata* has been eradicated.

MUNRO, 1998; MIENIS, 1999) that can be challenging to eradicate (FRANK, 2010). Although there is no precise documentation of the monetary losses suffered by the agricultural sector due to *O. punctata*, this sector is known to experience high financial losses with respect to the management of alien invasive species (PAINI *et al.*, 2016). *O. punctata* is known to thrive in various habitats, including rocky slopes (KERNEY *et al.*, 1983), gardens (MIENIS, 1999), wasteland, industrial land, disturbed land, docks (HERBERT & SIRGEL, 2001), coastal zones (FRANK, 2010), rural environments (DE MATTIA & MASCIA, 2011), vineyards, and walls (HERBERT, 2010).

The species is mostly nocturnally active (BARBARA & SCHEMBRI, 2008), particularly with dewfall, which facilitates mobility. *O. punctata* aestivates, sealing its aperture with an epiphragm (BARBARA & SCHEMBRI, 2008). During its active season, the snail feeds on foliage, favouring dense ruderal vegetation for grazing (WHITE-MCLEAN, 2011).

The climate and habitats of the Maltese Islands are suitable for the species, and invasion by *Otala punctata* may potentially pose a threat to native gastropod species, including rare and endemic forms (GIUSTI *et al.*, 1995; GUREVITCH & PADILLA, 2004). An *Otala* species was first recorded in the Maltese Islands by FEILDEN (1879), based on shells that were apparently deposited on the shore by waves and presumably transported from elsewhere. A shell of *Otala lactea* was reported to have been acquired from Malta (MACHIN, 1972), but this was likely a misidentification of *Eobania vermiculata* (BARBARA & SCHEMBRI, 2008), a common indigenous helicid which resembles to *O. lactea*. In 2003, a population of *O. lactea* was recorded from a horticultural nursery in the limits of Mosta, central Malta (MIFSUD *et al.*, 2003); however, BARBARA & SCHEMBRI (2008) showed this to be a misidentification of *O. punctata* and that this species had established a considerable breeding population covering an area of 50,000 m^2 around the nursery, with at least three generations occurring contemporaneously. BARBARA & SCHEMBRI (2008) hypothesized that the species had been accidentally imported into the nursery with plants and then escaped to the surrounding area. The population of *O. punctata* was found to be generally more abundant than that of indigenous helicid species; however, there was no evidence that the alien was limiting native helicids. Nevertheless, given its invasiveness potential, the authors recommended monitoring the population and possibly implementing an eradication programme, to prevent its further dispersal.

In April 2011, *O. punctata* was found in a second locality in Malta, at Baħrija valley, approximately 7.5 km from the site of the first record (Fig. 2); an empty juvenile shell and a live mature individual were collected from a vineyard (CILIA, 2012). Six months later, the same author collected eight

