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- 6 They are here to stay: The biology and ecology of lionfish (Pterois miles) in the Mediterranean Sea
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- 27 Abstract

28 The lionfish, Pterois miles, is one of the most recent Lessepsian immigrants into the Mediterranean Sea 29 and it poses a serious threat to marine ecosystems in the region. This study assesses the basic biology and 30 ecology of lionfish in the Mediterranean, examining morphometrics, reproduction and diet as well as population 31 structure and distribution. The population density of lionfish increased dramatically in Cyprus since the first 32 sighting in late 2012; by 2018 aggregations of up to 70 lionfish are found on rocky grounds with complex relief 33 and artificial reefs in depths of 0-50 m. Lionfish in Cyprus become mature within a year and adults are 34 spawning capable year-round, with peak spawning in summer when the sea surface temperature reaches 28.4 35 °C. The Cypriot lionfish grow faster and bigger than in their native range, and females are more common than 36 males. Lionfish are generalist predators in these waters, as also found in their native range, consuming a range 37 of teleost and crustacean prey, some of which are of high economic value (e.g. Spicara smaris and Sparisoma 38 cretense) or have an important role in local trophic webs (e.g. Chromis chromis). Overall, the reproductive 39 patterns, the presence of juveniles and adults throughout the year, the rapid growth rates and the generalist diet 40 indicate that lionfish are thriving and are now already well established in the region and could potentially 41 become the serious nuisance that they are in their temperate and tropical western Atlantic invasive range. 42 Key words:

43 Cyprus, growth rate, Lessepsian migration, marine invasive species, population structure, trophic relations

45 1. Introduction

46 Bioinvasions threaten marine environments as they can harm native species across all trophic levels 47 (Anton et al., 2019). Anthropogenic corridors are a major pathways for marine species introductions in the 48 Mediterranean Sea (Galil et al., 2017; Katsanevakis et al., 2013) and the number of recorded introductions is far 49 higher there than in other European Seas (Nunes et al., 2014). In fact, biological pollution in the Levant Sea is 50 greater than any other water body globally. More than 800 multicellular Non-Indigenous Species (NIS) have 51 been reported in the Mediterranean (Zenetos et al., 2017), with about two thirds known as Lessepsian 52 immigrants (i.e. species introduced through the Suez Canal; Galil et al., 2017). Successive enlargements of the 53 Suez Canal over time (Katsanevakis et al., 2013) have reduced the natural salinity barrier of the Bitter Lakes 54 and increased the influx of Lessepsian NIS, some of which are venomous or poisonous and a direct threat to 55 human health (Galil et al., 2015). These NIS can also act as pests causing profound changed in coastal biota, 56 structure, ecosystem functions and services (Galil et al., 2015, 2018).

57 One of the most recent Lessepsian fish invasions is the lionfish Pterois miles (Bennett 1828), also known 58 as devil firefish (Bariche et al., 2017; Dimitriou et al., 2019; Stern et al., 2018). Pterois miles is an Indian Ocean 59 species (Kulbicki et al., 2012), known from the Red Sea to eastern South Africa, Arabian Sea, Persian Gulf, 60 Gulf of Oman, Laccadive Sea, Bay of Bengal, Andaman Sea and Indonesian region (Kulbicki et al., 2012). At 61 the eastern extreme of its distribution P. miles and P. volitans populations overlap (Kulbicki et al., 2012). The 62 lionfish invasion of the western Atlantic is one of the fastest and most ecologically harmful marine fish 63 invasions to date (Côté and Smith, 2018). They are now an abundant and ubiquitous mesopredator throughout 64 subtropical and tropical parts of the western Atlantic where they have caused detrimental impacts to the native 65 biota and ecosystem (Albins and Hixon, 2013; Côté et al., 2013; Côté and Smith, 2018; Morris and Whitfield, 66 2009; Schofield 2009). The successful range expansion and proliferation of lionfish is attributed to a broad suite 67 of biological traits, including their wide ecophysiological tolerance, high fecundity and rapid growth, anti-68 predator defences, and generalist feeding habits (Côté and Smith, 2018). Lionfish are continuing to spread into 69 areas close to their thermal limits although their population density is in decline where they first invaded (Côté 70 and Smith, 2018).

71 Historically in the Mediterranean Sea, a single specimen of lionfish was first recorded in 1991 off the 72 coast of Israel (Golani and Sonin, 1992). Since then, it was not reported for two decades until two specimens 73 were caught off the coast of Lebanon in 2012 (Bariche et al., 2013) indicating a new invasion event (Bariche et *al.*, 2017). Following its reappearance, it has spread rapidly through the entire eastern Mediterranean basin,
including Cyprus (Jimenez *et al.*, 2016; Kletou *et al.*, 2016), Lebanon (Dailianis *et al.*, 2016), Syria (Ali *et al.*,
2016), Turkey (Özbek *et al.*, 2017), and Greece (Giovos *et al.*, 2018), reaching Tunisia in 2015 (Dailianis *et al.*,
2016) and Italy in 2016 (Azzurro *et al.*, 2017). Judging from its rapid westward expansion there is a general
consensus that the Mediterranean invasion will have widespread ecological impacts, causing alarm amongst
stakeholders such as fishermen (Kleitou *et al.*, 2019).

