So close so different: what makes the difference?

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Abstract. The introduction of alien fish species in wetland ecosystems could have a great impact on freshwater communities and ecological processes. Despite fish introduction has been noticed as one of the principal cause of freshwater extinctions, ecosystem processes alteration, and change in aquatic community assemblage, very few data about impact on freshwater reptiles are available. As study model we used two neighbour sub-populations of the endangered Sicilian pond turtle, *Emys trinacris*, inhabiting two small, close each other and very similar lakes, except for the presence of allocthonous fish, *Cyprinus carpio* and *Gambusia hoolbroki* in one of the two. The multi-year study allowed highlighting significant differences in abundance, growth and reproductive output between the two freshwater turtle sub-populations, suggesting their influence on phenotypic plasticity of the studied population. These results are discussed in the light of previous evidence about the impact of these alien species on abundance and assemblage of the invertebrate community with an evident impact on niche width, diet composition and therefore energy intake by *Emys trinacris*. These data may provide important information to address management strategies and conservation actions of small wetland areas inhabited by pond turtles, pointing out a threats never highlighted up to now.

Keywords. *Emys trinacris*, phenotypic plasticity, *Cyprinus carpio*, *Gambusia hoolbroki,* alien fishes impact.

INTRODUCTION

Phenotypic plasticity is an evolutionary adaptation to environmental variation often used to explain the intraspecific difference observed within various taxa, that could be influenced by local variation in abiotic and biotic factors associated with habitat type (Lubcke and Wilson, 2007). Nowadays, the introduction of alien species is one of the main stressor of freshwater ecosystem biodiversity (Genovesi, 2007), and it could have a great impact on freshwater communities causing cascading impacts on food webs and complex interactions among species (Ricciardi and MacIsaac, 2011). In the last decades, many species of fishes have been deliberately introduced worldwide to provide food or sport leisure, but also released from aquaria, bait buckets, and water gardens, as contaminants of fish intended for stocking, or in ballast water (Strayer, 2010). Despite fish introduction has been noticed as one of the principal cause of freshwater extinctions (Dextrase and Mandrak, 2006), ecosystem processes alteration (Pitcher and Hart, 1995) and change in aquatic community assemblage (Parkos et al.,

2003), very few data about impact on freshwater reptiles are available.

Within this group, freshwater turtles are known to vary in different natural history traits among conspecific populations both over broad geographic area (Lovich et al., 1998; Joos et al., 2017) as well as among adjacent sites (Tucker at al., 1998). They usually follow a growth pattern that involves rapid growth from hatching to sexual maturity, followed by little or no growth once maturity is attained (e.g., Wilbur, 1975; Bury, 1979). Growth can be influenced both by different ecological factors, such as hatching size, food availability and habitat suitability (Mahmoud, 1969; Bury, 1979). Moreover, different growth patterns can lead to the same size (Bury, 1979; Andrews, 1982) or to different size at the maturity (Stearns and Koella, 1986). These differences can have also a direct impact on reproductive output in a taxon where clutch size often increases with maternal body size (Zuffi et al., 2004).

In particular, Ottonello et al. (2017a) showed how the presence of two alien fishes, *Cyprinus carpio* Linnaeus, 1758 and *Gambusia holbrooki* Girard, 1859, can alter the abundance and the assemblage of the invertebrate community with a considerable impact on niche width and diet composition of an endangered turtle. Local food availability and quality may indeed play a key role to regulate feeding strategy with consequences on growth, reproductive output and population density (Dunham and Gibbons, 1990; Parmenter and Avery, 1990). For these reasons we have aimed at investigating if the presence of alien fishes can have an impact at local scale on a population of an endangered turtle using as case study two neighbour wild sub-populations under similar climatic and environmental conditions. As model species we selected *Emys trinacris* Fritz et al., 2005, the only native freshwater turtle of Sicily, a large island off the coast of Italy. The Sicilian pond turtle lives in wetlands and slow-moving water bodies (e.g., lagoons, deltas, inland waters and mountain lakes) with soft bottoms and abundant aquatic vegetation, especially on the banks, from the sea level up to 1036 m a.s.l. (Marrone et al., 2016). However, the presence of the species at about 1250 m a.s.l. was recently observed (F. Marrone and R. Scardino, pers. obs.). In view of the low number of sicilian indigenous fish (Zerunian, 2004) and the absence of specialized aquatic vertebrate predators (Aa. Vv., 2008), it is likely to assume that *Emys trinacris* is one of the top aquatic predators in many of these environments in Sicily, with an opportunistic and generalist pattern oriented mainly towards aquatic invertebrates (Ottonello et al., 2017a).

