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Contamination, parasitism and condition of *Anguilla anguilla* in three Italian stocks

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Abstract In conjunction with habitat loss and overfishing, pollution and parasitism are believed to be relevant causes of collapse of *Anguilla*, as these can affect eel swimming ability and the development of gonads and embryos. The present study investigated Persistent Organic Pollutant (POP) concentrations, infection levels of *Anguillicoloides crassus*, lipid content and gonad abnormalities in eels sampled in 2007–2008 in three Italian water bodies (Caprolace Lake, Lesina Lagoon and Tevere River) that vary in salinity, trophic condition, contamination level and fishing pressure. Our analysis revealed that low-to-moderate levels of contamination and parasitism were not associated with gonad abnormalities in Caprolace Lake and Lesina Lagoon. On the contrary, POP concentrations and abundances of swim bladder nematodes were remarkably high in eels from the heavily urbanized Tevere River and were associated with significant gonad and swim bladder alterations. Contamination and infestation levels were so high to potentially impair spawner successful migration and reproduction. POP concentrations in Tevere eels also exceeded levels considered safe for food consumption. Though marginally contaminated, eels from the oligotrophic Caprolace Lake were in critical health condition: their lipid reserve was so low as to be considered insufficient to sustain the energetic costs of the transoceanic migration. Lesina eel stock was the only one displaying relatively good quality but here spawner abundance is likely limited by overfishing. Our results suggest that multiple stressors may potentially affect eel reproductive success. More definitive studies are needed to assess whether health effects caused by these multiple stressors are additive, compensatory or synergistic.

Keywords *Anguilla anguilla* · POP bioaccumulation · Parasitism · Lipid content · Gonad alterations · Ecological and sanitary risk

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Introduction

In recent years increased attention has been given to the decline in recruitment, stock and yield of the European eel (*Anguilla anguilla* L. 1758) in most European and North African inland and coastal waters. Since 2000, the number of glass eels ascending European rivers has decreased down to 1–5 % of the pre-1980 levels (ICES 2007). A decrease in landings has also occurred, indicating a reduction of the continental stocks, possibly ten times lower than the pristine level (Feunteun 2002; Dekker 2003). In 2001 the International Council for the Exploration of the Sea (ICES) declared that eel status was below safe biological limits and that current harvesting rates were not sustainable (ICES 2001). In the following years, this led to several initiatives resulting in a number of measures aimed at eel conservation and sustainable management. In 2007, the European eel was listed in the Appendix II of the Convention on International Trade in Endangered Species (CITES 2007). In 2008 the International Union for Conservation of Nature (IUCN) included *A. anguilla* in the Red List as "critically endangered" (Freyhof and Kottelat 2008). The European Council Regulation 1100/2007 (EC 2007) demanded all Member States to adopt Eel Management Plans (EMPs) aimed at progressively removing the main causes of eel decline so as to guarantee the migration toward the sea of at least 40 % of the silver eel biomass from each catchment basin, with respect to reference conditions defined by the absence of anthropogenic impacts.

Besides a reduction in spawner abundance caused by overfishing and habitat loss, an additional driver of eel collapse could be a reduction in spawner quality due to infections by viruses and parasites and pollution by inorganic and organic chemicals (Belpaire et al. 2011a). The exotic nematode *Anguillicoloides crassus* can cause swim bladder dysfunction and thus influence the migration of mature eels (Möller et al. 1991; Sjoberg et al. 2009). The high lipid content, long life span, low depuration rate and autoecology of this semelparous carnivore species with benthic and rather sedentary behaviour (Larsson et al. 1991; Tesch 2003) make the European eel particularly prone to lipophilic contaminant accumulation with respect to other fish. Contamination by Persistent Organic Pollutants (POPs) in particular may affect eel physiology, migration and reproductive success (Geeraerts and Belpaire 2010).

So far only a small number of studies is focused on the joint effects of pollutants and parasites on eel health (Sures and Knopf 2004; Sures 2006; Sures et al. 2006). This is an area that requires further investigation, as contaminant exposure can lead to a decrease in growth or impair the physiology of the immune system, causing an increased sensitivity to infectious diseases and parasites. On the other hand, some parasites may bioconcentrate specific contaminants (Tenora et al. 1999; Eira et al. 2009), suppressing the detrimental effect of these chemicals on eel, or could influence the metabolism of pollutants (Geeraerts and Belpaire 2010). Pollution and parasites can thus interact in either synergistic or antagonistic ways (Sures 2006).