80 Most of the current knowledge about the ecological and biological characteristics of lionfish is derived 81 from research conducted in the western Atlantic, although a few studies have been carried out in its native 82 environment (e.g. Cure et al., 2012; Darling et al., 2011; Fishelson, 1975; Kulbicki et al., 2012). While these 83 serve as an important source for a preliminary risk assessment of its invasion in Europe, it is essential that 84 baseline information on several aspects of its biology and ecology are established for the Mediterranean case. 85 These will enable a thorough assessment and a better understanding of the lionfish invasiveness dynamics in the 86 already heavily disturbed marine ecosystems of the Mediterranean Sea (Boero, 2015; Micheli et al., 2013). The 87 overall objective of this study was to investigate some of the key biological characteristics of P. miles at the 88 European Union's (EU) first invasion point, Cyprus. We evaluate several aspects of its population structure (e.g. 89 population distribution, sex ratio, growth rates, age structure), characterise its reproduction patterns and finally 90 assess the morphometric relationships as well as the trophic ecology of lionfish in the Mediterranean.

92 2. Materials and Methods

93 The removals and sampling procedure of lionfish by scuba divers was approved and permitted by the

94 Department of Fisheries and Marine Research of Cyprus within the framework of European Union's

95 RELIONMED project (LIFE16 NAT/ CY/000832).

96 2.1. Study area

97 The study was conducted in Cyprus, which is located at the eastern Mediterranean Sea in the Levantine 98 Basin, the frontline of Lessepsian bioinvasions in the Mediterranean Sea. The area is characterised by 99 ultraoligotrophic waters (Krom et al., 2005) and a complex water circulation defined by seasonal changes, with 100 salinity values reaching ~39 PSU and sea surface temperature (SST) ranging from 17-28 °C throughout the year 101 (Mauri et al., 2019). Warm seawater occurs during the summer in the north, westernmost and easternmost sides 102 of the island, whereas the southwestern coastline experiences cooling events due to upwelling caused by north-103 westerly winds (Mauri et al., 2019; Zodiatis et al., 2003). The south to south-eastern coastline is predominantly a sand-gravel seafloor, whereas the south-western coastline and the south-eastern tip (i.e. Cape Greco) has rocky 104 105 substrata (Supporting Information Figure S1).

Sample collection was carried out from 2017-2018, at sites mainly on the eastern side of Cyprus
(Figure 1; Table 1), where sightings of large lionfish aggregations had been reported more frequently. More
precisely, lionfish collections were conducted following sighting information from citizen scientists. The citizen
scientists were mainly stakeholders such as recreational spear-fishers, professional fishermen,
recreational/professional divers, local authorities and scientists, all of whom were informed about the lionfish

111 invasion in a series of regional stakeholder meetings (Kleitou *et al.*, 2019). A total of 262 lionfish were sampled

by SCUBA divers using Hawaiian slings. Most of these were sampled from 15-30 m depth (n = 173), followed

113 by 5-15 m depth (n = 48), 30-50 m (n = 36) and 0-5 m (n = 5). Apart from two lionfish that were caught from

seagrass meadows of *Posidonia oceanica* (L.) Delile 1813, all the rest were collected from hard substrata; 146

from natural reefs and 114 from artificial reefs (concrete structures and shipwrecks).

116 Seasonal temperature data were derived from MODerate-resolution Imaging Spectroradiometer

117 (MODIS) instrument onboard both Aqua and Terra sun synchronous satellites. The MODIS SST products

118 provide per-pixel temperature and emissivity values (both with spatial resolution of 4630 m) in a sequence of

swath-based to grid-based global products. The MODIS Day and Night SST Thermal Monthly L3 Global,

120 configured on a 0.05 ° latitude/longitude climate modelling grid (CMG) were used for the period 2017 and 2018.

121 2.2. Morphometric analysis

Captured lionfish were either analysed right after sampling or frozen until subsequent analysis. Subsets of the total annual sample were used for the different analyses. For morphometry, individuals were numbered, photographed, counted, weighed (M_W , Wet Mass) to the nearest 0.01 g and Standard/Total Length (L_S/L_T) measured to the nearest mm. The Gape Area (A_G), also known as the mouth area, was measured as the distance (to the nearest mm) between the inside of the top jaw and inside of the bottom jaw (vertical mouth opening; MO_V) as well as across the width of the mouth (horizontal mouth opening MO_H). The A_G was then estimated based on an ellipsoid shape (Erzini *et al.*, 1997):

129
$$A_G = \pi(\frac{MO_V}{2}) \times (\frac{MO_H}{2})$$

130 where, $\pi = 3.14$.

