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

7 Ioannis Savva<sup>1\*</sup>, Niki Chartosia<sup>2</sup>, Charalampos Antoniou<sup>1</sup>, Periklis Kleitou<sup>1,3</sup>, Andreas Georgiou<sup>4,6</sup>, Nir Stern<sup>5</sup>,  
8 Louis Hadjioannou<sup>4</sup>, Carlos Jimenez<sup>4</sup>, Vasilis Andreou<sup>4</sup>, Jason M Hall-Spencer<sup>3</sup>, Demetris Kletou<sup>1</sup>

9 <sup>1</sup> Marine & Environmental Research (MER) Lab, 4533 Limassol, Cyprus

10 <sup>2</sup> Department of Biological Sciences, University of Cyprus, 1678 Nicosia, Cyprus

11 <sup>3</sup> School of Biological and Marine Sciences, University of Plymouth, PL4 8AA, Plymouth, United Kingdom

12 <sup>4</sup> Enalia Physis Environmental Research Centre, 2101 Nicosia, Cyprus

13 <sup>5</sup> National Institute of Oceanography, Israel Oceanographic and Limnological Research, Haifa 3108001, Israel

14 <sup>6</sup> KIOS Research and Innovation Centre of Excellence, University of Cyprus, 2109, Nicosia, Cyprus

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16 \*Corresponding author, Ioannis Savva: [isavva@merresearch.com](mailto:isavva@merresearch.com) | ORCID: 0000-0002-0336-2619

17 Niki Chartosia: [chartosia.niki@ucy.ac.cy](mailto:chartosia.niki@ucy.ac.cy)

18 Charalampos Antoniou: [cantoniou@merresearch.com](mailto:cantoniou@merresearch.com)

19 Periklis Kleitou: [pkleitou@merresearch.com](mailto:pkleitou@merresearch.com)

20 Andreas Georgiou: [a.georgiou@enaliaphysis.org.cy](mailto:a.georgiou@enaliaphysis.org.cy)

21 Nir Stern: [nirstern@ocean.org.il](mailto:nirstern@ocean.org.il)

22 Louis Hadjioannou: [l.hadjioannou@enaliaphysis.org.cy](mailto:l.hadjioannou@enaliaphysis.org.cy)

23 Carlos Jimenez: [c.jimenez@enaliaphysis.org.cy](mailto:c.jimenez@enaliaphysis.org.cy)

24 Vasilis Andreou: v.andreou@enaliaphysis.org.cy

25 Jason M. Hall-Spencer: jhall-spencer@plymouth.ac.uk

26 Demetris Kletou: dkletou@merresearch.com

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

44

## 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*

74 *al.*, 2017). Following its reappearance, it has spread rapidly through the entire eastern Mediterranean basin,  
75 including Cyprus (Jimenez *et al.*, 2016; Kletou *et al.*, 2016), Lebanon (Dailianis *et al.*, 2016), Syria (Ali *et al.*,  
76 2016), Turkey (Özbek *et al.*, 2017), and Greece (Giovos *et al.*, 2018), reaching Tunisia in 2015 (Dailianis *et al.*,  
77 2016) and Italy in 2016 (Azzurro *et al.*, 2017). Judging from its rapid westward expansion there is a general  
78 consensus that the Mediterranean invasion will have widespread ecological impacts, causing alarm amongst  
79 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.

91

## 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  
104 sand-gravel seafloor, whereas the south-western coastline and the south-eastern tip (i.e. Cape Greco) has rocky  
105 substrata (Supporting Information Figure S1).

106 Sample collection was carried out from 2017-2018, at sites mainly on the eastern side of Cyprus  
107 (Figure 1; Table 1), where sightings of large lionfish aggregations had been reported more frequently. More  
108 precisely, lionfish collections were conducted following sighting information from citizen scientists. The citizen  
109 scientists were mainly stakeholders such as recreational spear-fishers, professional fishermen,  
110 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  
112 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  
114 seagrass meadows of *Posidonia oceanica* (L.) Delile 1813, all the rest were collected from hard substrata; 146  
115 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

119 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

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

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

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:

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

134 where, a is the coefficient of shape and b is the power which fulfils the dimensional balance (Leonart *et al.*,  
135 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  
138 the gonads following Green *et al.* (2012a) and stomachs were collected and frozen before stomach content  
139 analysis.

