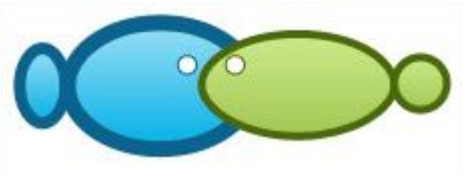

Anguillicoloides crassus (Nematoda: Dracunculoidea) infection in eels in Moroccan estuaries

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Abstract. This study assessed the prevalence and abundance of the invasive nematode *Anguillicoloides crassus* in 1138 wild European eel *Anguilla anguilla* sampled from two Moroccan estuaries between 2004 and 2009. Prevalence differed significantly between the two estuaries. The mean prevalence was 54% in Sebou and 34% in the Loukkos estuary. While the data suggested that the parasite is still spreading in Moroccan waters, there was a trend for stabilization or even decrease in prevalence values. The swimbladder degenerative index showed a significant positive relationship between the number of *A. crassus* and the damage observed in the infected organ. We discussed the possible mechanisms to explain the apparent stabilization in the infection levels.
Key Words: *Anguilla anguilla*, swimbladder degenerative index, prevalence, invasive species, Morocco.

Résumé. Le but de cette étude est l'évaluation de la prévalence et l'abondance du nématode invasif (*Anguillicoloides crassus*) dans 1138 anguilles (*Anguilla anguilla*) capturées entre 2004 et 2009 en deux éstuaires marocains. La prévalence est significativement différente entre les deux estuaires. La prévalence moyenne aux estuaires étudiés est de 54% à Sebou et de 34% à Loukkos. Alors que les données suggèrent que le parasite se propage toujours dans les eaux marocaines, il y avait une tendance à la stabilisation ou même à la diminution des valeurs de prévalence. L'indice du SDI (Indice de dégénrescence da la vessie natatoire) montre une correlation positive entre le nombre des parasites d' *A. crassus* et le niveau d'endomagement de la vessie natatoire infectée. Cette étude propose aussi des explications de la stabilité des niveaux d'infection de la vessie natatoire.
Mots clés: *Anguilla anguilla*, l'indice de la dégénerescence de la vessie natatoire, prévalence, espèces invasives, Maroc.

Introduction. European eel (*Anguilla anguilla*) constitutes the largest wild eel stock of the world (Dekker 2003). The European eel is found in an area spanning the whole of Europe, northern Africa, and Mediterranean Asia (Dekker 2000). The stock has shown a steep decline during the past decades in almost all of its distribution area. Additionally, the market for eels has recently become a world market: freshly caught eels as well as processed end products are now transported all over the world (Dekker 2003). In Morocco, the extreme southern limit of eel distribution (Qninba et al 2011), European eel still remains one of the most considered overexploited by fisheries species (Sabatié & Fontenelle 2007). There are many potential and synergetic causes for this decrease decline in Morocco including: poaching, overfishing, hydraulic equipment alterations, pollution by PAH metabolites (Wariaghli et al 2015) and heavy metals (Wariaghli et al 2013) and other pollutants.

Adding to this causes, there are various diseases, especially the infection by a very successful parasitic invader *Anguillicoloides crassus* found in Moroccan waters (El Hilali et al 1996). *A. crassus* is a swimbladder nematode. This inhabiting haematophagous parasite is thought to be native in the Japanese eel (*Anguilla japonica*) in East Asia (Nagasawa et al 1994). The first report of *A. crassus* in European waters was from *A. anguilla* in the Weser-Ems River in Northern Germany in 1982 (Neumann 1985). The parasite was probably introduced into Europe with

commercial importation of Japanese eels in the early 1980s (Peters & Hartmann 1986). Repeated infections of *A. crassus* result in hemorrhages and hyperplastic, oedemic and fibrotic changes in the swimbladder wall of *A. anguilla* that impair the function of the swimbladder as a hydrostatic organ (Molnár et al 1991; Würtz & Taraschewski 2000). That can reduce swimming performance in silver eels and increase energy consumption, thus compromising the ability of eels to migrate to spawning grounds in the Sargasso Sea and most likely diminishing reproductive fitness (Palstra et al 2007; Bary et al 2014).