Because of the introduction of non-native fish species, that are now the most represented non-indigenous taxon occurring in Sicily (Marrone and Naselli-Flores, 2015), new dynamics that can significantly alter and threaten the structure of the native biota have been established (Naselli-Flores and Barone, 2012) and top predators are likely to be valuable indicators of ecosystem health (Landres et al., 1988). Although some data and experimental studies highlighted the poorly competitive abilities of the genus *Emys* against some alien species, like *Trachemys scripta* (Cadi and Joly, 2003, 2004) and *Micropterus salmoides* Lacepède, 1802 (Lacomba and Sancho, 2004; Ayres and Cordero, 2007), robust evidence of interactions in the wild is still lacking (but see Polo-Cavia et al., 2010; Lambert et al., 2019). Moreover, Rakausas et al. (2016), studying the predator-prey interactions between a recent invader in Lituania, the Chinese sleeper (*Perccottus glenii* Dybowski, 1877) and the European pond turtle stated that this fish does not directly contribute to the decline of *E. orbicularis* because hatchlings turtles are resistant to *P. glenii* predation and, adults of *E. orbicularis* consumed juvenile *P. glenii,* but no data about indirect impacts (e.g., trophic resource competition) have been evaluated. Therefore, we assumed that Sicilian pond turtles in a habitat without alien fishes would have access to wider food availability than would individuals in a habitat with introduced alien fishes. We therefore hypothesized that this would affect their rates of energy intake and we predicted that turtles in a fish-less habitat would: (1) exhibit different patterns of growth, (2) have higher annual reproductive output, and (3) have higher abundance. To test this hypothesis in the wild, we compared the parameters of two *Emys trinacris* sub-populations inhabiting a fish-inhabited and a fish-less lakes during a multi-year study. The evidence of the negative impact of the presence of introduced fish fauna can provide additional information to further understand the effect of alien species on native fauna as well as promote adequate management plans.

MATERIALS AND METHODS

Study area

The Nature Reserve of the "Lake Preola and Gorghi Tondi" is a protected area of south-western Sicily, established in 1998 and currently managed by the Italian Association for the World Wildlife Fund. The Nature Reserve (medium point at 37°36'36.78"N 12°39'8.19"E) has a surface of 335 hectares and is surrounded by a homogeneous agricultural landscape (vineyards and olive groves). The Nature Reserve protects four distinct wetlands (Pantano Murana, Lake Preola, Gorghi Alto-Medio and Gorgo Basso), originating in a karstic depression. The basins do not have tributaries and the groundwater table is

located in the calcarenites and is fed by local meteoric recharge (Cusimano et al., 2006). Lake Preola is the widest water body of the area, with a surface of 33 ha approximately, filled with a thin layer of water occasionally deeper than two meters. The "Gorghi" are lake environments in a mature euthrophic status, with an average surface area of approximately 3 ha for the lake Gorgo Basso (altitude: 6 m a.s.l) and 5 ha for the lake Gorghi Alto-Medio (altitude: 3 m a.s.l.), and a maximum depth of 12 m. They are set on sink-holes and they are therefore immediately deep and surrounded by riparian helophytes [*Phragmites australis* (Cav.) Trin. ex Steud., *Typha latifolia* L. and *Cladium mariscus* (L.) Pohl], with some peripherals areas with a maximum depth of about 2 m. Comparison of water chemistry and benthic samples showed no significant difference between the two lakes and both showed high concentration of lead and arsenic (Bellante et al., 2015; Arpa Sicilia, 2016).

