Reproductive potential of silver European eels (Anguilla anguilla) migrating from Vistonis Lake (Northern Aegean Sea, Greece)

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Abstract

The European eel (*Anguilla anguilla*), once abundant throughout much of Europe and North Africa, has recently been classified as critically endangered. Information on its biology from the eastern Mediterranean is lacking, especially in relation to spawner quality. Therefore, silver eels were sampled during their seaward spawning migration from Vistonis Lake in Greece. Characteristics linked to reproductive output and success (i.e. body size and condition, sex ratio, silvering, *Anguillicola crassus* infection, fecundity and oocyte diameter) were examined. The lake produced large (687–1138 mm), exclusively female silver eels, 61.7% of which were infected by *A. crassus*. Silver eel fecundity, the first estimates from the southern part of the species range, was positively related to body length ($R^2 = 0.693$; $P < 0.001$) and body weight ($R^2 = 0.731$; $P < 0.001$). Fecundity did not differ between *A. crassus* infected and uninfected silver eels, but Greek silver eels were significantly more fecund than those in northwest Europe. The reproductive potential of Vistonis Lake silver eels and their contribution to the *A. anguilla* spawning stock is discussed.

Keywords: *Anguilla*, eastern Mediterranean, spawner quality, fecundity, *Anguillicola crassus*, gonad development.

Introduction

European eels (*Anguilla anguilla*) begin life in the Sargasso Sea area of the western North Atlantic, but the continental distribution (as resident yellow eels) encompasses the coastal and inland waters of Europe and North Africa (Dekker, 2003; Tesch, 2003). Towards the end of the lifecycle, yellow eels metamorphose into migratory silver eels, which return to the Sargasso Sea to reproduce and die (Tesch, 2003). As a result of the widespread population collapse (e.g. Limburg & Waldman, 2009), *A. anguilla* has recently been classified as critically endangered (Freyhoff & Kottelat, 2010). Along with other temperate anguillid eels, the species is currently the focus of much conservation-orientated research (e.g. Righton & Walker, 2013).

During the continental residency, yellow eels are subject to numerous factors which determine the likelihood of successful migration and reproduction as silver eels. These include, for example, infection by the swimbladder nematode *Anguillicola crassus*, bioaccumulation of lipophilic contaminants, and insufficient energy reserves (see Belpaire *et al.*, 2011). Hence, the investigation of

'spawner quality' (according to ICES (2006): "the capacity of silver eels to reach spawning areas and to produce viable offspring") has become an important research topic. However, studies are primarily focused on eel subpopulations migrating from western and northern Europe, the Baltic region and the western Mediterranean (e.g. MacNamara & McCarthy, 2012; Marohn *et al.*, 2013; Quadroni *et al.*, 2013; Amilhat *et al.*, 2014). The eastern Mediterranean (defined here as east of 20° E) is distinctly under-represented, even in 'pan-European' type studies (Jakob *et al.*, 2009; Belpaire *et al.*, 2011). Eel fisheries data is limited for this region (Dekker, 2003), and available biological data (from Greece, Former Yugoslav Republic of Macedonia, Turkey, Israel and Egypt) generally relates to the glass and yellow eel life-stages (see e.g. Golani *et al.*, 1988; Cakić *et al.*, 2002; Genç *et al.*, 2005; Zompola *et al.*, 2008; Abdelmonem *et al.*, 2010). Therefore, the aim of this study was to obtain baseline information on characteristics linked to reproductive output and success in silver eels migrating from the eastern Mediterranean (Vistonis Lake, Greece), to assess their potential contribution to the spawning stock.