131 The relationship between A_G and M_W to L_T were further examined and constructed using the allometric 132 model:

133
$$M_W \text{ or } A_G = a \times L_T^{b}$$

where, a is the coefficient of shape and b is the power which fulfils the dimensional balance (Lleonart *et al.*,2000).

136 During dissection, sagittal otoliths were extracted, cleaned with distilled water and stored dry in labelled

137 Eppendorf tubes for age and growth rate determination. Individuals were sexed via macroscopic examination of

the gonads following Green *et al.* (2012a) and stomachs were collected and frozen before stomach content

analysis.

140 2.3. Reproduction

Gonads were removed from most of the specimens, and were macroscopically examined (shape, volume,colour, presence or absence of oocytes and sperm or egg shedding), weighed when possible (to the nearest 0.001

g) and the developmental stages were classified based on the categories adapted by Green *et al.* (2012a). The approximate size of first maturity was identified as the smallest size of mature individuals. Reproduction was examined over time for both genders using the Gonadosomatic Index (I_G ; n = 160) and the macroscopic maturity stages (n = 208). The I_G was calculated as follows:

147
$$I_{G} = \frac{gonad \; mass\;(g)}{body\; mass\;(g) - gonad\; mass\;(g)} \times 100$$

148 2.4. Population structure and growth rate

Population structure was assessed based on the sex-ratio, size composition, population density and age structure. Size composition of *P. miles* was characterised by L_T frequency distribution of specimens, while the age structure and growth rate were determined by using Length Frequency Analysis (LFA) and otolith ring readings.

153 2.4.1. Population density and distribution analysis

Herein, the analysis provides a preliminary census of the spatio-temporal density/distribution patterns of lionfish in Cyprus. This was based on sightings of lionfish from the published literature such as sighting record publications (e.g. Kletou *et al.*, 2016; Jimenez *et al.*, 2016); via citizen science e.g. utilising groups of marine Mediterranean biodiversity or groups created specifically for lionfish sightings within social media platforms; through four spearfishing competitions; and by personal contact (e.g. emails, telephone, etc.). Data obtained from the present study were also fitted within the population density analysis, in view of the fact that lionfish samplings were principally driven by sighting reports.

161 KERNEL density algorithm was used as a non-parametric spatial analysis method to present the
approximate distribution of lionfish in Cyprus and the probability density per 10 km radius per year since 2013,
as well as all years combined. The data were normalised from 1-70, where the range represents the minimum
and maximum lionfish observed from a single sighting. The analysis was carried out using ArcGISTM V.10.1
provided by ESRI.

166 2.4.2. Age and growth rate analyses

167 Length Frequency Analysis was performed on all 262 specimens collected during the study. The age
168 modal class analysis was computed via the Bhattacharya's method (Bhattacharya, 1967) but prior to that, all the

assumptions were verified – (1) Length Frequency Distribution (LFD) consists of a number of components, (2)
each component corresponds to a cohort, (3) the LFD of each component has a normal distribution. The
estimated means for lengths at age were then used to calculate the Von Bertallanfy growth parameters following
the Gulland and Holt (1959) method, complemented by the Pauly (1979) to determine length at time zero.

173 Of 217 pairs of sagittal otoliths extracted, 53 were chosen randomly from both sexes and all size ranges 174 for analysis. The selected otoliths were embedded in epoxy resin, mounted and sectioned using a low speed 175 diamond wheel saw [South Bay Technology (SBT), model 650]. Sections were gently grinded with a lapping 176 and polishing machine (SBT, model 920). Polished sections of approximate 150 µm were then mounted on glass 177 slides and covered with Glycerin, to improve the visibility of the growth rings. Last, the prepared sections of the 178 otoliths were inspected under a light stereoscope and photographed for further evaluation of the growth rings. 179 Sections without the centrum of the otolith were discarded from the analyses. Both otoliths of five specimens, 180 were inspected to verify consistency of our readings and for quality control. Data from otolith analyses were 181 then used to express the fish length as a function of age, following the Von Bertalanffy Growth Function 182 (VBGF):

183
$$L_{(t)} = L_{\infty}(1 - exp^{-K[t-t_0]})$$

where, $L_{(t)}$ is the length of a fish at age t, L_{∞} is the asymptotic maximum length, K is the Brody growth coefficient, and t₀ is the theoretical time at which a fish was length 0.

186 *2.5. Diet and stomach analyses*

187 A subset (n = 81) of the total lionfish sample was chosen randomly from all available seasons for 188 stomach analysis. The stomachs were weighed full and emptied. The food items in each stomach were identified 189 to the lowest possible taxon using stereoscope and light microscopy. When identification was not possible due 190 to advanced digestion, the prey items were classified as 'unidentified'. The number of empty stomachs was also 191 recorded. Prey items were grouped into major taxa categories to facilitate diet comparisons and eliminate biases 192 associated with comparisons based on variable levels of identification (Cortés, 1997). Remains of P. oceanica 193 leaves, as well as small plastic particles were considered as "accidental items". Parasites found in the stomachs 194 were also recorded.