In 2007, the Working Group On Eels started the set up of the European Eel Quality Database to collect recent data on lipid content, body burden of chemicals—such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs)—and epidemiological parameters such as infection level of *A. crassus* (Belpaire et al. 2011a). While a remarkable quantity of field evidence regarding eel contamination has been gathered in Central and Northern Europe (Macgregor et al. 2010; Belpaire et al. 2011b), information is still scarce and incomplete for eels from the Mediterranean countries (ICES 2011). In the case of France, Spain and Italy, only few works have been recently published about the status of local eel stocks (Bordajandi et al. 2003; Buet et al. 2006; Ferrante et al. 2010) and little is known about the past decades (Bressa et al. 1997). *A. crassus* has been extensively monitored in the Camargue Lagoon and in other Mediterranean inland and coastal waters (Lefebvre et al. 2002, 2003; Lefebvre and Crivelli 2004; Maillo et al. 2005; Genc et al. 2005; Esteve and Alcaide 2009). Other than a few notable exceptions, the quality of eels fished and farmed in the coastal areas of the rest of the Southern Europe and Northern Africa is almost unknown (Maamouria et al. 1999; Rahhou et al. 2001; Gargouri Ben Abdallah and Maamouria 2006).

In a previous work, Bettinetti et al. (2011) reported the levels of PCBs, DDTs (dichlorodiphenyltrichloroethane), HCHs (hexachlorocyclohexanes) and HCB (hexachlorobenzene) in eels collected from three different water bodies in Italy (Caprolace Lake, Lesina Lagoon and Tevere River) to investigate the possible use of this species for POP biomonitoring in European aquatic ecosystems. In this study we integrated the dataset used by Bettinetti et al. (2011) with contamination data from additional eel specimens and extended the POP analysis to other chlorinated compounds, i.e. β-HCH, dioxins, furans and dioxin-like PCBs (DL-PCBs). Moreover, we used the same specimens to investigate the abundance of *A. crassus* and the degree of gonad alteration. The goal of this study was twofold. First, we wanted to present an updated and comprehensive analysis of contamination and infection levels of *A. anguilla* in three Italian coastal environments that vary for natural factors (i.e. salinity and primary production) and anthropogenic pressures associated with a wide range of farming, fishing and industrial activities. Second, we intended to assess potential effects on eel health by analysing possible associations between pollution, parasitism, lipid content, condition, swim bladder damage and

reproductive organ alterations. On the basis of these results, we discussed whether the observed levels of contamination and infection may reduce eel reproductive success either directly by affecting gonad development or indirectly by impairing eel migration to the spawning area. Finally, we elaborated on the possible risk to human health caused by eel consumption, as the widespread use of *A. anguilla* as a gastronomic delicacy in many traditional recipes in Europe may represent an important pathway of human exposure to POPs (Harrad and Smith 1999)—many of which are considered potential carcinogens (Miller and Sharpe 1998).

Materials and methods

Study area and sample collection

Samplings were carried out on three coastal water bodies in Central-Southern Italy (Fig. 1) with different levels of salinity and human mediated pressures. Eels were collected randomly in all sites among commercial fishery catches with fyke netting (mesh size 10 mm).

In February–March 2008, 22 eels were caught in Caprolace Lake (Latium; length = 3.7 km; mean width = 0.6 km; area = 2.3 km^2; mean depth = 1.3 m; maximum depth = 2.9 m; salinity = 34.6–41.1 ‰; water temperature = 8.7–29.1 °C; Fig. 1; 41°21′N–12°59′E), located within Circeo National Park in the Tyrrhenian coast between two other coastal lakes. These lakes are the remnants of a reclamation performed in the 1930s and were declared "Wetlands of International Importance" under the Ramsar Convention in 1978. In the early 1980s, freshwater inputs were cut off due to high levels of nutrients flowing in Caprolace Lake from the surrounding area, where intensive agriculture as well as cattle farming is present. The lake is connected to the sea by one artificial channel (250 m long) and has artificial banks. The park authority exerted moderate fishing pressure until 2007, after which any exploitation of the local eel stock was prohibited.

In October-December 2007, 28 eels were caught in Lesina Lagoon (Apulia; length = 22.2 km; mean width = 2.5 km; area = 51.4 km^2; mean depth = 0.7 m; maximum depth = 1.5 m; salinity = 10.6–28.4 ‰; water temperature = 9.0–27.0 °C; Fig. 1; 41°53′N–15°51′E), located within Gargano National Park along the Adriatic coast. The lagoon is connected to the sea by two artificial tidal channels (3 and 0.8 km long), endowed with sluices to regulate water exchanges. The catchment basin (460 km^2) includes some livestock farms as well as seed and irrigated crops. Several fish farm treated waters and the partially treated urban waste waters of three municipalities, with a cumulated population amounting to 30,000 inhabitants, flow into the lagoon. The salinity variations are pronounced and show a permanent E–W gradient due to the freshwater inputs in the eastern part of the lagoon. Eel fishing is a