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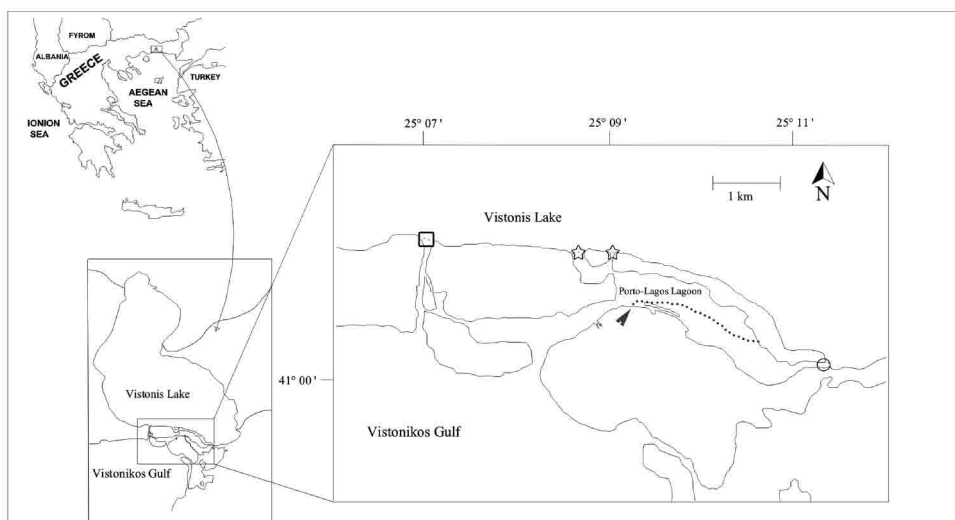
Fig. 1: Map of the Vistonis Lake and Porto Lagos Lagoon system. The channel which connects the lake directly with Vistonikos Gulf (square) and the two channels between the lake and lagoon (stars) have 10 mm mesh screens installed. The fourth channel, between the lake and lagoon (circle), is permanently open. Gridded screens in the lagoon (dotted line) guide migrating eels towards the entrapment device (arrow).

Materials and Methods

Study area and sampling

Vistonis Lake (41° 00' N, 25° 09' E) in the north-east of Greece is a shallow (2 m mean depth), hypereutrophic lake, which covers an area of ca. 4000 ha (Markou *et al.*, 2007). During the year, salinity in the lake varies between 0.6 and 14.0 (Markou *et al.*, 2007). As shown in Figure 1, seaward migrating eels from Vistonis Lake are guided through Porto Lagos Lagoon (ca. 275 ha area, 1 m mean depth) to a specially designed entrapment device similar to the French 'bordigue' (described by Pauly & Yáñez-Arancibia, 1994). Silver eel migration from the lake typically occurs between October and March. Eel samples were collected from the entrapment device in January 2013, as this was the peak migration period during the 2012 / 2013 season: 63.4% (1553 kg) of the total season (October to March, 2450 kg) eel production was obtained during this month. Eels were transported to the laboratory, sacrificed and stored frozen at −18° C.

Body measurements

After thawing, body length and body weight were measured (to the nearest mm and g respectively). Measurements were corrected for shrinkage according to Wickström (1986): by 2.4% for body length and by 2.7% for body weight. Condition factor (K) was calculated as:

$$(body\ weight / body\ length\ ^3)\ x\ 10\ ^5.$$

Dissection

A randomly selected sub-sample was retained for detailed morphological examination and dissection. Pectoral fin length and eye diameter were measured (to the nearest 0.01 mm) to classify eels into silvering stages according to Durif *et al.* (2009a). Eels were dissected ventrally and the swimbladder was removed. If present, *A. crassus* were removed from the swimbladder lumen and counted. Parasitological terms were in accordance with Bush *et al.* (1997): prevalence (number of hosts infected / number of hosts examined), abundance (number of parasites in a single host) and mean intensity (number of parasites / number of hosts infected). The gonads were examined macroscopically following Tesch (2003) to determine the sex.

Fecundity

The gonads were carefully removed and weighed to the nearest 0.01g. Gonadosomatic index (GSI) was calculated as:

$$(gonad\ weight / body\ weight\ )\ x\ 100$$

and eye index (Pankhurst, 1982), based on the relationship between body length and the mean size of both eyes, was calculated as:

$$[\ \{\ (\ right\ horizontal\ eye\ diameter + right\ vertical\ eye\ diameter\ )\ /\ 4\ \}\ x\ (\ left\ horizontal\ eye\ diameter + left\ vertical\ eye\ diameter\ )\ /\ 4\ \}\ x\ (\ \pi\ /\ body\ length\ )\ ]\ x\ 100$$

To ensure that eels were sufficiently mature, and to facilitate comparison with other studies (i.e. MacNamara & McCarthy, 2012), only eels with an eye index >6.5 (Pankhurst, 1982) and GSI >1.2% (Durif *et al.*, 2005) were considered for fecundity analysis.