The Gorghi Alto-Medio is situated only 200 m away from the Gorgo Basso, but it is separated by an asphalt road and with a slight difference in elevation. Despite their proximity the two sites can be considered inhabited by two distinct sub-populations of *Emys trinacris*. Indeed, although no genetic data are available, capture-recapture data showed a very low migration rate between these lakes confirming what observed over the last fourteen years (D'Angelo, unpubl. data), when for instance only five individuals migrated between the two sites, three from the Gorgo Basso to the Gorghi Alto-Medio and two conversely.

Different non-indigenous species are reported for this territory, like the invasive Red swamp crayfish *Procambarus clarkii* Girard, 1852 (D'Angelo and Lo Valvo, 2003), and Eastern mosquitofish *Gambusia holbrooki* and Wild common carp *Cyprinus carpio* (Ottonello et al., 2017a). Red swamp crayfish is widespread in all the basins without significant difference in abundance and distribution (Maccarone et al., 2016), while fishes are limited only to the Gorghi Alto-Medio (Ottonello et al., 2017a). In the latter, fish presence affects the food web and the assemblage and abundance of the potential preys of *Emys trinacris* (Table 1; Ottonello et al. 2017a), in accordance with available results on the negative impacts that allocthonous species produce on local invertebrates communities (*Cyprinus carpio*: Parkos et al., 2003; as well as *Gambusia holbrooki*: Pyke, 2008).

Sampling

Individuals were captured by six fyke nets and 30 baited funnel traps in Gorgo Basso and Gorghi Alto-Medio located 50 m apart of each other. Fieldwork was carried out from 2014 to 2016 with 10 sampling occasions during the active season (March-October). Date of capture, sex, age (adult/juvenile), straight carapace length (SCL, sliding callipers to the nearest 0.1 mm), width (CW), height (CH) and body mass (BM, digital balance ± 1 g) were recorded on each capture according to a standardized procedure, and only individuals with evident sexual characters were considered as adults (Zuffi and Gariboldi, 1995). All captured turtles were individually marked by unique notch on their carapace (Servan et al., 1986) as a part of a long-term study on the ecology of the species (Ottonello et al., 2017b).

Abundance

The abundance of turtles in each site was measured indirectly by means of the Catch Per Unit Effort (CPUE) method. CPUE was calculated for each wetland and sampling occasion with effort measured as trap-day, and the number of traps on a site multiplied by the number of days set. Because animals were released after each capture we considered, within the same sampling occasion, only the first event for each individual to avoid pseudoreplication (Hurlbert, 1984). We used only data collected in 2015 and 2016 because the sampling was not carried out in Gorghi Alto-Medio in 2014. This method, usually applied in fisheries management (Maunder et al., 2006), can be used also for chelonians to infer the relative abundance and compare different groups (Selman et al., 2014).

Size and growth

Data and sites differences (Gorgo Basso *vs* Gorghi Alto-Medio) in size and body mass of turtles were firstly checked for normality with Shapiro-Wilk normality test and then analysed using a Student's *t* test. Females and males were analysed separately due to their difference in size and mass (Ottonello et al., 2017b). Recaptured animals were excluded from the analysis to avoid pseudoreplication. Population structure was analysed using a graphical approach by dividing SCL data in size classes of 10 mm. Individual variation in growth was assessed by the

Table 1. Comparative descriptive data of the two studied sites.

	Gorgo Basso	Gorghi Alto-Medio
Surface (ha)	3.1	4.9
Open deep water (ha)	2.1	4.4
Shallow water dominated by helophytes (ha)	1.0	0.5
Maximum Depth (m)	11	12
Fish	Absent	*Gambusia holbroki Cyprinus carpio*
Invertebrate alien species	*Procambarus clarckii*	*Procambarus clarckii*
Temperature (°C)[1]	27.9	26.6
Dissolved Oxygen (%sat)[1]	66.4	77.5
Chlorophyll alfa (µg/L)[1]	0.11	1.36
Total phosphorus (µg/L)[1]	6.9	13.3
Total nitrate (mg/L)[1]	2.21	0.87
N° of invetebrate sampled[2]	346	103
N° of invertebrate *taxa* sampled[2]	9	6
Food Niche Breadth of *E. trinacris*[2]	1.36	0.97
Average number of prey per faecal sample of *E. trinacris*[2]	15	1