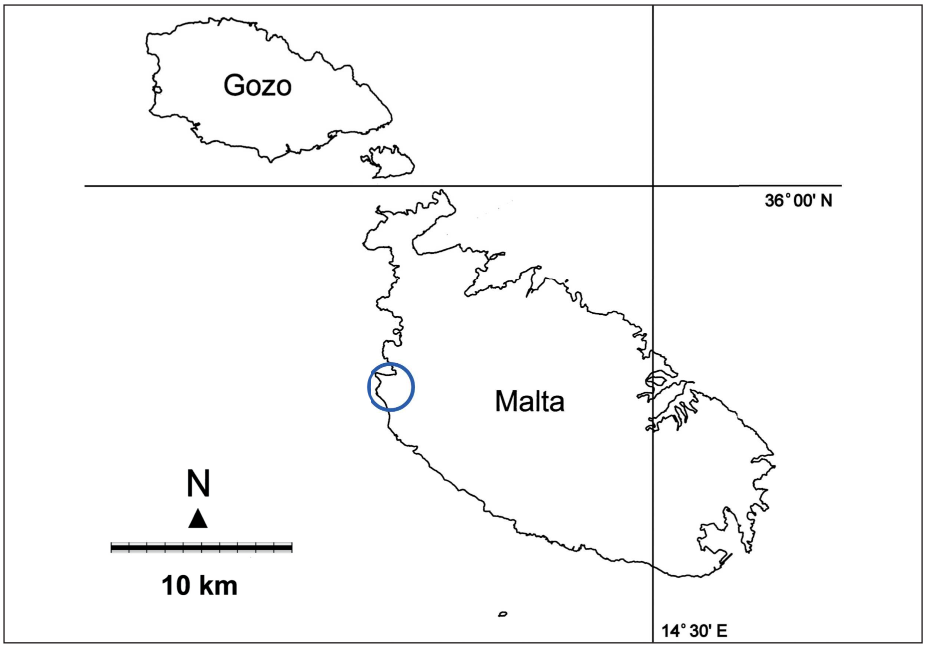

Fig. 2 — Map of Malta indicating the general area of Baħrija.

empty shells and 14 live mature individuals from Baħrija. Twelve of the latter were grazing on cultivated Jerusalem artichoke (*Helianthus tuberosus* L.), while the other two were attached to the stems of two grapevines (*Vitis vinifera* L.). CILIA (2012) hypothesised that the species had likely been introduced into Baħrija from the Mosta nursery, possibly with seedlings of Jerusalem artichoke or other vegetation and/or with soil. In May 2018, we were shown two unusual snails found in a cabbage (*Brassica oleracea*) bought at Paola, Malta, which we identified as adult individuals of *O. punctata*.

In the present study we assessed this second population of *O. punctata*, particularly its distribution and abundance in the upper segment of the Baħrija valley system, paying particular attention for any sign of potential impact, given that Baħrija is one of the most productive agricultural areas on the islands and that it is also a protected area listed in the Habitats Directive (92/43/EEC), mainly due to the presence of perennial running water along the Baħrija valley watercourse, which is a very rare habitat in the Maltese Islands. Given that produce from Baħrija is sold all over Malta, if the alien has established a large breeding population, there is a real danger that it will be widely dispersed. Therefore, we also investigated if *O. punctata* may potentially disperse through produce.

Materials and Methods

The distribution of *O. punctata* at Baħrija was mapped based on field surveys carried out between early October 2018 and late May 2019, corresponding to the wet season and thus the period during which individuals were expected to be active and more noticeable. The area from where *O. punctata* was recorded by Cilia (2012) was surveyed first, and surveying was then extended outwards from this location, both up and down the valley, until no further specimens of *O. punctata* were found; in total, approximately 2 km^2 of land were surveyed. Surveying was carried out by walking along pathways, roadsides and field boundaries, actively searching for the species. Both live individuals and empty shells were noted. Where permission from landowners was granted, snails were searched for also in cultivated fields. Physical barriers blocking the movement of the alien helicid were noted. The spatial extent of *O. punctata* was then mapped and the rate of spatial spread was calculated in terms of area of occupancy at the end of a time period (T) divided by the number of years since first occurrence (Y), with T/Y therefore giving the rate of aerial expansion per year.

Abundance was determined along two Baħrija valley tributaries (Wied iż-Żebbuġ and Wied tal-Marġa) (Fig. 2) in October 2018 through use of 50 m-long contiguous belt transects. The starting point of the two transects was very close to the locality of the Cilia (2012) record. The number of active *O. punctata* present within a band 1 m wide along the belt transect was recorded, until no further individuals were observed. Data collection was carried out over a period of 12 days and always during rain shower episodes, in order to ensure similar field conditions.

Since the extent of distribution of *O. punctata* was more than one kilometre along the valley system, a plot sampling method (Cornell University & Penn State University, 2009) was used to estimate the abundance of the helicid in the valley. Three sampling plots were established along the two valley channels, each sampling plot measuring 150 m by 100 m (Fig. 3). These plots were then subdivided into smaller 10 m by 10 m squares. A random number generator was then used to select squares for quadrat sampling, with a 1 m^2 quadrat randomly thrown within each selected square. The number of live *O. punctata* and *E. vermiculata* individuals in each quadrat was recorded, together with details of the microhabitat in which the molluscs occurred. Mean density per square metre was calculated for each micro-habitat type for each of the three sampling plots. The number of empty shells belonging to these species was also recorded. All data collection from plots took place during the dry season (August–September 2018).