Gonads were treated according to the protocols described by Barbin & McCleave (1997) and MacNamara & McCarthy (2012). Each pair of gonads were thawed and placed in a container of 250 mL 2% acetic acid. After settling for one day, each container was shaken every second day. All oocytes and ovarian tissue were separated within seven days. Prior to oocyte counts, each solution was diluted, depending on the gonad weight (i.e. <15 g = 2 L, 15–20

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g = 4 L, 20–25 g = 5 L, 25–30 g = 6 L, 30–35 g = 7 L and >35 g = 8 L). After stirring to obtain a uniform suspension of oocytes, a 1 mL sub-sample was taken from the centre of the container. Counts were made in a Sedgewick-Rafter counting cell at 40 x magnification. This was repeated four times in total, and the mean number of oocytes counted was multiplied by the dilution factor to give an estimate of fecundity (i.e. the number of oocytes produced). All counts were completed within 7–9 days of the gonads being placed in the 2% acetic acid.

Oocyte diameter

A sample of the undiluted acetic acid / gonad solution (< 500 oocytes) was placed on a slide immediately prior to dilution, covered with a cover-slip and photographed at 60 x magnification with a Nikon DS-Fi1 camera. A Nikon Digital Sight DS–L2 system was used to measure the diameter of 100 randomly selected oocytes, calculated as the mean value of minimum and maximum oocyte diameters (in µm).

Statistical analysis

The relationships between body length, body weight, fecundity and oocyte diameter were assessed using regression analysis. Eels were grouped according to presence or absence of *A. crassus*, and differences between groups were tested using *t*-tests (body length and body weight) and Mann Whitney *U*-tests (K, GSI, and eye index). Difference in fecundity between groups was tested using analysis of covariance (ANCOVA), with body weight controlled as the covariate. Levene's test was used to ensure equality of variances between the groups and homogeneity of the regression slopes was used to ensure there was no interaction between the covariate and the groups. Statistical analysis was performed using IBM SPSS Statistics 21.

Results

Morphometrics, silvering and parasitology

A total of 188 eels captured migrating from Vistonis Lake were measured, ranging in body length from 687–1138 mm (mean ± Standard Deviation (SD) = 905 ± 69 mm) and in body weight from 743–2762 g (1788 ± 408 g). The body length frequency distribution is shown in Figure 2. All eels were considered female, as all exceeded the typical male size range of 350–460 mm described by Tesch (2003). The body length–body weight relationship is described by the equation:

$\log_{10}$ body length = (0.290 x $\log_{10}$ body weight) + 2.015 (R^2 = 0.811, $P < 0.001$, $N = 188$).

Of the initial sample, 25% ($N = 47$) were retained for detailed morphological examination and dissection (Fig. 2, Table 1). Macroscopic examination of the gonads confirmed the 1:0 female:male sex ratio. According to the silvering classification, 95.7% of eels were stage IV migrants, while 4.3% were stage III premigrants. No stage V migrants were observed. Examination of the swimbladder revealed 61.7% of eels were infected by *A. crassus*. Parasite abundance varied from 0 to 21 individuals, and the mean intensity of infection was 3.41 (Table 1). No significant difference was observed between *A. crassus* infected ($N = 29$) and uninfected ($N = 18$) eels in terms of body length ($P = 0.950$) or body weight ($P = 0.687$). Furthermore, K ($P = 0.457$), GSI ($P = 0.948$) or eye index ($P = 0.965$) were not significantly different between infected and uninfected eels.