## 140 2.3. Reproduction

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

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

$$147 \quad I_G = \frac{\text{gonad mass (g)}}{\text{body mass (g)} - \text{gonad mass (g)}} \times 100$$

#### 148 2.4. Population structure and growth rate

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

##### 153 2.4.1. Population density and distribution analysis

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

161 KERNEL density algorithm was used as a non-parametric spatial analysis method to present the  
162 approximate distribution of lionfish in Cyprus and the probability density per 10 km radius per year since 2013,  
163 as well as all years combined. The data were normalised from 1-70, where the range represents the minimum  
164 and maximum lionfish observed from a single sighting. The analysis was carried out using ArcGIS™ V.10.1  
165 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



169 assumptions were verified – (1) Length Frequency Distribution (LFD) consists of a number of components, (2)  
170 each component corresponds to a cohort, (3) the LFD of each component has a normal distribution. The  
171 estimated means for lengths at age were then used to calculate the Von Bertalanffy growth parameters following  
172 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):

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

184 where,  $L_{(t)}$  is the length of a fish at age  $t$ ,  $L_{\infty}$  is the asymptotic maximum length,  $K$  is the Brody growth  
185 coefficient, and  $t_0$  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.

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

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

198 
$$I_{FO} = \frac{n}{Ns} \times 100$$

199 **Percentage of prey ( $I_P$ )**

200 
$$I_P = \frac{n'}{Np} \times 100$$

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

206 
$$I_V = \frac{E}{T} \times 100$$

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

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

## 212 2.6. Statistical analyses

213 All the biological and collection data were categorised into season, gonadal stage and sex, for use in  
214 statistical analysis. Maximum, average, minimum and median values were calculated for each of the  
215 morphometric parameters (i.e.  $L_S$ ,  $L_T$ ,  $A_G$ ,  $M_W$ ) for each gender separately and for the total sample. Statistical  
216 comparisons for each morphological parameter and the  $I_{SF}$  were performed between the two genders using a  
217 Mann-Whitney test. For the  $A_G$ -to- $L_T$  and  $M_W$ -to- $L_T$  relationships, data were log-transformed to achieve linearity,  
218 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  
221 entire sexed annual sample size as well as for each distinct size class (i.e.  $\leq 19$ , 20-25, 26-30 and  $\geq 31$  cm  $L_T$ )  
222 using a Chi-square test. Furthermore, a two-Proportions test was further carried out to examine differences in  
223 proportions of juvenile ( $< 15$  cm  $L_T$ ) and large lionfish ( $> 30$  cm  $L_T$ ) between seasons. The  $I_G$  was compared for  
224 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  
226 all statistical analyses, the level of significance  $\alpha$  was adjusted to 0.05. All statistical analyses were carried out  
227 in R-Studio v3.5.1, and graphics were produced via the package ggplot2 (Wickham, 2016).

228

### 229 3. Results

#### 230 3.1. Morphometrics

231 Lionfish morphometric analysis was conducted on 262 specimens, unless stated otherwise (Table 2;  
232 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),  
233 with males achieving higher morphometrics than the females. Despite lionfish's recent colonization, some of the  
234 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,  
238 slope = 3.29, SE = 0.06, t-value = 53.77,  $p < 0.001$ ) and  $R^2$  was found to be equal to 0.77 and 0.92, respectively.  
239 The  $A_G$ -to- $L_T$  relationship in females ( $b = 1.963 \pm 0.119$ ,  $n = 119$ ) did not vary significantly (Welch modified 2-  
240 sample t-test,  $t = 1.398$ ,  $df = 192$ ,  $p > 0.05$ ) to that of males ( $b = 1.717 \pm 0.114$ ,  $n = 75$ ). 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 ( $b = 3.164 \pm 0.085$ ,  $n = 119$ ) to that of males ( $b = 3.125 \pm 0.158$ ,  
243  $n = 75$ ).

244

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  
248 0.017 to 1.33 for males. Female  $I_G$  values varied significantly by season (Kruskal-Wallis,  $\chi^2 = 28.45$ ,  $p < 0.001$ ;  
249 Figure 3). Summer had higher female  $I_G$  values than winter (Dunn's test,  $Z = 5.2$ ,  $p < 0.001$ ), autumn (Dunn's  
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.