During plot sampling, it was noted that many individuals were aestivat-

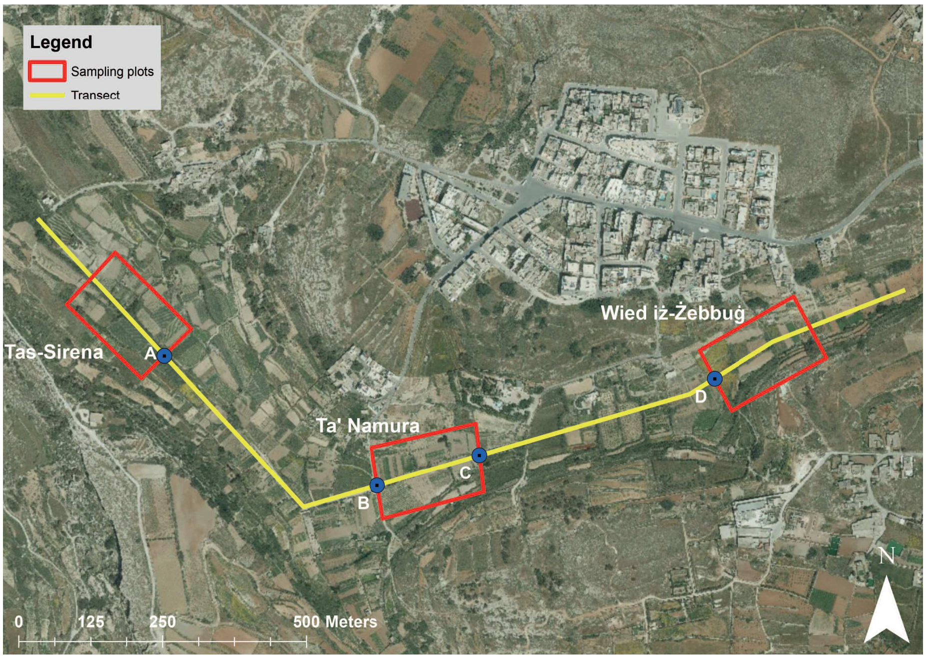

Fig. 3 — The location of the contiguous transects (in yellow) and the three sampling plots (red boundaries), point A to point B and point C to point D measure 350 metres each. (Base map: SIntegraM, 2017).

ing attached to the trunk and branches of trees; the methodology was therefore modified to also include sampling of trees. Large trees (at least 1.7 m high and of 1 m circumference) within the randomly selected squares were surveyed for presence of *O. punctata* and *E. vermiculata*. If more than one large tree occurred in the selected square, one was randomly selected. The number of *O. punctata* and *E. vermiculata* attached to the tree from base to eye level (1.58 m) were recorded. Trees were assumed to be of cylindrical shape and the circumference of the tree was recorded to the nearest 0.1 m to enable an estimation of the area of trunk sampled.

The maximum shell width of each individual *O. punctata* occurring in the quadrats or on sampled trees was measured to the nearest 0.1 mm using digital Vernier callipers as a proxy for age, since the width/diameter of a helicid's shell is proportional to its age (LAZARIDOU-DIMITRIADOU & KATTOULAS, 1981). Shell measurements were grouped into 5 mm size-classes.

Cabbages were used to quantify the species' dispersal potential via produce. Cabbages in crates ready to be transported to the main produce market on the island of Malta were sampled; eight cabbages selected randomly from each of two different consignments (consisting of 16 and 19 cabbages respectively) were examined minutely for any *O. punctata* individuals, and the number of snails per cabbage was recorded.

Finally, landowners, local farmers, bird-trappers/hunters, and other land-users encountered by chance during fieldwork sessions, were shown empty shells of *O. punctata* and asked if they recognised the species. If these individuals were familiar with the species, they were further asked (i) when they first come across it at Baħrija and (ii) where, within the area, they had encountered it.