Fecundity and oocyte diameter

For the fecundity analysis, three eels were excluded: one eel due to insufficiently high eye index (6.13); one eel due to insufficiently high GSI (0.82%); and one eel due to spillage of the gonad / acetic acid solution. Therefore, oocyte counts were undertaken for 44 eels in total (body size range 687–994 mm (873 ± 69 mm), 743–2543 g (1642 ± 407 g)). Based on the mean of the four sub-samples (coefficient of variation for individual eels ranged from 0.3 to 9.6%, except for one (34.1%)) and the corresponding dilution factor, fecundity was estimated to range from 3 287 500 to 10 832 000 oocytes (6 413 250 ± 1 719 874 oocytes). Relative fecundity (i.e. oocytes per kg of body weight) was 3 906 153. The $\log_{10}$-transformed regression equations of fecundity–body length and fecundity–body weight are given in Fig. 3a & b. After controlling for the effect of body size (i.e. body

Fig. 2: Frequency distribution of eel body lengths: all eels measured from Vistonis Lake (grey bars, $N = 188$), all eels retained for detailed morphological examination and dissection (black bars, $N = 47$) and all eels retained for examination of oocyte diameter (white bars, $N = 30$).
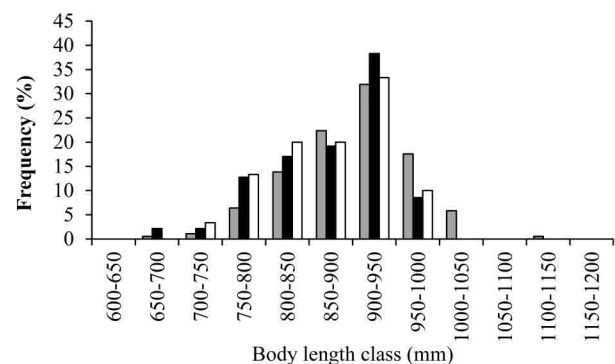
Table 1. Morphometric and parasitological details of the 47 eels examined.

	Body length (mm)	Body weight (g)	Condition factor (*K*)	GSI (%)	Eye index	*Anguillicola crassus*	
Mean ± SD	878 ± 70	1672 ± 414	0.242 ± 0.026	1.72 ± 0.31	8.43 ± 1.25	Prevalence (%)	61.7
Min.	687	743	0.202	0.82	6.13	Abundance	0–21
Max.	994	2543	0.292	2.77	11.20	Mean intensity	3.41

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weight as the covariate), fecundity was not significantly different between *A. crassus* infected ($N = 27$) and uninfected ($N = 17$) silver eels (ANCOVA, $F_{1, 41} = 0.007$, $P = 0.934$).

Oocyte measurements were undertaken for 30 silver eels (body size range 748–994 mm (877 ± 65 mm), 953–2543 g (1677 ± 410 g), Fig. 2). Overall, oocyte diameter ranged from 89 to 434 μm (241 ± 61 μm). The mean oocyte diameter for individual eels ranged from 181 to 341 μm (Fig. 4) and was inversely related to body length ($R^2 = 0.153$, $P = 0.033$, $N = 30$). However, when analysed by group, a significant inverse relationship between body length and oocyte diameter was only found for *A. crassus* infected eels ($R^2 = 0.294$, $P = 0.017$, $N = 19$).

Discussion

There is a growing awareness that eel spawner quality must be fully assessed if stock recovery efforts are to be successful (e.g. ICES 2006; Belpaire *et al.*, 2011; Marohn *et al.*, 2013; Amilhat *et al.*, 2014). Consequently, researchers are investigating various components of this complex topic, but data from the eastern Mediterranean part of the species range is extremely limited. This study is the first on the spawner quality and reproductive ecology of silver eels migrating from Greece. In addition to the study of Rad *et al.* (2013) on silver eels migrating from Turkey, and to a lesser extent some artificial maturation trials on Egyptian silver eels (see Amin, 1998 and references therein), this new data represents an important biological reference point for eastern Mediterranean *A. anguilla*.