In order to analyse the data that resulted from the stomach content analysis, the methods described by
Hyslop (1980), and Kelleher *et al.* (2000) were used, and the following indices were calculated:

197Frequency of occurrence (*I_{FO}*)

$$I_{FO} = \frac{n}{Ns} \times 100$$

199Percentage of prey (IP)

$$I_P = \frac{n'}{Np} \times 100$$

where, *n* is the number of stomachs containing a certain prey, *Ns* is the total number of stomachs examined, *n'* is the total number of individuals of a certain prey, *Np* is the total number of prey individuals. According to I_P values, prey categories were distinguished as preferential ($I_P > 50\%$) and secondary (10% < $I_P < 50\%$). Feeding activity was evaluated with the vacuity index (I_V), meaning the percentage of empty stomachs according to the equation:

$$I_V = \frac{E}{T} \times 100$$

207 where *E* is the number of empty stomachs and *T* is the number of stomachs examined (Hureau, 1970).

208The stomach-filling index (I_{SF}) was calculated by the sum of weights (g) of all the prey items in a209stomach divided by the L_T (cm) of fish. This approach is a useful estimator of the feeding intensity that excludes210the effect of fish size and avoids subjectivity problems expected from visual stomach fullness scaling methods211(Bachiller and Irigoien, 2015).

212 2.6. Statistical analyses

All the biological and collection data were categorised into season, gonadal stage and sex, for use in statistical analysis. Maximum, average, minimum and median values were calculated for each of the morphometric parameters (i.e. L_S , L_T , A_G , M_W) for each gender separately and for the total sample. Statistical comparisons for each morphological parameter and the I_{SF} were performed between the two genders using a Mann-Whitney test. For the A_G -to- L_T and M_W -to- L_T relationships, data were log-transformed to achieve linearity, and a linear regression analysis was computed to examine the significance of each relationship and the 219 coefficient of determination. The coefficient of determination of each relationship was also compared between

220 genders via a Welch's test for unequal variances. The proportion of females and males was compared for the

entire sexed annual sample size as well as for each distinct size class (i.e. $\leq 19, 20-25, 26-30$ and ≥ 31 cm L_T)

- 222 using a Chi-square test. Furthermore, a two-Proportions test was further carried out to examine differences in
- proportions of juvenile (< 15 cm L_T) and large lionfish (> 30 cm L_T) between seasons. The I_G was compared for
- each gender against seasons via a Kruskal-Wallis test, followed by a Dunn's post hoc test with Bonferroni
- 225 correction. Finally, gonadal stages were tested for differences between seasons using a Kruskal-Wallis test. For
- all statistical analyses, the level of significance α was adjusted to 0.05. All statistical analyses were carried out
- in R-Studio v3.5.1, and graphics were produced via the package ggplot2 (Wickham, 2016).
- 228

229 **3. Results**

230 *3.1. Morphometrics*

Lionfish morphometric analysis was conducted on 262 specimens, unless stated otherwise (Table 2; Figure 2). Overall the L_S , L_T , M_W and A_G of the sampled population exhibited a wide range of values (Table 2), with males achieving higher morphometrics than the females. Despite lionfish's recent colonization, some of the specimens already reached a considerable L_T and M_W , characterised by large A_G (Table 2).

235 Computation of relationships showed that A_G (b = 1.8; n = 249) and M_W (b = 3.2; n = 249) increase 236 relatively fast with L_T (Figure 2). Both log-transformed relationships were highly significant (A_G -to- L_T : Linear 237 regression analysis, slope = 1.93, SE = 0.07, t-value = 28.13, p < 0.001; M_W -to- L_T : Linear regression analysis, slope = 3.29, SE = 0.06, t-value = 53.77, p < 0.001) and R² was found to be equal to 0.77 and 0.92, respectively. 238 239 The A_G -to- L_T relationship in females (b = 1.963 ± 0.119, n = 119) did not vary significantly (Welch modified 2-240 sample t-test, t = 1.398, df =192, p > 0.05) to that of males (b = 1.717 ± 0.114, n = 75). The same outcome 241 appeared in the M_W -to- L_T relationship (Welch modified 2-sample t-test, t = 0.236, df = 192, p > 0.05), where 242 females exhibited similar allometric coefficient (b = 3.164 ± 0.085 , n = 119) to that of males (b = 3.125 ± 0.158 , 243 n = 75).