Results

All nineteen respondents were familiar with *O. punctata* at Baħrija; 18 of these linked its introduction in the area to the planting of trees within a specific property, located approximately 180 m away from the site of Cilia's (2012) record. Through analysis of a time series of aerial photos obtained from the Malta Planning Authority's public Geoserver (http://geoserver.pa.org.mt/publicgeoserver), it was determined that the trees in question were likely planted between 1999 and early 2004. Considering that the first record of *O. punctata* at the Mosta plant nursery dates from the same time period, it is possible that the trees in question may have been sourced from this nursery. If this is the case, it would mean that the helicid was most probably introduced to Baħrija at approximately the same time as it was introduced at the Mosta site.

At Baħrija, *O. punctata* was found to range over a total area of approximately 0.6 km^2, with the two most distant individuals observed located 1.87 km apart. The largest linear distance separating an individual from the possible source of origin identified above was 1.4 km. Populations of *O. punctata* were found within three disjunct areas (Fig. 4), with particular prevalence on agricultural land, especially that irrigated and cultivated with leaf vegetables. The largest area of distribution (Area 1, ~ 535,000 m^2) incorporated the possible point of origin and the area of the Cilia (2012) record. Area 2 (~65,000 m^2) is downstream of Area 1 and almost entirely separated from it by a large expanse of abandoned agricultural land within which no *O. punctata* were observed. The two areas are approximately 200 m apart. Area 3 (~ 800 m^2) is further downstream of Area 2 and separated from it by a distance of at least 275 m. Here, the species was limited to just two small fields. Several barriers may possibly be impeding further spread of *O. punctata* (Fig. 4). The roads on the northern and downstream borders of Areas 1 and 2, respectively, may completely halt further dispersal of the helicid. Expanses of abandoned agri-

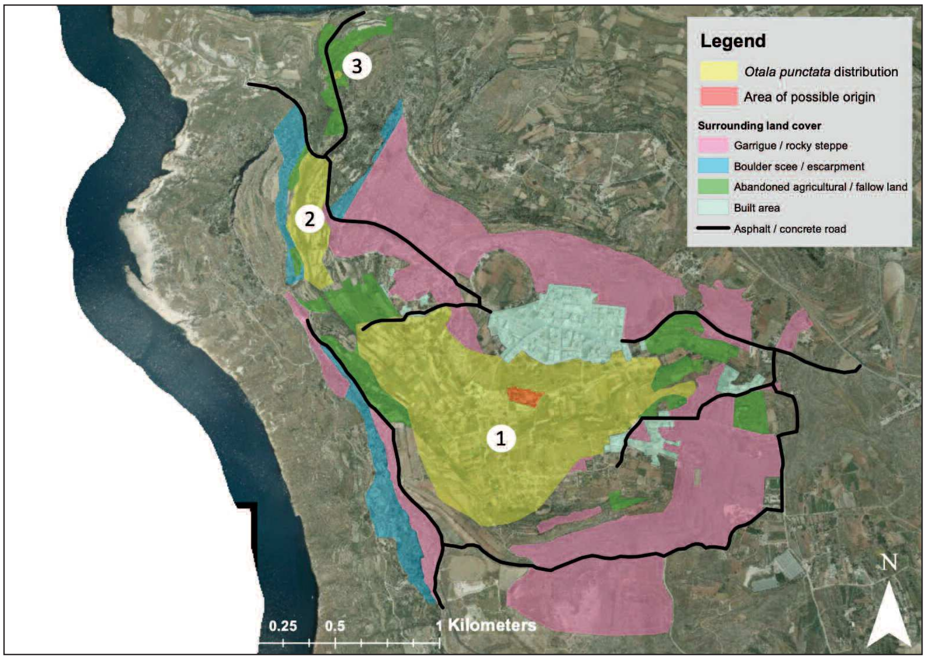

Fig. 4 — The three areas of distribution of *O. punctata* and barriers restricting further spread. (Base map: SIntegraM, 2017).

cultural land also appear to act as containment buffers, as do escarpments and boulder screes. No *O. punctata* were observed in the built-up area, probably due to lack of food sources. The alien has a limited spatial distribution and reduced abundance on rocky steppe, garrigue and phrygana habitats, possibly because it prefers more tender vegetation than the woody species that dominate these habitats.