Silver eel sub-populations from the western Mediterranean are often characterised by male-skewed sex ratios and / or low mean female eel size (e.g. Quadroni *et al.*, 2013; Amilhat *et al.*, 2014). However, available data suggests this may not typically be the case in the eastern Mediterranean; Vistonis Lake eels were all large females (905 mm mean body length), as were those from the nearby River Nestos in Greece (656 mm mean body length in 2008; Koutrakis *et al.*, unpublished data) and from the Göksu Delta, Turkey (729 mm mean body length between 2007 and 2010; Rad *et al.*, 2013). The majority (>95%) of eels examined from

Vistonis Lake were classified as silver-stage migrants according to established indices (Pankhurst, 1982; Durif *et al.*, 2005; Durif *et al.*, 2009a), and their condition factor exceeded that of female silver eels from French Atlantic drainages (Durif *et al.*, 2005) and the Baltic Sea (Sjöberg *et al.*, 2009). Inferences from artificial maturation trials suggest that these characteristics (i.e. large body size, high condition factor and advanced silvering) may be important for successful reproduction (Durif *et al.*, 2006), although Mordenti *et al.*, (2013) found that large body size and high condition factor did not increase reproductive performance.

One aspect of eel spawner quality that has received surprisingly little attention is fecundity. Some *A. anguilla* fecundity estimates have been obtained from hormone treated eels (e.g. Amin, 1998, see also Table 2 in MacNamara & McCarthy, 2012), but estimates from wild silver eels are limited to north-west Europe (from Ireland, Northern Ireland and England) and this study from the eastern Mediterranean (Greece). Fecundity estimates of 400 g silver eels from north-west Europe ranged between ca. 1.4 million oocytes (MacNamara & McCarthy, 2012; Massey, 2013) and 1.6 million oocytes (Bark *et al.*, 2007). In contrast, fecundity of identically-sized Greek silver eels was estimated to be 1.8 million oocytes (Fig. 3b). As fecundity is size-related, the large body size and female dominated sex ratio suggests high population fecundity of Vistonis Lake silver eels. According to the data given by MacNamara & McCarthy (2012), which is directly comparable with this study due to the identical sampling and count protocols, the expected individual fecundity of Vistonis Lake silver eels would have ranged from 2.2 to 7.6 million oocytes. However, counts indicated that the fecundity ranged from 3.3 to 10.8 million oocytes. This is significantly higher than that of the Irish silver eels ($N = 38$) when the effect of body length is controlled for (ANCOVA, $F_{1, 79} = 24.753$, $P < 0.001$), possibly reflecting differences in environmental conditions between these sub-populations during the yellow eel life-stage. In addition to their high fecundity, Lake Vistonis silver eel oocytes were typically in the early vitellogenic stage (after Adachi *et al.*, 2003) and had diameters (means for individual eels ranging from 181 to 341 μm, Fig. 4) comparable with those of other wild *A. anguilla* silver eels at the beginning of the spawn-
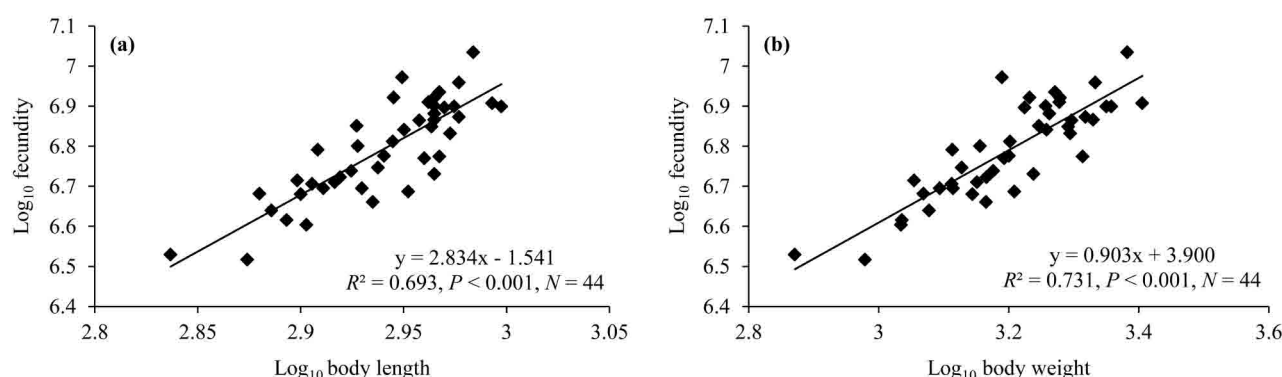
Fig. 3: (a) Log$_{10}$-transformed fecundity–body length and (b) fecundity–body weight relationships.