[1] Arpa Sicilia 2016; [2] Ottonello et al. 2017a.

mean of the Relative Growth Rate (RGR) equation modified from Brody (1945) by Cox et al. (1991):

$$RGR = \frac{lnSCL_2 - \ln SCL_1}{t_2 - t_1}$$

where SCL_1 represents straight carapace length at first capture, SCL_2 represents straight carapace length at last recapture, and $t_2 - t_1$ represents the time interval in months between the two measurements. Juveniles at first and last capture were used in both groups (males and females), based on the assumption that early growth trajectories of the sexes do not differ (Dunham and Gibbons, 1990). Moreover, we considered only growing individuals (class 1, 2, 3) excluding aged (class 4) animals (see Olivier, 2002 for class definition), to avoid the possible bias due the presence or dominance of no or slow growing old animals in the two areas (Bury, 1979).

RGR analyses assume that growth is exponential, but this assumption is rarely valid (Cox et al., 1991). However, the regression between RGR and SCL_1 gives an estimate of growth rate at a specific carapace length, allowing direct comparison of growth rates both among individuals with different carapace lengths and among individuals with similar carapace lengths (Cox et al. 1991). We tested the effect of the site (Gorgo Basso *vs* Gorghi Alto-Medio) on the RGR using the Analysis of covariance (ANCOVA), separately for males and females. RGR has been identified as the dependent variable, SCL_1 as the independent variable and the site as the categorical variable.

The growth has been modelled for each site (Gorgo Basso and Gorghi Alto-Medio) using the von Bertalanffy Growth Model (VBGM), as previously used to estimate size-age relation in different species of chelonians (Lovich et al., 1990; Litzgus and Brooks, 1998; Çiçek et al., 2016). The general von Bertalanffy equation is:

$$SCL = a \cdot (1 - be^{-kt})$$

where SCL is straight carapace length, a is asymptotic carapace length, b is a parameter related to length at hatching, e is the base of the natural logarithm, k is the intrinsic growth factor, and t is age in years. Since this is a non-linear model for the parameter estimation, non-linear statistic was used, by the estimation of parameters and their confidence interval (95%) with non-linear least-square regression method using the FSA package (Ogle, 2012) in R. The function "predict" was used to estimate the age at sexual maturity assuming the SCL of the smallest gravid female and of the smallest male showing secondary sex characteristics for each site. The age of individuals was estimated with the count of plastral growth rings (Keller et al., 1998), a reliable method for young European pond turtles as showed by Çiçek et al. (2016) in a comparative study with skeletochronology. The maximum number of growth rings that was possible to count was nine, after which the plastron abrasion does not allow a reliable estimate of the age of individuals. Because no wild new born of Sicilian pond turtle were captured, we used data of hatchlings from a local breeding centre – where eggs are incubated naturally – for the estimate of parameter b. Juveniles were used in both groups (males and females), based

on the assumption that early growth trajectories of the sexes do not differ (Dunham and Gibbons, 1990).

Reproductive biology

The presence of oviductal eggs was verified by palpation of the inguinal region (Zuffi et al., 1999) directly at the capture site. Gravid females were transferred temporarily to the veterinary clinic of the University of Messina for X-ray examinations. In our experiment the exposure was made at 50 mA, 50 kV, 0.1 min, similar to that used by Zuffi et al. (1999) for *Emys orbicularis* (Linnaeus, 1758). As these latter authors, we presumed the absence of significant injuries at the gonadal level in our sampled females and in hatchlings (Hinton et al., 1997), although we recommended suspending this type of analysis within this population in next years as a precautionary measure. All individuals were released at the point of capture after the end of the procedures.