The highest and lowest counts of *O. punctata* recorded within transects were of 99 and 1 individual, respectively, with corresponding densities of 1.98 and 0.02 individuals per m^2. Abundance varied widely across the transect (Fig. 5), with highest abundance very close to the site of the CILIA (2012) record. This area of highest abundance and that of the second highest abundance are both situated within active agricultural land. Other areas of high abundance were in sites where the species was aestivating on trees. Comparatively, very low abundances were recorded in transect intervals 12 and 13, although these were closest to the possible area of origin; this may be due to these sites being located on ploughed agricultural land without cultivated produce (and thus with no food source for the snails). Moreover, adjacent valley slopes were covered with xerophytic vegetation, which *O. punctata* does

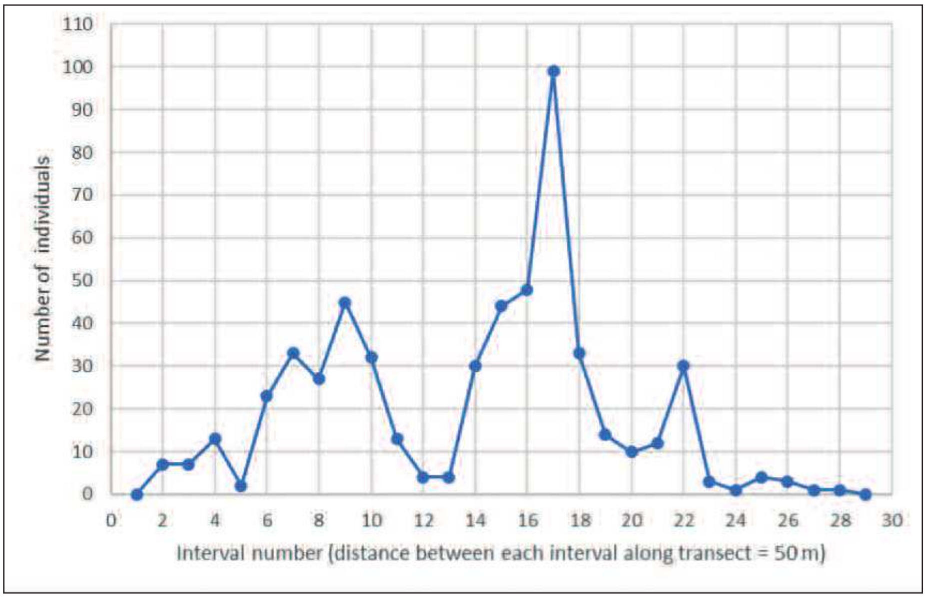

Fig. 5 — The number of individuals of *O. punctata* occurring in contiguous 50-metre segments of a line transect along distribution Area 1 (see Fig. 3).

not seem to feed on. A very similar habitat occurred at interval 5 and the low abundance here may be explained in the same way. Low abundances registered from interval 19 onwards may be due to the use of molluscicides, as noted from pellet residues next to crops and dead *O. punctata* observed along this span of the transect. This was confirmed by interviewing farmers in this part of the valley, who reported that the snail had proliferated to such an extent that it was consuming large quantities of their crop and they therefore had to resort to using a molluscicide in an attempt to control it.

Of the three plot areas (all located in Distribution Area 1), the Ta' Namura area had the highest abundance of *O. punctata*, followed by Wied iż-Żebbuġ and Tas-Sirena (Fig. 6). The Ta' Namura area is situated closest to the site of possible origin of the alien and in the middle of the largest area of occurrence of the species. Abundance in Wied iż-Żebbuġ was notably higher than in Tas-Sirena, possibly due to the Wied iż-Żebbuġ sampling plot being situated closer to the site of possible origin (shortest distance, 225 metres) when compared to the Tas-Sirena area (shortest distance, 375 m).