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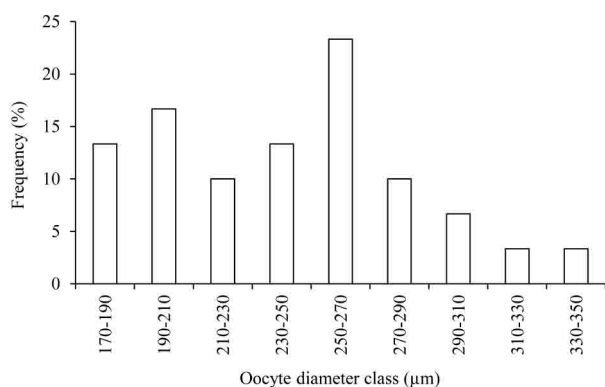
Fig. 4: Frequency distribution of the mean oocyte diameter of 30 eels migrating from Vistonis Lake.

ing migration (e.g. Durif *et al.*, 2009b). However, over 87% of the oocytes examined were smaller than the minimum diameter (i.e. 316 μm) described by Palstra *et al.* (2005) for hormone treated *A. anguilla*.

The invasive nematode *A. crassus* causes damage to the swimbaldder, which may manifest itself during the oceanic migration, meaning infected silver eels may not be able to reach the spawning ground or contribute to reproduction (Palstra *et al.*, 2007; Sjöberg *et al.*, 2009). *Anguillicola crassus* was first reported from Greece as early as 1988 (Moravec, 1992) and is now found throughout much of the eastern Mediterranean. Prevalence in the region is highly variable, ranging from 10.7% in Egypt (Abdelmonem *et al.*, 2010), 39.7% in the Former Yugoslav Republic of Macedonia (Cakić *et al.*, 2002), 61.7% in Greece (this study), to 78.1% in Turkey (Genç *et al.*, 2005). The original introduction pathway to Greece appears to be through escape of its native host, the Japanese eel (*A. japonica*), from eel aquaculture facilities (see review by Perdikaris *et al.*, 2010). It is not known how *A. crassus* was introduced to Vistonis Lake, but commercial eel activity such as live transport or aquaculture escapes seems most likely. Future monitoring in Vistonis Lake will be needed to determine if infection levels since the initial invasion have stabilised according to the carrying capacity of the system (e.g. Bernies *et al.*, 2011).

In this study, at the beginning of the spawning migration, female silver eel body or gonadal parameters (with the possible exception of oocyte diameter) did not differ between *A. crassus* infected and uninfected individuals. Weak relationships (for the overall sample, and for *A. crassus* infected eels only) were observed between body length and oocyte diameter, suggesting that larger (i.e. more developed) oocytes were found in smaller silver eels. Mordenti *et al.* (2013) noted that smaller eels with higher silver index showed more regular gonad development, leading eels to spontaneous spawning in response to hormone treatment. However, the extent of the relationship between *A. crassus* infection and oocyte diameter in Vistonis Lake silver eels is difficult to determine, due to the small sample size. Quadroni *et al.* (2013) proposed that *A. crassus* induced stress may affect gonad development, although their observations

mainly refer to yellow eels and male silver eels, and bioaccumulation of lipophilic contaminants may have been a contributory factor. Nonetheless, body size and the influence of *A. crassus* could be important considerations for future *A. anguilla* artificial maturation programmes.

Conclusions

Accurate and representative biological data for *A. anguilla* is fundamental for successful stock recovery. Regional conservation actions can be prioritised by identifying areas of high spawner quality (Belpaire *et al.*, 2011). Despite the possible negative effects of *A. crassus*, silver eels from Vistonis Lake in Greece could be important contributors to the spawning stock; the female dominated sex ratio, large body size and high fecundity are indicative of the reproductive potential of the lake's silver eels. In future, spawner quality data can be integrated with demographic studies to enable development of 'effective spawner escapement' throughout this region.

Acknowledgements

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