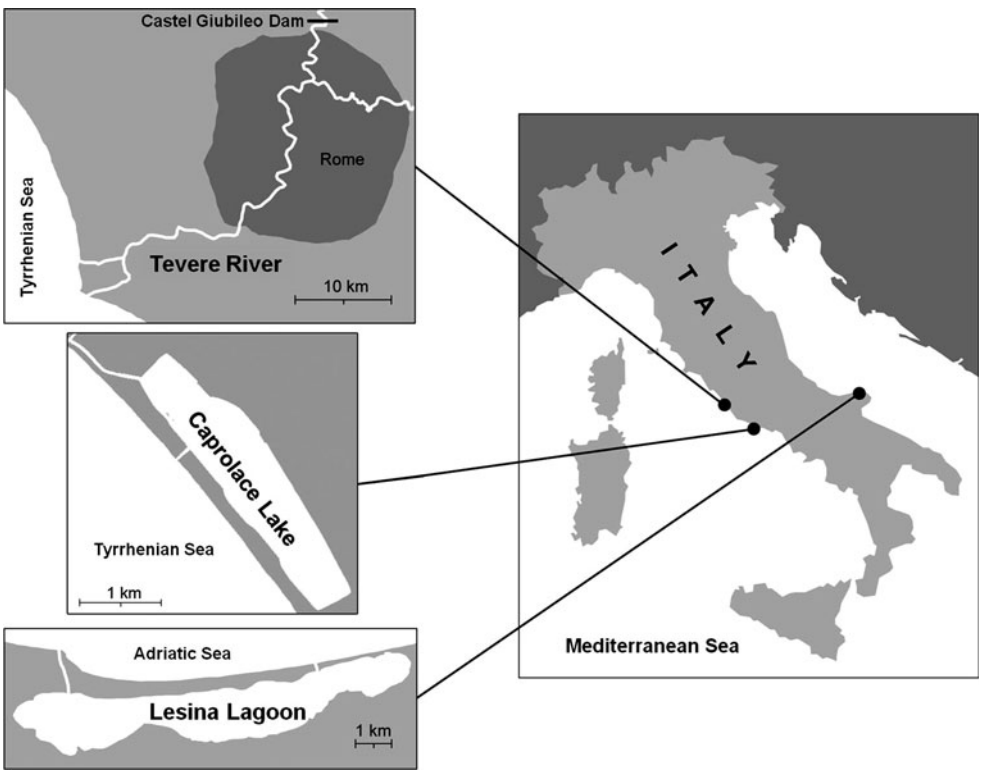

Fig. 1 Study area and sampling stations: Caprolace Lake, Lesina Lagoon and Tevere River

traditional activity, carried out with particular net setting, called *paranza*. Most of the catch occurs between October and March and is composed of migrating silver eels, which are sold for direct consumption.

In October–November 2007, 24 eels were caught at 20 km from the mouth of Tevere River (Latium; length = 405 km; catchment area = 17,375 km^2; water temperature = 10.0–23.7 °C; Fig. 1; 41°48′N–12°25′E). The dam of Castel Giubileo, located upstream from the city of Rome, represents the first obstacle to the upward migration of eels. Between this dam and the mouth there is the Aniene inlet, an important eel reservoir and a source of pollution that, along with the high urbanization of Rome and many industries, exerts a high pressure on the lower Tevere River. In this stretch the fishery mainly targets yellow eels less than 40 cm long that are sent to aquaculture facilities.

Each individual was anaesthetized with 2-phenoxyethanol 1 %, painlessly killed, weighed and measured. Otoliths were extracted, embedded in a low viscosity resin and stained with 3 % toluidine blue following grinding of the convex side (ICES 2009). Individual age was determined from reading annual otolith rings (annuli) starting from the first ring after the marine nucleus edge using a light stereomicroscope. The assigned age of the animal corresponds to the number of years of colonization from the continental water body. The developmental stage was assigned on the basis of livery (yellow or silver eel) and confirmed by the Ocular Index (OI) calculated according to Pankhurst (1982). Sex was macroscopically assessed whenever possible by gonad examination during dissection according to Colombo et al. (1984). Alternatively, sex was determined by histological analysis of the gonad structure according to previously established criteria of gonad differentiation (Colombo and Grandi 1996; Grandi and Colombo 1997). Fulton's condition factor (K) was calculated according to the equation $W*100/L^3$, where W is the weight expressed in g and L the length in cm (Nash et al. 2006).

Chemical analysis

Due to budget limitations eels less than 40 cm long from Lesina Lagoon and Tevere River were pooled in two size classes for POP analysis, i.e. a first pool for eels smaller than 30 cm ($n = 5$ and 9, respectively)—mostly young and undifferentiated eels—and a second pool for eels between 30 and 40 cm ($n = 13$ and 8, respectively)—mostly sexually differentiated yellow and silver eels. Eels over 40 cm from Lesina Lagoon and Tevere River and all Caprolace specimens, regardless of their size, were individually analyzed.