The aim of the present study was to keep monitoring infection levels of *A. crassus* in Moroccan eel in Atlantic estuaries: Sebou and Loukkos. They are considered as the main areas for commercial eel fisheries in Morocco. This was done by combining new data with those previously collected (El Hilali et al 1996; Kheyyali et al 1999; Rahhou et al 2001; El Hilali et al 2004; Yahyaoui et al 2004; Loukili & Belghyti 2007) to review the dynamics of anguillicolosis in Morocco from 1994 until 2009 and assess the health state of eels in Moroccan waters.

Material and Method

Study sites. This study was conducted at Sebou and Loukkos estuaries, Morocco. The Sebou Estuary (34°16'N, 6°34'W) is located in the northwest of Morocco on the Atlantic coast, and receives water from the Sebou drainage basin which has a surface area of 40000 km^2. Its water flows from the north-western slopes of the Middle Atlas range to the Atlantic Ocean (Aberkan 1989). The Loukkos Estuary (35°09'N, 6°05'W) flows into the Atlantic Ocean through the city of Larache. Its water originates from the Rif Mountains and the surface area of this drainage basin is 3730 km^2 (Figure 1). The salinity, measured in practical salinity units (psu), in Sebou, Loukkos estuaries ranges respectively between 13-33 and 22-34 psu (Snoussi 1980).

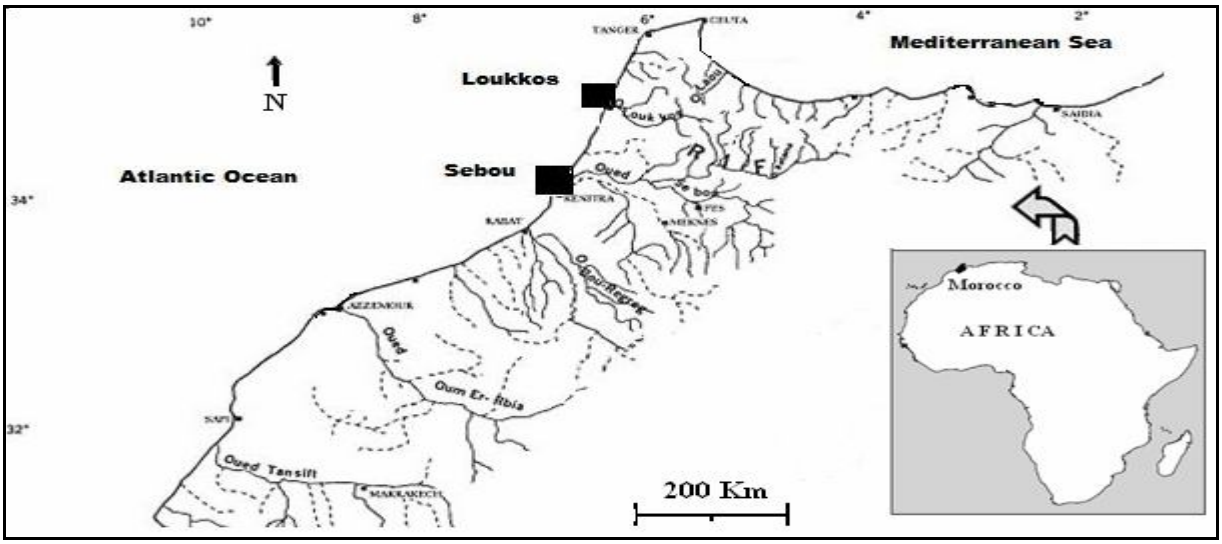

Figure 1. Map showing the locations of the sampling sites: Sebou and Loukkos estuaries.

Capture and examination of eels. Eels were caught between 2004 and 2009 by fishermen using fyke nets (5 mm mesh). A total of 1138 eels were sampled from Sebou Estuary (2004-2009) and 100 eels from the Loukkos Estuary (2008-2009).