The densities of *O. punctata* and of *E. vermiculata* within the three areas are given in Fig. 7. At Ta' Namura, there was a higher density of both living and dead *O. punctata* compared to *E. vermiculata.* However, at Wied iż-Żebbuġ and Tas-Sirena, the density of *E. vermiculata* was higher than that of the

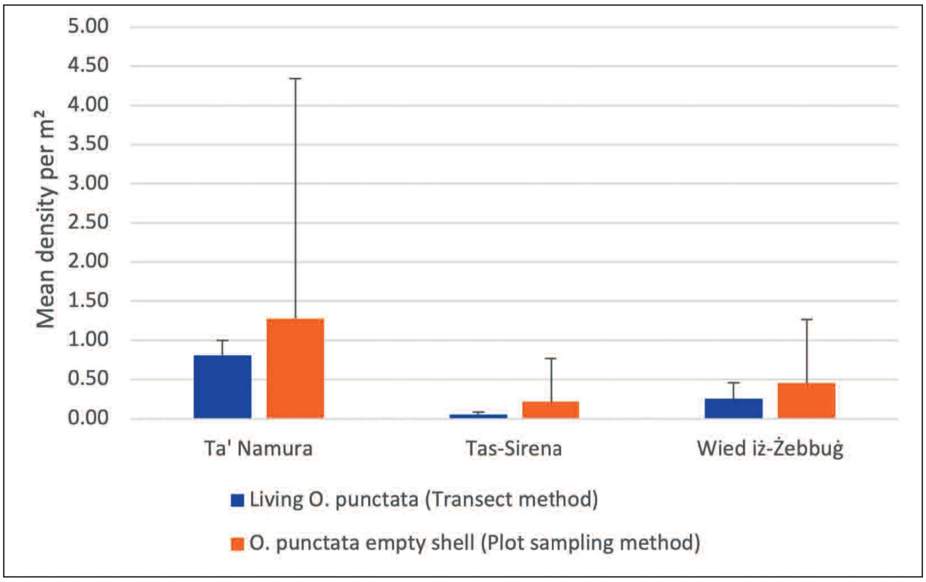

Fig. 6 — Mean density of living *O. punctata* in the transect (blue) and the empty shells in plots (orange), all in distribution Area 1. Error bars are + 1 SD.

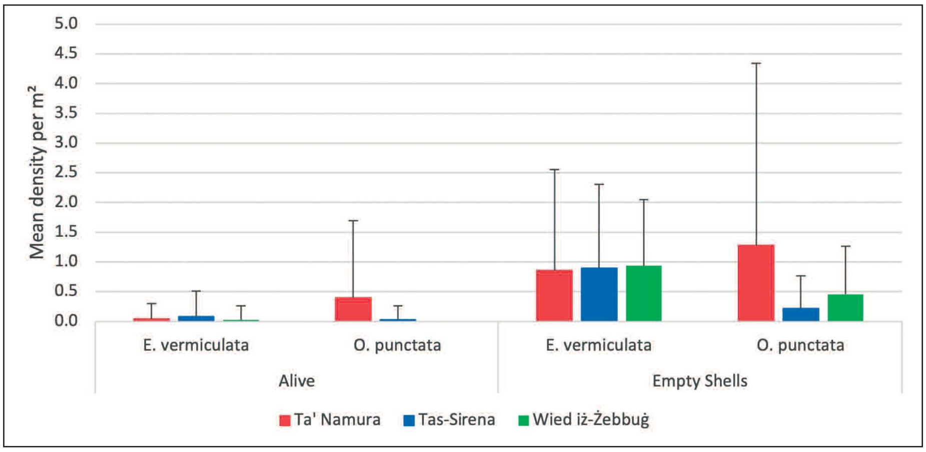

Fig. 7 — Density of *E. vermiculata* and *O. punctata* (individuals per m²) in the three areas of study (see Fig. 3). Error bars are + 1 SD.

alien. Densities of *E. vermiculata* were more or less the same in all the three areas, whereas those of *O. punctata* fluctuated markedly.

The microhabitats occurring within quadrats were classified into five categories: ploughed soil, dry grass, low virescent vegetation, vines, and trees.