245 3.2. Reproduction

246 Gonadosomatic indices were calculated for 160 lionfish, which comprised 104 females and 56 males 247 captured between September 2017 and June 2018. The I_G values ranged between 0.059 to 15.36 for females and 0.017 to 1.33 for males. Female I_G values varied significantly by season (Kruskal-Wallis, $\chi^2 = 28.45$, p < 0.001; 248 Figure 3). Summer had higher female I_G values than winter (Dunn's test, Z= 5.2, p < 0.001), autumn (Dunn's 249 250 test, Z= -4.61, p < 0.001) and spring (Dunn's test, Z= -2.9, p < 0.001). Male I_G values also varied significantly 251 by season (Kruskal-Wallis, $\chi^2 = 25.93$, p < 0.001; Figure 3), exhibiting the lowest I_G values during the winter, 252 which were only statistically different when compared to autumn (Dunn's test, Z = 4.73, p < 0.001) and summer 253 (Dunn's test, Z = 2.99, p < 0.001). Overall, the highest female I_G values occurred during the summer period 254 coinciding with sea warming (Figure 3), whereas the I_G for males seems to remain relatively the same 255 throughout the year, except in the winter, whereby the observed I_G drop coincides with the lowest mean SST.

Gonad activity was assessed visually in 126 females and 82 males (Figure 4). The smallest lionfish found capable of spawning was L_T 15.4 cm and was a male, whereas the smallest spawning capable female was L_T 15.8 cm. The gonadal stages differed between seasons for both females (Kruskal-Wallis, $\chi^2 = 8.92$, p < 0.05) and males (Kruskal-Wallis, $\chi^2 = 19.80$, p < 0.001). Both males and females were spawning capable throughout the year (Figure 4). Males showed to enter the resting/spent stage during the winter, concurring with the I_G drop (Figure 3 and 4). The resting/spent stage of females was only visible in spring, after which, 43% of the sample was actively spawning, matching with the summer I_G peak (Figure 3 and 4).

263 *3.3. Population structure and growth rate*

264 3.3.1. Population density and distribution

Lionfish were found around 63% of the entire coastline of Cyprus (Figure 5). The first confirmed record was found in Limassol in late 2012 (Jimenez *et al.*, 2016). In 2013 a few sightings were made in two main locations, the Limassol and Larnaca districts (Figure 5). From 2015, lionfish sightings increased within the Famagusta district in the form of small groups, notably in Cape Greco (a Marine Protected Area) and Nissia area (a Natura 2000 site). In 2017 to 2018, lionfish appeared in large numbers either sparsely or in aggregations along the entire Famagusta region, including Cape Greco, Nissia, Pernera and Famagusta Bay (Figure 5). In about 98.7% of the total sightings (n = 202), lionfish were found on hard substrata, notably on rocky reefs

- 272 (75.4%), artificial reefs (19.8%) and caves (3.4%). In the remaining sightings, lionfish were detected on sand
- 273 (1.1%) and *P. oceanica* meadows (0.2%). Most of the sighting locations were characterised by habitat mosaics
- of rocky reefs, seagrass meadows (*P. oceanica*) and sand.

275 3.3.2. Sex ratio

- 276 Of the fish caught, 206 were sexed and comprised 125 females and 81 males (Chi-squared test, $\chi^2 =$
- 9.3981, df = 1, p < 0.01; Figure 6), resulting to a sex ratio (M:F) of 1:1.54. A much lower proportion of males to
- females was observed in the 20-25 cm size class (Chi-squared test, $\chi^2 = 28.6$, df = 1, p > 0.05) acquiring a ratio
- of 1:3.25. The proportion of females to males at size classes ≤ 19 cm (Chi-squared test, $\chi^2 = 0.04$, df = 1 p >
- 280 0.05), 26-30 cm (Chi-squared test, $\chi^2 = 0$, df = 1, p > 0.05) and ≥ 31 cm (Chi-squared test, $\chi^2 = 3$, df = 1, p > 0.05)
- **281** 0.05) was not significantly different (Figure 6).

282 3.3.3. Size composition

The lionfish caught ranged from 8-37 cm long, representing a unimodal distribution with peak frequency in the 20-25 cm size class (Figure 7). Lionfish sampled in the summer had the highest frequency of juvenile lionfish (2-Proportions test, $\chi^2_{autumn} = 20.1$, $\chi^2_{winter} = 11.9$, $\chi^2_{spring} = 5.9$, p < 0.05) and large individuals when compared to winter (2-Proportions test, $\chi^2 = 8.6$, p < 0.05) and spring catches (2-Proportions test, $\chi^2 = 4.9$, p < 0.05; Figure 7).

288 3.3.4. Age structure and growth rate

Four age modal classes were distinguished by the Bhattacharya's method (Figure 7). At one year old lionfish reached 16.3 cm long and could already be capable of spawning. Lionfish in the 20-25 cm size class were presumably around 1.5-2.5 years old, these were the most frequent age class caught (Figure 7).