Eels were frozen until they could be examined and dissected in the laboratory. The length (mm) and weight (g) were recorded for each eel and the swimbladder was removed and macroscopically examined for the presence of adult and pre-adult *A. crassus.* The prevalence and the mean intensity were calculated according to Bush et al (1997). For eels captured in the period 2008-2009, the swimbladder degenerative index (SDI) was used to describe the health state of the infected organ. The SDI quantifies the observed damages into an index of parasite pressure, ranging from 0, where no pathological signs of infection were observed (thin and transparent swim

bladder wall), to 6 which indicates an extremely damaged swim bladder (Lefebvre et al 2002).

Statistical analysis. Epidemiological parameters were compared between groups (years, sites) using General Linear Model (GLM) or the non-parametric Kruskal-Wallis test when necessary. Correlations were assessed using the Spearman rank test. Significance of differences was defined at $p < 0.05$. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software.

Results. Eel size, total number of parasite, prevalence, intensity (number of parasites per infected eels) and the SDI factor are presented in Table 1.

Table 1

Epidemiology of infection of *Anguilla anguilla* by *Anguillicoloides crassus* in Moroccan waters between 1994-2009

Sources	*Date*	*Site*	*No of eels*	*Eel size (mm)*	*No of parasite*	*SDI±SD*	*Prevalence (%)*	*Mean intensity*
El Hilali et al (1996)	10/94	Loukkos	60	168.6	59	NR	51.6	1.9
	11/94	Sebou	60	170.3	49	NR	43.3	1.9
El Hilali et al (2004)	02/02	Sebou	86	181.6	27	NR	12.7	2.4
	03/02	Sebou	62	197.9	75	NR	37.1	3.2
	04/02	Sebou	60	170.3	49	NR	43.3	1.9
	05/02	Sebou	45	157.3	27	NR	40.0	1.5
	06/02	Sebou	41	153.5	26	NR	43.9	1.4
	07/02	Sebou	58	169.9	47	NR	46.5	1.7
	09/02	Sebou	61	144.8	48	NR	47.5	1.6
	10/02	Sebou	60	168.7	59	NR	50.0	1.9
	12/02	Sebou	59	208.6	46	NR	38.9	2.0
	01/03	Sebou	56	188.7	63	NR	55.3	2.0
Loukili & Belghyti (2007)	07/04	Sebou	86	340.2	335	NR	65.1	3.9
Present study	04/04	Sebou	107	342.0	148	NR	42.0	3.3
	05/04	Sebou	131	307.0	381	NR	58.7	4.9
	10/04	Sebou	63	305.0	155	NR	79.3	3.1
	11/04	Sebou	58	341.0	91	NR	41.3	4.0
	12/04	Sebou	39	290.4	75	NR	56.4	3.4
	01/05	Sebou	66	327.4	123	NR	56.0	3.3
	05/05	Sebou	60	323.0	73	NR	58.3	2.0
	04/05	Sebou	96	328.1	38	NR	63.5	0.6
	01/06	Sebou	60	367.9	173	NR	56.6	5.1
	02/06	Sebou	51	339.7	108	NR	58.8	3.6
	03/06	Sebou	41	340.1	97	NR	73.1	3.2
	05/06	Sebou	23	347.9	65	NR	78.2	3.6
	04/08	Loukkos	33	330.2	35	1.3±1.4	36.3	1.6
	03/08	Loukkos	40	320.8	18	0.9±1.5	24.0	1.8
	04/08	Sebou	30	358.83	37	1.9±2.0	39.0	3.1
	05/09	Loukkos	10	460.1	8	1.9±1.8	40.0	2.0
	10/09	Loukkos	17	504.8	37	3.0±1.5	65.0	3.3
	03/09	Sebou	17	411.7	20	2.4±1.3	51.0	2.2
	05/09	Sebou	37	387.5	44	1.4±0.7	58.0	2.0
	10/09	Sebou	94	312.1	144	2.2±1.2	51.0	3.0
	11/09	Sebou	20	398.1	3	1.8±0.7	30.0	0.5

NR: not recorded.

Sebou. Eel size in the Sebou estuary differed significantly between 2004 and 2009 (Kruskal-Wallis ANOVA: $H4.1138 = 34.83$; $p < 0.05$) but all size classes of eel were infected with *A. crassus*. The smallest infected eel was 14 cm total length (2 parasites) and the highest prevalence detected in eels < 60 cm. The mean yearly

prevalence (%) and mean intensities of *A. crassus* in the eels in Sebou estuary are shown in Figure 2.