For the last category, only live gastropods attached to trees were recorded. In all three sampled sites, abundancies of empty *O. punctata* shells were highest in low living vegetation. Conversely, *E. vermiculata* shell abundances were higher in dry grass. Both gastropods showed a particular preference for fields with *Vitis vinifera*. The least favourable habitat was ploughed soil, with this accounting for the lowest number of individuals of both species.

Figure 8 shows the size-frequency distribution of living and empty shells of *O. punctata*. Two main size classes occur within the sampled areas, corresponding to adults and juveniles. For live *O. punctata*, all the three sites are dominated by adults. At Wied iż-Żebbuġ and Tas-Sirena a considerable number of empty juvenile shells were recorded.

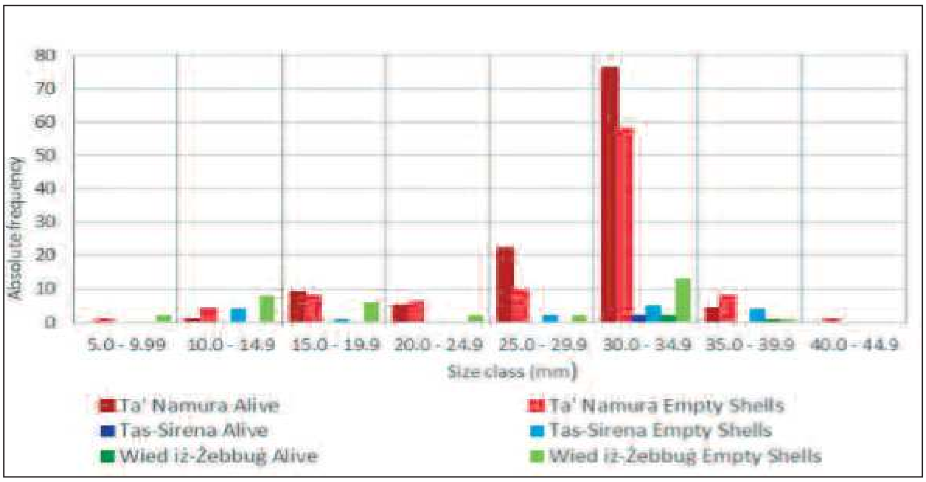

Fig. 8 — The size-frequency distribution of live and empty shells of *O. punctata*, in the three areas of occurrence.

Table 1 shows results of the analysis of cabbages prepared for transport to the central market. Almost 69% of the cabbages examined had at least one individual of the alien species in them, and some had up to eight. The individuals found were mostly juveniles.

Discussion

O. punctata occurred in three areas at Baħrija. The different abundances in these seem likely linked to distance from the possible point of original introduction of the alien. Area 1 surrounds the presumed point of origin,

Table 1
Number of individuals of Otala punctata *found within each randomly chosen cabbage (*Brassica oleracea *var.* capitata*) prepared for transport to the market in two areas of occurrence at Baħrija.*

Ta' Namura		Wied iż-Żebbuġ	
Cabbages	*Otala punctata* found	Cabbages	*Otala punctata* found
1	2	1	3
2	0	2	4
3	0	3	8
4	4	4	2
5	2	5	3
6	0	6	0
7	1	7	8
8	4	8	0
Mean:	1.6	Mean:	3.5
Standard Deviation:	1.7	Standard Deviation:	3.1

while Areas 2 and 3 are more peripheral. Based on the present results there are indications that snails have dispersed downstream along the valley from Area 1 to Areas 2 and 3. This dispersal may have been achieved under their own steam or the snails could also have been transported through human agency, for example with harvested crops (especially those providing good hiding places such as cabbages and cauliflowers), by agricultural vehicles, with agricultural equipment (especially irrigation pipes, since the helicid was occasionally observed to aestivate inside irrigation pipes that are not in use), and through transfer of eggs with soil adhering to the roots of seedlings. Human-mediated transport is suggested by the discontinuity between the three areas. In downstream Area 2, the helicid appears to be moving upstream, colonizing the terrain between Area 2 and Area 1 where it presently does not occur. According to a local resident who works the fields in the downstream part of Area 2, the mollusc had already established itself in downstream part of Area 2 in 2015 (J. Dimech, pers. comm, May 19, 2019). On the other hand, it only more recently colonised the upstream section of Area 2 as indicated by the distribution of living/empty specimens; empty *O. punctata* shells were abundant in the downstream part of Area 2, and decreased further upstream until they were absent altogether, although some living individuals were still present. Given the intensity of agricultural cultivation in the area and considering that many farmers farm plots of land in different sections within the valley system, it is very likely that farmers have accidentally dispersed the alien from Area 1 to both Areas 2 and 3. Further spread along the valley is likely, both through human agency as well as due to the limited extent of natural barriers to movement (such as abandoned agricultural land and roads) at the southern extremity of Area 1.