256 Gonad activity was assessed visually in 126 females and 82 males (Figure 4). The smallest lionfish found  
257 capable of spawning was  $L_T$  15.4 cm and was a male, whereas the smallest spawning capable female was  $L_T$   
258 15.8 cm. The gonadal stages differed between seasons for both females (Kruskal-Wallis,  $\chi^2 = 8.92$ ,  $p < 0.05$ ) and  
259 males (Kruskal-Wallis,  $\chi^2 = 19.80$ ,  $p < 0.001$ ). Both males and females were spawning capable throughout the  
260 year (Figure 4). Males showed to enter the resting/spent stage during the winter, concurring with the  $I_G$  drop  
261 (Figure 3 and 4). The resting/spent stage of females was only visible in spring, after which, 43% of the sample  
262 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*

265 Lionfish were found around 63% of the entire coastline of Cyprus (Figure 5). The first confirmed record  
266 was found in Limassol in late 2012 (Jimenez *et al.*, 2016). In 2013 a few sightings were made in two main  
267 locations, the Limassol and Larnaca districts (Figure 5). From 2015, lionfish sightings increased within the  
268 Famagusta district in the form of small groups, notably in Cape Greco (a Marine Protected Area) and Nissia area  
269 (a Natura 2000 site). In 2017 to 2018, lionfish appeared in large numbers either sparsely or in aggregations  
270 along the entire Famagusta region, including Cape Greco, Nissia, Pernera and Famagusta Bay (Figure 5). In  
271 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  
274 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,  $\chi^2 =$   
277 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  
278 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  
279 of 1:3.25. The proportion of females to males at size classes  $\leq 19$  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  $\geq 31$  cm (Chi-squared test,  $\chi^2 = 3$ ,  $df = 1$ ,  $p >$   
281 0.05) was not significantly different (Figure 6).

### 282 3.3.3. Size composition

283 The lionfish caught ranged from 8-37 cm long, representing a unimodal distribution with peak frequency  
284 in the 20-25 cm size class (Figure 7). Lionfish sampled in the summer had the highest frequency of juvenile  
285 lionfish (2-Proportions test,  $\chi^2_{\text{autumn}} = 20.1$ ,  $\chi^2_{\text{winter}} = 11.9$ ,  $\chi^2_{\text{spring}} = 5.9$ ,  $p < 0.05$ ) and large individuals when  
286 compared to winter (2-Proportions test,  $\chi^2 = 8.6$ ,  $p < 0.05$ ) and spring catches (2-Proportions test,  $\chi^2 = 4.9$ ,  $p <$   
287 0.05; Figure 7).

### 288 3.3.4. Age structure and growth rate

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

292 In total, 53 otoliths were inspected for growth rings (Supporting Information Figure S3), belonging to 28  
293 females, 23 males and two immature juveniles. About eight different age categories were identified, four based  
294 on an annual interval (Figure 8). The range of total length sizes was between 15.3 cm to 39 cm (average  $26.1 \pm$   
295 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  
296 was four years old, corroborating the LFA method, and was represented by two individuals (3.7% of total  
297 inspected individuals) caught in late 2017, constituting one male and a female. The most frequent age class was  
298 1.5 years old, which seems to reflect the LFA's 2-year-old age class. The two methods mismatched by

299 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  
301 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  
314 *Sparisoma cretense* (L. 1758) / *Scorpaena* sp. L. 1758 (Table 3). Only four individuals of *P. miles* had  
315 completely empty stomachs ( $I_V = 6.1$ ). Plastic debris were also present (1.7% of the total stomach items), while  
316 one third of the individuals examined had parasites in their stomachs, mainly belonging to Trematoda and in two  
317 cases Nematoda.

318

319 **4. Discussion**

320 Since late 2012 Cyprus has become a hotspot of the Mediterranean lionfish invasion. Using simple  
321 handheld slingshots we were able in late 2017 and 2018 to collect 262 specimens for analysis, mainly from  
322 rocky habitats and artificial reefs in the eastern part of the island. Our analysis of these specimens reveals  
323 important biological traits of *P. miles* including morphometric relationships, reproduction, population structure  
324 and feeding habits, providing strong evidence of acclimation and establishment of lionfish to its new  
325 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).

345 The lionfish were spawning capable year-round, despite having low  $I_G$  during the winter and spring. The  
346 highest  $I_G$  was observed during the summer coinciding with high seawater temperature, when most females were  
347 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 °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 habitat  
378 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*  
405 *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 al.*  
410 *et 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  
421 shown for other species (Kousteni *et al.*, 2017). This is consistent with the optimal foraging theory (Gerking,  
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.

444

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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  
459 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.

464

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