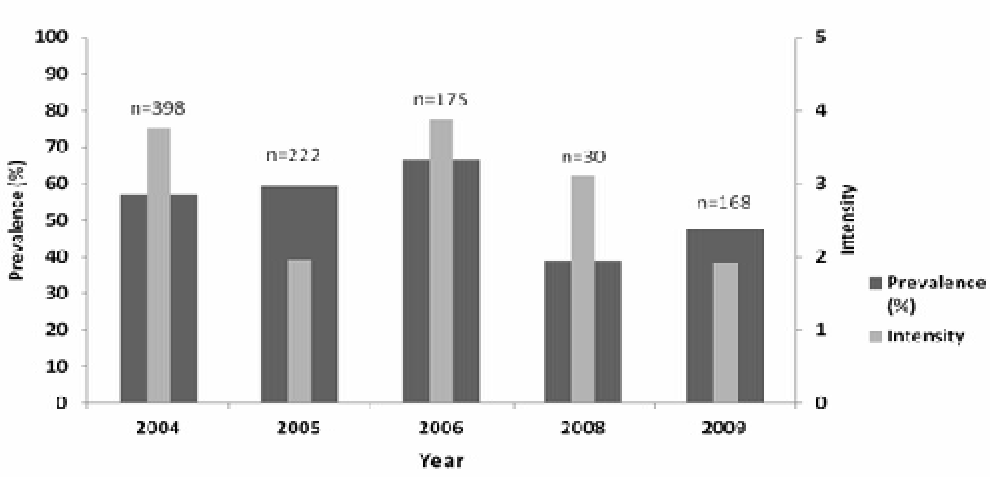

Figure 2. Prevalence and mean intensity of *A. crassus* infections in eels in Sebou estuary in 2004-2009 (n = number of eels examined).

Prevalence varied between 39 and 66.7% with a maximum of 66.7(%) (n = 175) intensity attained in 2006. The mean intensity was 2.93 parasites per infected eel in Sebou.

Loukkos. Prevalence values ranges from a minimum of 12% in May 2009 in Loukkos to a maximum of 79.36% in October 2004 in Sebou. The highest mean intensity was observed in January 2006 in Sebou.

The majority of eels were uninfected or with low of number of parasites (45.9% of eels uninfected; 41.8% with 1-5 parasites), while a small number is heavily infected (Figure 3).

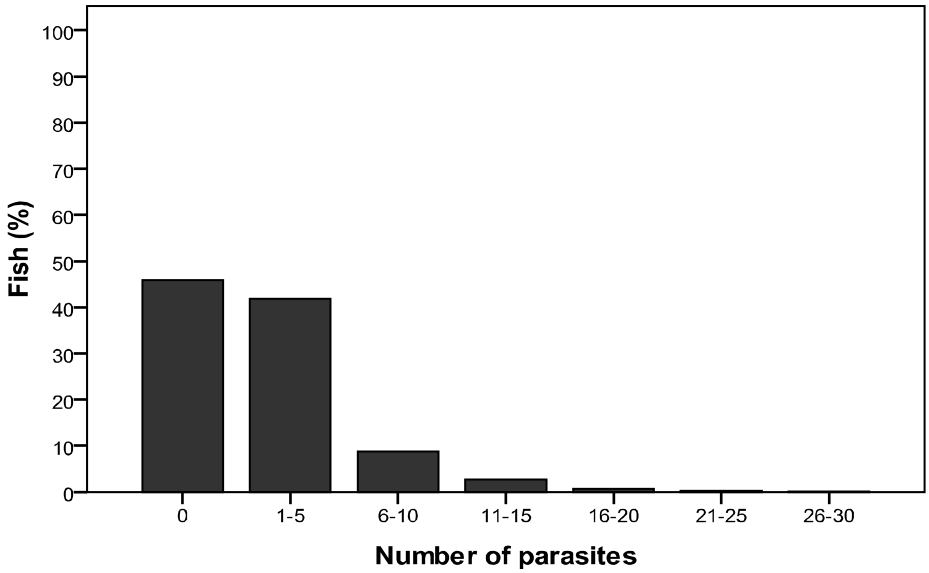

Figure 3. Frequency distribution of *Anguillicoloides crassus* in eels caught in Sebou, and Loukkos estuaries, Morocco.