In total, 53 otoliths were inspected for growth rings (Supporting Information Figure S3), belonging to 28 females, 23 males and two immature juveniles. About eight different age categories were identified, four based on an annual interval (Figure 8). The range of total length sizes was between 15.3 cm to 39 cm (average 26.1 \pm 5.2 cm), with an average age of 1.92 \pm 0.66 years for females and 2.15 \pm 0.80 for males. Maximum age detected was four years old, corroborating the LFA method, and was represented by two individuals (3.7% of total inspected individuals) caught in late 2017, constituting one male and a female. The most frequent age class was 1.5 years old, which seems to reflect the LFA's 2-year-old age class. The two methods mismatched by approximately half a year (at length-at-age between 0.5-2.5 years old; Supporting Information Table S2), with

300 LFA being ahead of otolith readings. The von Bertalanffy growth curve (Figure 8) fitted well the data (Lack of

fit test, F = 0.43, p > 0.05), but the computed growth parameter estimates were lower than those generated by

302 LFA (Bhattacharya's method; Figure 7).

303 *3.4. Diet and stomach analyses*

304 Of 81 stomachs examined, 25 belonged to males, 38 to females and 15 to unidentified sex (Supporting 305 Information Table S3). The L_T ranged from 7.6 cm to 33.2 cm. The mean L_T was 17.76 cm for unidentified 306 individuals, 24.27 cm for males, and 22.72 cm for females. The heaviest individual assessed was a 417 g female. 307 The I_{SF} was positively correlated with L_T (Pearson's Correlation, $\rho = 0.267$, p < 0.05) and was similar between 308 males and females (Mann-Whitney Test, W = 431, p > 0.05).

309 Stomach content analysis yielded 214 stomach items of which 119 were considered as prey items 310 belonging to 14 prey categories (Table 3). The dominant prey were fish ($I_{FO} = 95.06\%$, $I_P = 87.39\%$) and the rest 311 were crustaceans ($I_{FO} = 13.58\%$, $I_P = 12.61\%$). Among fish, unidentified Pisces was the dominant category in 312 terms of frequency and abundance (Table 3). Chromis chromis (L. 1758) was one of the most frequent prey 313 items, followed by Spicara smaris (L. 1758), Apogon imberbis (L. 1758), Thalassoma pavo (L. 1758) and Sparisoma cretense (L. 1758) / Scorpaena sp. L. 1758 (Table 3). Only four individuals of P. miles had 314 315 completely empty stomachs ($I_V = 6.1$). Plastic debris were also present (1.7% of the total stomach items), while one third of the individuals examined had parasites in their stomachs, mainly belonging to Trematoda and in two 316 317 cases Nematoda.

319 4. Discussion

Since late 2012 Cyprus has become a hotspot of the Mediterranean lionfish invasion. Using simple
handheld slingshots we were able in late 2017 and 2018 to collect 262 specimens for analysis, mainly from
rocky habitats and artificial reefs in the eastern part of the island. Our analysis of these specimens reveals
important biological traits of *P. miles* including morphometric relationships, reproduction, population structure
and feeding habits, providing strong evidence of acclimation and establishment of lionfish to its new
environment in the eastern Mediterranean Sea.

326 The presence of both juveniles and adults throughout the sampling period combined with higher 327 sightings and abundance than earlier years, suggests that the population of P. miles is thriving and well 328 established. In contrast to the 1:1 sex ratio reported from studies of the western Atlantic (Edwards et al., 2014; 329 Fogg et al., 2013), the Mediterranean population had female predominance, especially in the 20-25 cm size 330 range. This lower male-to-female ratio may allow rapid population growth, assuming, one male mates with 331 multiple females. The significance of sustaining a male-predominant sex ratio has been previously stressed by 332 Edwards et al., (2014) in the context of lionfish population management. Otherwise, the removals may prove 333 unsuccessful partly because of sustained reproductive output. The sizes, and weights were found within the 334 margins reported in the literature from several geographic zones of the western Atlantic, including the Gulf of 335 Mexico, Caribbean Sea (Fogg et al., 2013; Sabido-Itzá et al., 2016; Toledo-Hernández et al., 2014). The 336 allometric relationship A_G -to- L_T estimated here exhibits a relatively fast increase of A_G with length, a common 337 characteristic of carnivorous fish (Karachle and Stergiou, 2011). The coefficients for such relationship have not 338 been reported yet for the lionfish in published literature, thus here, we provide for the first time the allometric 339 estimates. The M_W -to- L_T allometric relationship showed an extremely fast increase of weight with length, which 340 indicates that as the lionfish grows it becomes heavier, relatively stouter or deeper-bodied. The results of our 341 study were similar to studies in the Atlantic invasive range (Sabido-Itzá et al., 2016 and references therein) and 342 the slight variation observed among studies is usually attributed to sample size, time of the year, feeding habits, 343 maturity stage, environmental factors and the rapid growth in early life stages (Sandel et al., 2015; Toledo-344 Hernández et al., 2014).