The size of the eggs has been recorded directly from the radiographic images. Due to the possibility that the eggs were not placed parallel on the radiographic plane we considered only the minimum diameter (D_{min}, ± 0.1 mm) in subsequent statistical analyses. The non-parametric Mann-Whitney U test was used to check any difference in the number of eggs between the two sites (Gorgo Basso *vs* Gorghi Alto-Medio). We tested the effect of the site on the number of eggs using the Analysis of covariance (ANCOVA) to avoid the "size effect". The number of eggs has been identified as the dependent variable, SCL as the independent covariate and the site as the categorical variable. The data collected in different years were treated uniquely to increase the accuracy of the statistical sample since a no significant inter-annual variability is known for the congeneric *Emys orbicularis* (Zuffi and Foschi, 2015). Only those females with oviductal eggs were considered certainly sexually mature. The reproductive phenology was considered as a whole, merging data of the two groups.

All above analyses were carried out with R 3.2.5 (R Development Core Team, 2015).

RESULTS

Abundance

A total of 1405 captures of 579 different turtles (493 in the Gorgo Basso, 86 in the Gorghi Alto-Medio) were made in 2015 and 2016. A strong site fidelity was confirmed as only two individuals were captured in both sites, and this happened only once during all sampling occasions. The sub-population of the Gorgo Basso was more abundant and reached a significant higher CPUE than the sub-population of the Gorghi Alto-Medio (Mann-Whitney U test = 42, $N_1 = N_2 = 6$, P < 0.01), respectively with an average of 3.02 and 0.80 turtles/trap/day.

Size and growth

The sub-population of the Gorgo Basso was characterized by a dominance of turtles (72%) with an SCL between 110 and 129.9 mm while the sub-population of Gorghi Alto-Medio is characterized by a dominance of turtles (76%) with an SCL between 100 and 119.9 mm (Fig. 1).

Females and males of Gorgo Basso were heavier (t-test$_{females}$= 8.73, df = 45.17, P < 0.001; t-test$_{males}$= -9.60, df = 68.20, P < 0.001) and bigger than individuals of Gorghi Alto-Medio, both in terms of carapace length (t-test$_{females}$= 9.61, df= 41.89, P < 0.001; t-test$_{males}$= 10.09, df= 66.31, P < 0.001), width (t-test$_{females}$= 5.50, df= 35.37, P < 0.001; t-test$_{males}$= 7.67, df= 77.63, P < 0.001), and height (t-test$_{females}$= 4.22, df= 28.13, P < 0.001; t-test$_{males}$= 6.48, df= 65.89, P < 0.001) (Table 2).

We used data on 79 males (61 from Gorgo Basso and 18 from Gorghi Alto-Medio) and 41 females (27 from Gorgo Basso and 14 from Gorghi Alto-Medio) to estimate relative growth rate (RGR). Individual variation in growth rate was high (range 0.0 – 0.0176), but
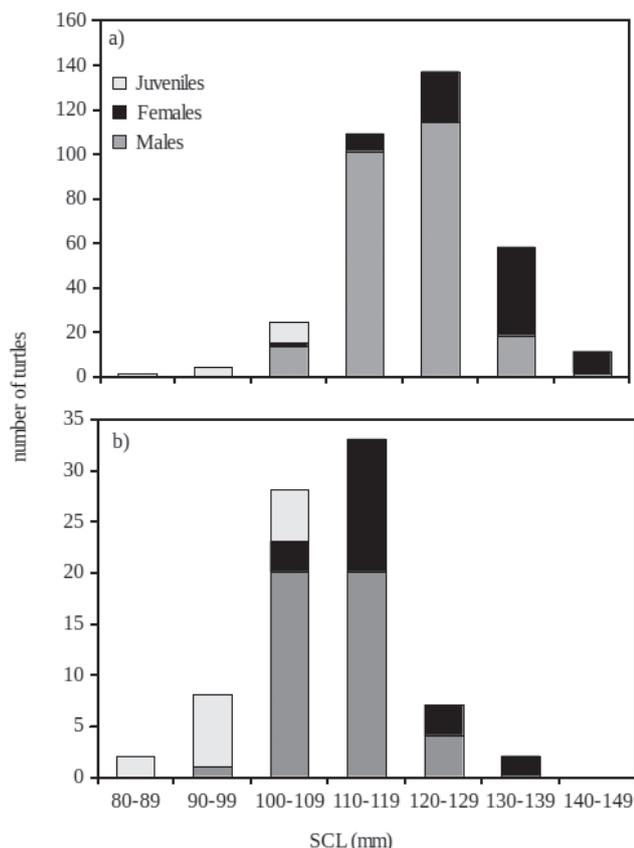
with no significant difference between the sexes (t-test = -0.59, P = 0.5564). Considering independently the two sites, the models showed for both sexes (models 1 and 3, Table 3) that the interaction between the independent variable (SCL) and the categorical variable (site) was not significant, indicating that the slopes of