The results of the GLM demonstrated that prevalence was dependent on estuary ($p < 0.05$) and the year of assessment ($p < 0.05$), but was independent of host length and season (Table 2).

Table 2

Effects of site, year, season and host length on the prevalence and intensity of *Anguillicoloides crassus* in eels using a general linear model (ANOVA) for the Sebou and Loukkos estuaries

Effect	*Df*	X^2	*P*
Prevalence			
Site	1	3.264	< 0.05
Year	5	7.44	< 0.05
Season	3	0.29	0.83
Host length	1	0.28	0.92
Mean intensity			
Site	1	1.029	0.32
Year	5	0.882	0.49
Season	3	1.316	0.29
Host length	1	176.765	< 0.05

Swimbladder degenerative index. The variation of SDI factor between 2008 and 2009 of samples in Loukkos estuary were respectively 1.13±0.23 (2008) and 2.45±0.77 (2009), whereas in Sebou the levels of SDI did not present big change: 1.9±2.04 (2008) and 1.97±0.44 (2009) (Figure 4).

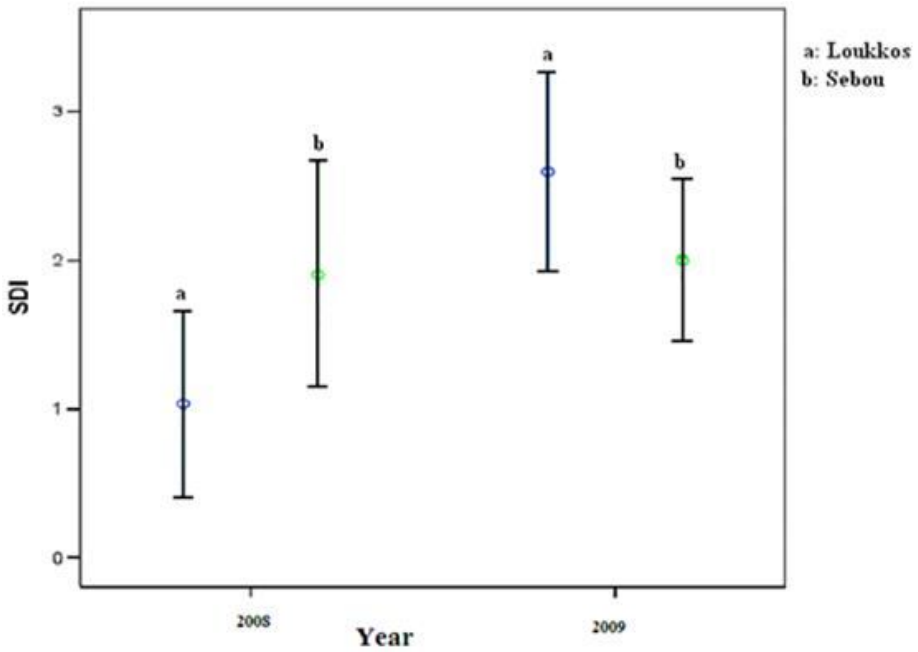

Figure 4. Mean SDI±SD of eels caught in Sebou and Loukkos estuaries in 2008 2009.

In fact, there is a significant variation between 2008 and 2009 for the swim bladder degenerative index (SDI) of eels from Loukkos (Kruskal-Wallis H1.100 = 14.28; $p < 0.001$). The number of parasite has revealed high correlation with the degree of damage of the swimbladder in both Loukkos and Sebou samples, Spearman's correlation were respectively 0.77 and 0.67.