The lionfish were spawning capable year-round, despite having low I_G during the winter and spring. The highest I_G was observed during the summer coinciding with high seawater temperature, when most females were actively spawning. In the same period, almost the entire male sample size was also spawning capable. These 348 results are not unexpected since temperature is a key environmental cue on metabolic processes, and has a 349 strong effect on reproduction (Brown et al., 2004; Lambert et al., 2003) as also shown by a number of other 350 factors including, prey availability (Tyler and Stanton, 1995), female size (Fitzhugh et al., 2012), lunar cycle 351 (Domeier and Colin, 1997) and photoperiod (Bromage et al., 2001). Subsequently, fish of tropical affinity such 352 as *P. miles* are anticipated to display a reproductive peak in warmer temperatures, particularly within the 353 prevailing ranges of their native distribution (Kulbicki et al., 2012; Locarnini et al., 2018), which are only met 354 during the summer and the autumn in the eastern Mediterranean Sea. The lionfish in the size range 12-18 cm 355 detected in highest abundances during summer of 2018, are probably the result of a long peak spawning event 356 from the previous summer/autumn (2017). The scarcity of individuals of the same size class during the winter 357 and spring may be the product of a low reproductive effort that occurred the previous winter/spring. The 358 reproductive pattern observed herein, resembles the one from the northern Gulf of Mexico (Fogg et al., 2017) 359 and Bermuda (Eddy et al., 2019), where both regions exhibit strong seasonality. These however, do not mirror 360 the results from the tropical Caribbean (Gardner et al., 2015), where lionfish reproduction is defined by two 361 major spawning peaks during periods of stable cool (27 °C; December-February) and stable warm seawater 362 temperatures (30 °C; July-August). Thus the colder waters and the ultraoligotrophic character of the Levantine 363 basin may constrain the reproductive peak season, particularly for females, which need to meet high energetic 364 demands for gonad development and spawning (McBride et al., 2015).

365 Judging from our findings and knowledge of lionfish larvae dynamics (Ahrenholz and Morris, 2010), 366 lionfish probably occur in small numbers all around the island of Cyprus and have certainly become common at 367 several locations within just five years. This could be attributed to high summer seawater temperatures (29.32 368 $^{\circ}$ C), which reduce the duration of the pelagic phase of lionfish larvae so that they settle early and near the 369 spawning area (Côté and Green, 2012), particularly where currents are weak. The number of lionfish sightings 370 and individuals increased rapidly in Cyprus since the 2012 first record and the probability density by 2018 was 371 higher on the eastern side of the island, where it is generally warmer and has structurally complex rocky reefs 372 together with numerous artificial structures. Habitat complexity is associated with higher densities of lionfish 373 (Côté and Smith, 2018; Rojas et al., 2016), and could be the single most important factor affecting their 374 distribution (Lara and Gonzalez, 1998; Rojas et al., 2016). The higher densities observed in the eastern side of 375 Cyprus could be a bias as it attracts more visitors including tourists, recreational fishermen and divers. However, 376 in contrast to the eastern side, reports from the western side never recorded >10 lionfish per SCUBA dive.

377 Despite our preliminary findings, it is deemed essential for further work to better understand the habitat378 preference and densities of lionfish in Cyprus.

379 The lionfish size composition showed seasonal fluctuation. In particular, the most noticeable examples 380 were the presence of size class < 20 cm predominantly during the warmer seasons of the year (summer and 381 autumn) and the low number of larger-sized lionfish during the cold seasons (i.e. winter and spring). The former 382 example could be attributed to the undetectability of this cohort at their earlier life stages (fry and juvenile) 383 during winter and spring when they are still small-sized following summer reproductive peaks, combined with 384 other factors such as the sampling frequency, the fishing gear selectivity, a preceding low reproductive output, 385 the overwinter mortality at age 0 (Lankford and Targett, 2001), and settlement in seagrass meadows (Claydon et 386 al., 2012) that were not thoroughly investigated. Whereas, the latter may reflect an ontogenetic shift to deeper 387 waters in the winter and reappearing during the summer and the fall (Johnson and Swenarton, 2016), possibly to 388 breed while taking the advantage of feeding bounties. Ontogenetic shifts are not rare in marine fishes (Frank et 389 al., 2018), and have been hypothesized to explain the presence of larger lionfish at greater depths in the 390 Levantine Sea (Jiménez et al., 2019) and the Atlantic Ocean (Claydon et al., 2012; Lesser and Slattery, 2011).

391 The LFA growth rate parameters reported here were higher than those generated by otolith ring readings, 392 but both estimates were found in line with several studies carried out in the Atlantic Ocean, including North 393 Carolina, Florida and Little Cayman (Barbour et al., 2011; Edwards et al., 2014; Johnson & Swenarton, 2016; 394 Pusack et al., 2016; Rodríguez-Cortés et al., 2015). The higher growth rates observed in some regions of the 395 western Atlantic (Côté and Smith, 2018 and references therein) may suggest better conditions than the 396 Levantine Sea, associated to the absence of a strong seasonality effect and greater availability of food resources 397 in more productive waters. Furthermore, as in the Atlantic invasive range, lionfish appear to grow faster in 398 Cyprus than those living in their native range (Pusack et al., 2016), and attain considerably larger sizes (Darling 399 et al., 2011). While the reasons behind this difference are speculative (e.g. predator pressure, foraging 400 frequency, prey size selectivity and reduced parasite loads), lionfish in the Mediterranean and Atlantic region 401 potentially have a greater advantage in their successful propagation and population growth, since greater sizes 402 reflect higher reproductive output, survival and better-quality larvae (Birkeland and Dayton, 2005).