Table 2. Mean values of biometric measurements for *Emys trinacris* in the two studied sites. SCL = straight carapace length; CW = carapace width; CH = carapace height; BM = body mass; n = number of individuals; S.E. = Standard Error.

Biometric measurement		GT Basso	GT Alto-Medio
SCL (mm) Adult females	Mean	131.5(n =144)	116.5 (n = 21)
	S.E.	0.7	0,05
	Min	110.0	4,42
	Max	150.9	5,5
SCL (mm) Adult males	Mean	120.1 (n = 291)	111.1 (n = 45)
	S.E.	0.4	0.9
	Min	101.3	99.7
	Max	141.1	124.9
CW (mm) Adult females	Mean	101.7 (n = 116)	93.7 (n = 21)
	S.E.	0.8	1.3
	Min	77.6	84.0
	Max	121.3	102.0
CW (mm) Adult males	Mean	94.0 (n = 282)	87.6 (n = 45)
	S.E.	0.4	0.7
	Min	69.4	71.4
	Max	112.0	101.5
PH (mm) Adult females	Mean	50.9 (n = 124)	47.0 (n = 21)
	S.E.	0.4	0.8
	Min	38.0	40.2
	Max	62.6	55.0
PH (mm) Adult males	Mean	42.6 (n = 271)	39.9 (n = 45)
	S.E.	0.2	0.4
	Min	25.4	34.6
	Max	53.5	46.3
BM (g) Adult females	Mean	401.1 (n = 144)	294.7 (n = 21)
	S.E.	7.7	12.8
	Min	217.0	221
	Max	658.0	422
BM (g) Adult males	Mean	281.2 (n = 290)	223.2 (n = 45)
	S.E.	3.4	5.7
	Min	143.0	154.0
	Max	453.0	310.0

Fig. 1. Structure of sub-populations of *Emys trinacris* inhabiting the Gorgo Basso (a) and the Gorghi Alto-Medio (b).

the regressions were homogeneous. Therefore, in both sites, the growth rate is reduced progressively as the individual grows. The other models (models 2 and 4, Table 3) showed that the categorical variable (site) has a significant effect on the dependent variable (RGR), due to the significant difference of the intercept regression lines between the two sites. These models showed that individuals, despite having a growth rates that is inversely related to size, display a different growth rate: turtles from the Gorgo Basso are bigger than those of Gorghi Alto-Medio. The von Bertalanffy growth models showed that the Sicilian pond turtle of Lake Preola and Gorghi Tondi Nature Reserve appeared to mature sexually at approximately six years for males of Gorgo Basso (SCL_{min} = 102.8 mm) and of Gorghi Alto-Medio (SCL_{min} = 99.5 mm) and between seven and eight years

for females of both sites, with a SCL_{min} of 112.1 mm and 106.2 mm respectively.

Allometry and reproductive biology

A total of 312 captures of 178 different females (152 in the Gorgo Basso and 26 in the Gorghi Alto-Medio) was made between 2014 and 2016. We collected 28 individuals with detectable eggs, 21 from the Gorgo Basso and seven from the Gorghi Alto-Medio. Reproductive females were found between May and July, with a peak in the first half of June (Fig. 2). In this period, the percentage of reproductive females collected over two-weeks interval ranged from 20% to 58.3%. We noticed also a double egg deposition of a female (SCL = 132.2 mm) captured on 10 June 2015 with four shelled eggs and on 7 July 2015 with five shelled eggs.