Additionally, there is the potential for island-wide anthropogenic dispersal given the number of *O. punctata* found in harvested cabbages ready to be transported to the central agricultural market, from where individual sellers buy produce in bulk for sale at outlets all over the island. Just 16 randomly sampled cabbages had 41 *O. punctata* individuals on them. Juveniles, in particular, are better able to hide within produce and are thus less likely to be spotted and removed by farmers or sellers. The species also has the potential to be deliberately transported from the area as a result of its culinary value; this large edible species is already consumed by some farmers at Baħrija (T. Camilleri, pers. comm, July 9, 2019) and the practice may become more widespread.

The size-frequency results show that at the periphery (Wied iż-Żebbuġ and Tas-Sirena) of the main area of occupancy juveniles are more abundant, which may mean that they are moving further away from the main area, possibly in response to lack of food and other resources. The number of empty juvenile shells suggests a high juvenile mortality in these peripheral areas; this may be due to natural causes but also to the application of molluscicides. However, empty shells accumulate and persist for many years, so these results represent a larger temporal spread than those for live individuals.

While the preferred habitat for *E. vermiculata* was found to be dry grass, *Otala punctata* evidently preferred low-growing vegetation. The native and the alien thus have different microhabitats, and possibly, food preferences. However, both species showed an affinity for fields cultivated with *Vitis vinifera*, possibly due to the presence of year-round vegetation in vineyards, as well as because *Vitis* stems offer adequate elevation from the ground for aestivation. However, while *O. punctata* appears to prefer to aestivate in vineyards, *E. vermiculata* seems to prefer trees.

The area of distribution of *O. punctata* at Baħrija is approximately twelve times that observed at Mosta (BARBARA & SCHEMBRI, 2008), likely due to the longer time period the species has had to establish itself there; this assumes that the species was introduced at Baħrija at approximately the same time as it was introduced at Mosta. However, the rate of colonization appears to differ between the two sites. Based on the time that elapsed between the first record from Mosta in 2003 and the subsequent study by BARBARA and SCHEMBRI (2008), the yearly aerial expansion rate was calculated to be 0.017 km^2 per year, compared to a yearly aerial expansion rate of 0.075 km^2 per year at Baħrija. It is reasonable to hypothesize that the higher expansion rate at Baħrija is the result of increasing propagule pressure. Given that the helicid is not a very active animal, the rate of colonization of the species is quite rapid in both areas.

While *O. punctata* still has a relatively limited distribution in the Maltese Islands, this study has confirmed the successful establishment of a second population at Baħrija and the potential for further spread, particularly through anthropogenic dispersal. It is not yet known whether small populations of *O. punctata* have already been established elsewhere in the Maltese Islands, but it would not be surprising if they have. This is of concern given the potential for the species to become an agricultural pest, as is already the case at Baħrija. Further research is needed to determine whether the species is already present elsewhere, to assess its likely impact on agriculture, to better understand the species' food preferences, and to identify appropriate management strategies.

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Authors' Addresses — J. CAMILLERI, L.F. CASSAR*, Institute of Earth Systems, University of Malta, Msida, MSD 2080 Malta; P.J. SCHEMBRI, Department of Biology, University of Malta, Msida, MSD 2080 Malta; e-mail: justin.camilleri.16@um.edu.mt, louis.f.cassar@um.edu.mt, patrick.j.schembri@um.edu.mt

* Corresponding author.