403 Most of previous studies in the Atlantic invasive range recorded ages averaging around three years (Fogg
404 *et al.*, 2015; Johnson and Swenarton, 2016; Potts *et al.*, 2010) with a maximum age of nine years old (Eddy *et al.*, 2019). Considering that lionfish can achieve a life span of at least 30 years (Potts *et al.*, 2010), the four years

406 old population of Cyprus is assumed young and coincides with the year of their first appearance in the island, 407 which is estimated around late 2012 to early 2013. Combining the results of LFA and otoliths, the lionfish in 408 Cyprus may reach the first year at the size of 16.3 or 20.8 cm respectively, when it can be already capable of 409 spawning. In the Atlantic, conspecific P. volitans reaches first year at the size between 15-22 cm (Edwards et 410 al., 2014; Potts et al., 2010), and becomes sexually mature at the size of 16.6-19 cm (Fogg et al., 2017; Gardner 411 et al., 2015). Such an early maturity development is believed to be another contributing factor to the increased 412 densities observed in the Caribbean relative to their native environment (Green and Côté, 2009), resulting to 413 recruitment disruption of many local ichthyofauna.

414 The diet of lionfish in Cyprus was found similar to that of lionfish from other locations of both invasive 415 and native environment, consisting mainly of a range of teleost and crustacean prey (e.g., Sandel et al., 2015). 416 However, probably due to low sampling size, the contribution of teleosts by number, i.e. abundance (I_P) to the 417 diet of lionfish in Cyprus appears to be higher and more frequent in the stomachs compared to other regions 418 (71.2% *I_P* in the Bahamas, 74.4% *I_P* in the Mexican Caribbean, and 84.1% *I_P* along the southeastern US coast; 419 Eddy et al., 2016 and references therein). The I_{SF} was influenced by size, with adult individuals showing higher 420 feeding intensity, probably because of the high energy demands required for reproduction and growth as also shown for other species (Kousteni et al., 2017). This is consistent with the optimal foraging theory (Gerking, 421 422 1994), suggesting that lionfish modify their structural capacity as they grow (e.g. enlarged A_G -to- L_T) to reduce 423 unsuccessful feeding attempts (Karachle and Stergiou, 2011) and potentially targeting larger prey (Green et al., 424 2012b; Karachle and Stergiou, 2011), thus to ensure that the increased energetic demands are met with less 425 energy expenditure. The identified prey categories found in the stomachs of lionfish, consist mainly of native 426 species to the Mediterranean, one of which, although common in the Mediterranean, has never been previously 427 reported in Cyprus - the shrimp Stenopus spinosus Risso 1827. Among the main prey categories participating in 428 lionfish's diet, there are some very important species in terms of socio-economic (e.g. S. smaris, S. cretense) and 429 ecological value e.g., C. chromis, whereby the latter serves as an important prey as for the native mesopredators. 430 This similarity in feeding preferences could give rise to interspecific competition, which would be expected 431 when two or more predators of the same trophic level share the same resources (Kalogirou et al., 2012) e.g. 432 lionfish vs. dusky grouper (Epinephelus marginatus). Depending on local fishing pressures and predator 433 communities, lionfish predation could increase competition on stressed populations.

434 In conclusion, this study strengthens available knowledge on the presence and biology of lionfish in the 435 eastern Mediterranean. This information is useful for researchers, managers and policymakers, enhancing the 436 understanding of lionfish invasive dynamics in the region. Our morphometric analyses, reproduction findings 437 and the population structure, confirm that lionfish are thriving and in just 5 years became well established 438 around Cyprus. Lastly, the increasing densities observed over time, combined with its generalist diet and 439 consumption of ecologically and socio-economically important fish, may result in competition of native 440 predators of the same trophic level and further disruption of local marine communities within an already 441 anthropogenically stressed marine environment. While the lionfish cannot be eradicated, the development of a 442 lionfish fishery could provide a management tool to ameliorate impacts to coastal ecosystems of the 443 Mediterranean Sea.

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454 6. Conflict of Interest

455 The authors have no conflict of interest to declare.

456 7. Authors Contribution

457 I.S. prepared the first draft. N.C. carried out stomach contents analysis and developed the trophic ecology

- 458 section. A.G. carried out GIS analysis and produced the maps. I.S. developed the remaining sections, analysed
- the data on morphometrics, reproduction, sex ratio, LFA, otolith readings and produced the graphs and tables.
- 460 I.S., C.A., P.K., L.H., C.J., and D.K. participated in the field work. I.S., C.A., P.K. and V.A. carried out
- 461 laboratory work, including dissection, otolith extraction, as well as data collection on morphometrics and
- 462 reproduction. N.S. carried out the otolith readings. All authors helped write the paper. J.H-S, P.K. and D.K.
- 463 reviewed the final version.

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