The average clutch size was 4.14 ± 0.23 (range 2 – 7, n = 28), with a positive correlation with the mother carapace length (F-statistic = 39.19, df = 27, P < 0.001) and a significant difference between the sites (Mann-Whitney U Test = 132.5, N_1 = 21, N_2 = 7, P < 0.01). The difference in the clutch size between the sites was significantly independent of the size of the females (Table 4). The average clutch size was 4.57 ± 1.07 (range 2 – 7, n = 21) for the sub-population of the Gorgo Basso and 2.86 ± 0.69 (range 2 – 4, n = 7) for the sub-population of the Gorghi Alto-Medio.

The average minimum diameter (D_{min}) was 19.46 ± 1.21 mm (range 16.0 – 21.5 mm, n = 89), with a not significant difference (t-test = 0.15, df = 24.23, P = 0.8843) between females of Gorgo Basso (D_{min} = 19.47 ± 0.94, n = 69) and those of Gorghi Alto-Medio (D_{min} = 19.42 ± 1.40, n = 20). The average minimum diameter (D_{min}) was positively correlated with the carapace length of the females ($r_{Pearson}$ = 0.70; t-test = 4.45, df = 21, P < 0.001), since the increase of the size allows a greater pelvic aperture width ($r_{Pearson}$ = 0.89; t-test= 8.75, df = 21, P < 0.001), that is a mechanical limiting factor for egg size in turtles.

Table 3. Site effect on growth rate. RGR (relative growth rate) = dependent variable, SCL_1 (first capture straight carapace length) = independent variable, Site = categorical variable.

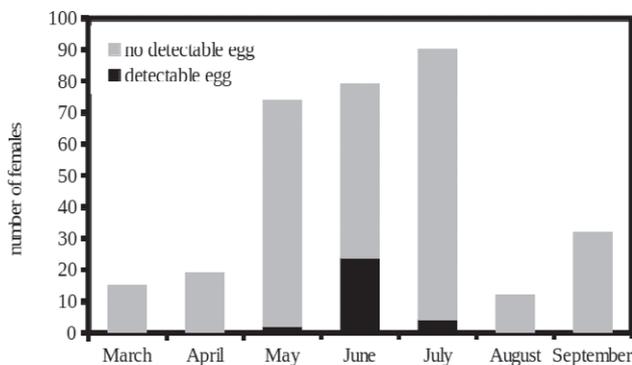|		F	d.f.	P
Male	Model 1 (interaction test)			
	SCL_1	20.03	1	< 0.001
	Site	13.17	1	< 0.001
	$SCL_1 \times$ Site	1.50	1	0.22
	Model 2 (no interaction)			
	SCL_1	19.89	1	< 0.001
	Site	13.08	1	< 0.001
Female	Model 3 (interaction test)			
	SCL_1	14.39	1	< 0.001
	Site	19.84	1	< 0.001
	$SCL_1 \times$ Site	0.69	1	0.41
	Model 4 (no interaction)			
	SCL_1	14.51	1	< 0.001
	Site	20.00	1	< 0.001

Fig. 2. Number of females of *Emys trinacris* with and without shelled eggs in the oviduct determined by inguinal palpation.

Table 4. Site effect on number of eggs per female. Number of eggs = dependent variable, SCL (straight carapace length) = independent variable, Site = categorical variable.

	F	d.f.	P
Model	5.11	5	< 0.05
SCL	0.81	1	0.38
Site	0.48	2	0.63
SCL × Site	0.55	2	0.59

DISCUSSION

The growth of the Sicilian pond turtles in the Lake Preola and Gorghi Tondi Nature Reserve followed the general pattern described for other freshwater turtles: juveniles display a fast growth until sexual maturity, followed by a slowdown during ageing and at much larger size (Bury, 1979). The population studied seems to fall in the general rule noticed for the genus *Emys* (Zuffi et al., 2011) and for many others Emydidae (Iverson et al., 1993) as a typical southern ecotype with an advance in age at maturity at a smaller body size with respect to the northernmost populations, although regional fluctuations of temperature and precipitation are better predictors of body size than latitudinal temperature clines, especially in females of *Emys orbicularis* (Joos et al., 2017).