Discussion. The first studies done in Moroccan estuaries were in Sebou and Loukkos (El Hilali et al 1996), where the prevalence was respectively 52 and 43%. The prevalence decreased from 60% in 2004 to 47% in 2009 (Sebou). In Loukkos the infestation rate was more stable, 30% (2008) and 38% (2009). In both sites, the degree of infestation in the last years is in a decrease, which suggests that the infestation by this swimbladder nematode will be stabilized as has already been suggested from studies in brackish lagoons in France (Lefebvre et al 2002) and other European countries like the Netherlands: Van Willigen & Dekker (1989), Haenen et al

1994, 1996; England: Ashworth (1994); Hungary: Székely et al (1991), Molnár et al (1994); Germany: Koops & Hartmann (1989), Würtz et al (1998); Belgium: Audenaert et al (2003); Austria: Schabuss et al (2005), Weclawski et al (2013).

The possibility of host adaptation to explain the stabilization of the infestation may be taken in consideration. Indeed, the adaptation to the resistance is undoubtedly acquired after a long host-parasite co-evolutionary period (Kirk 2003). We should consider as well that the salinity in these estuaries is also high. Indeed this factor is limiting the spreading of *A. crassus* over all the Moroccan waters. Under experimental conditions, Kirk et al (2000) observed maximal egg-hatching, survival and infectivity of larvae in freshwaters and a decline with increasing salinity. Nevertheless, adult nematodes were able to survive and reproduce in brackish as well as marine waters. Our data showed a correlation between the count of parasite and the SDI values, the more the swimbladder harboured parasites the more it showed sign of degradation.

Considering the inter-annual seasonal variation (from 2004-2009) in Sebou, there is a global decrease of the infestation rate in spring, summer and autumn but no big variation in winter. In Loukkos the monthly variation is showing the same decrease except in October 2009 where a high rate was recorded (65%) as well as in Sebou during the same month (51%). This cannot be related to the reproductive migration of larger and heavily infected eels, since a similar seasonal drop is still observed when only considering small size classes (i.e., < 200 mm, data not shown). Rather, this decrease may indicate either a seasonal host regeneration of the swimbladder tissue, or a seasonal host mortality of heavily affected eels (parasite-induced mortality) as it was suggested by Lefebvre et al (2002). The seasonal fluctuation of the rate of parasitism may also be related to changes in the period of eel feeding. The eels undergo two critical periods during which they do not feed: once in the winter, when the temperature is lower than 10°C, and again in the summer, when the temperature may rise above 30°C (Deelder 1984; Tesch 2003). These fluctuations of temperature and salinity explain, firstly, the low prevalence recorded in comparison with those in fresh water (prevalence up 100%) in different regions of Europe (Kennedy & Fitch 1990), and secondly, the high prevalence (83%) mentioned in 1997 by Kheyyali et al (1999) in Sebou which might be due to a strong freshwater inflow during the winter period, facilitating larval dispersal in free phase.

The way of introduction of *A. crassus* is still unknown, since Morocco has never imported live eels but does only export. As it was mentioned above, the first data recorded of this parasite was in 1994 in Loukkos and Sebou (El Hilali et al 1996). In 1987 and 1993, fingerlings of Cyprinids fish from Bulgaria were introduced in Moroccan rivers in order to fight against the eutrophication problem. This fish is considered as paratenic host of this nematode (Moravec & Konecny 1994). In 1991, Székely et al (1991) publish the first occurrence of *A. crassus* in Hungary and Csaba et al (1993) described the mortality of eels in Balaton lake caused by this nematode. The Hungary waters shares many rivers systems with Bulgary, then the hypothesis which appears most convincing is that this nematode was introduced by these cyprinids into Moroccan waters inland, and dispread over these hydrodynamic ecosystems. *A. crassus* is found in the main sites of fisheries of eel especially in Sebou and Loukkos. The mean intensity has started to decrease, so there may be hope of an evolutionary equilibrium of this parasite.

Conclusions. This study shows that eels from Sebou and Loukkos estuaries are infested by *Anguillicoloides crassus*; this parasitic nematode spread easily in Moroccan waters (Sebou and Loukkos estuaries); the swim bladder of eels collected from Sebou were significantly more infected than those from Loukkos estuary and the SDI (swim bladder degenerative index) was positively correlated to the count of parasite that harbored eel's swim bladder. Nevertheless, the seasonal fluctuation of the rate of *A. crassus* in Moroccan eels is suggesting an apparent stabilization in their infection levels. Therefore, a long-term monitoring programme is suggested to evaluate the degree of infestation of eels (endangered species) by this parasite.

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