Moreover, the average clutch size is lower than that of similar sized population of the related *Emys orbicularis* in Italy (Zuffi et al., 1999; Zuffi et al., 2015), while no substantial variation in the nesting period and in the clutch frequency was noticed.

Despite these natural history traits, if we analysed separately the two sub-populations, a significant difference is noticeable: turtles in the Gorgo Basso reached higher body size, produced more eggs per clutch and had higher density than individuals in the Gorghi Alto-Medio.

Excluding both the presence of environmental and genetic effects due to the proximity and similarity of the sites, all these elements suggest the that phenotypic plasticity, driven by a stressor, may explain the observed difference. Furthermore, this hypothesis is supported by the different size at maturity reached in the two wetlands, following the pattern "five" described by Stearns and Koella (1986), where animals, that are forced to grow slowly by an inhibiting factor, mature at a same age, but a smaller size with respect to unstressed con-specific. This type of response is not uncommon in freshwater turtles at a local scale, such as noticed by Gibbons et al. (1981) for females of *Trachemys scripta* (Thunberg in Schoepff, 1792)*,* while at a larger scale it can take over an adaptive process involving environmental and genetic components (Bernardo, 1993).

Indeed, turtles for the most part used energy resources, depending on age or period of year, for growth, maintenance of baseline metabolism and reproduction (Kuchling, 1999). In particular, during the juvenile stage of chelonians, almost all resources are dedicated to rapid growth to reach a body size and a strength of the shell that make them less susceptible to predation (Kuchling, 1999) and this is regulated mainly by the temperature and by the availability and qual-

ity of trophic resources (Kuchling, 1999) as well as by local factors related to the presence of contaminants that can alter the metabolic processes (Burger et al., 1998). Censi et al. (2013) speculated that smaller body mass of *Emys trinacris* living in Lake Preola and Gorghi Tondi Nature Reserve with respect to another Sicilian site can be associated to the supposed reduction of food disposal induced by lanthanide pollution of aquatic environment that influences the growth of aquatic micro-organisms, but no differences was observed within our study sites (D'Angelo, unpubl. data). In the same way, there are no substantial differences in the concentration of lead in the sediment - an inhibitor of growth in turtles (Burger et al. 1998) - between the two wetlands (Arpa Sicilia, 2016).

Therefore, the differences found could be explained in the light of the alteration of abundance and assemblage of the invertebrate community induced by the presence of alien fishes (Ottonello et al., 2017a) possibly exacerbated by the ubiquitous presence of *Procambarus clarkii.* In our opinion these differences in prey availability can definitely alter the energy intake by turtles affecting both the growth of individuals in the two sites, as already observed in other American Emydidae (Cagle, 1946; MacCulloch and Secoy, 1983; Dunham and Gibbons, 1990) and in *Chelydra serpentina* Linnaeus, 1758 (Brown et al., 1994) as well as the abundance (Congdon et al., 1986; Galbraith et al., 1988). These differences are likely to impact both the genetic variability of the population because males of *Emys* mate preferably with larger females (Poschadel et al., 2006), as well as the reproductive output, with females of the Gorgo Basso that laid twice as many eggs of the Gorghi Alto-Medio. These observations are concordant with the optimal egg size theory, according to which the major variation in the reproductive output due to different environmental conditions is in clutch size rather than in egg size, which has been optimized by natural selection (Brockelman, 1975).

In our opinion, these results highlighted how the introduction of non-native fish species can affect the ecology of the Sicilian pond turtle, also focusing on a often overlooked aspect, the problem of the interactions between alien fishes and turtles, on which data are lacking, in contrast to the more studied competition with non-native freshwater turtles (Cadi and Joly, 2003; 2004). Although further analyses are needed to confirm our finding with other wild populations, we believe that our data may provide important information for the management strategy of the Nature Reserve and for other similar areas and cases, in order to improve the conservation status of *Emys trinacris* and the quality of habitat in which it lives.

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