All the animals were stored frozen at −20 °C; a portion of the dorsal muscle tissue above the gastrointestinal cavity was freeze-dried and homogenized. 0.5 g of each sample (either from a single fish or a pool) were extracted with 50 mL of an acetone-*n*-hexane (pesticide analysis grade, Carlo Erba, Italy) 1:1 (v/v) mixture in glass microfibre thimbles (19 × 90 mm, Whatman, England) using a modified Soxhlet apparatus (Velp Scientifica, ECO 6 Thermoreactor, Italy). The gravimetric determination of lipids was performed after solvent evaporation. Lipids were suspended in 2 mL of *n*-hexane and destroyed with 5 mL of H_2SO_4 (98 %, Carlo Erba, Italy). Chlorinated compounds were then recovered by several *n*-hexane washings. Hexane extracts were concentrated under vacuum to about 2 mL and cleaned-up on a Florisil column (7 × 40 mm, Merck, Germany), eluting with 25 mL of a *n*-hexane-dichloromethane (pesticide analysis grade, Carlo Erba, Italy) 85:15 (v/v) mixture according to a procedure used in an intercalibration exercise (Galassi et al. 1981). The purified extracts (1 μL) of each sample were introduced by on-column injection into a gas chromatograph (TOP 8000, Carlo Erba, Italy) equipped with a capillary column (50 m × 0.25 mm, film thickness 0.25 μm, WCOT fused silica CP-Sil 8CB, Varian, USA) and a ^{63}Ni electron capture detector (ECD 80, ThermoQuest Carlo Erba, Italy), heated at 320 °C. The temperature program used was: from 60 to 180 °C at 20 °C/min, followed by a run from 180 to 200 °C at 1.5 °C/min, a further run from 200 to 270 °C at 3 °C/min, and a final isotherm at 270 °C for 20 min, with helium as carrier gas (1 mL/min) and nitrogen as auxiliary gas (30 mL/min). For OCP quantification an external standard was prepared with pure compounds (Pestanal, Sigma-Aldrich, Germany) dissolved in iso-octane (pesticide analysis grade, Carlo Erba, Italy), while for PCB quantification Aroclor 1260 (Alltech, USA) with the addition of PCB 28, 52 and 118 was used. The sum of PCB 28, 52, 101, 118, 138, 153 and 180 is reported as indicator PCBs since these 7 congeners are the most representative on the basis of their persistence in food webs and tendency for bioaccumulation.

Good laboratory practices were tested on the standard reference materials BCR-598 and BCR-349 (Community Bureau of Reference, Belgium) for OCP and PCB residues, respectively, analysing samples in triplicate. The percentage of recovery of pp′DDE (1,1′-dichloro-2,2′-bis-(4-chlorophenyl)-ethylene) was 107.5 (±4.0 %), of pp′DDD (1,1′-dichloro-2,2′-bis-(4-chlorophenyl)-ethane) 106.2 (±4.0 %), of pp′DDT (1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane) 106.2 (±3.0 %), of HCB 105.2 (±9.9 %), of HCHs varied between 89.5 (±9.1 %) and 107.7 (±7.2 %) and of PCBs between 91.3 (±1.1 %) and 102.2 (±1.6 %). The detection limit was 0.01–0.05 ng/g wet weight (w.w.) or 0.1–0.5 ng/g lipids (lip.) depending on the chlorinated compound.

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and DL-PCBs were determined in three silver eels for each study site by High

Resolution Gas Chromatograph/Mass Spectrometer (HRGC/MS) (these analysis were performed by the accredited Laboratory of Environmental Chemistry and Toxicology, Mario Negri Institute for Pharmacological Research, Milan, Italy). The detection limit was 0.10-0.58 pg/g w.w. depending on the dioxin-like compound. World Health Organization-Toxic Equivalent Factors (WHO-TEFs) for human (van den Berg et al. 2006) were used for calculating Toxic Equivalents (TEQs) with respect to 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzodioxin).

Parasitology investigation

Each individual was dissected and the swim bladder was removed, maintained damp in distilled water and dissected in a longitudinal sense to identify and single count *A. crassus* nematodes using a binocular stereomicroscope. Parasites were weighted and the swim bladders were analyzed in order to detect the presence of encapsulated larvae in the wall. Classical epidemiological parameters—i.e. prevalence (percentage of infected individuals on the total examined eels), abundance (number of parasites in a specimen) and mean intensity (average number of parasites in the infected eels)—were calculated according to Bush et al. (1997) for each sampling site. Following Székely et al. (2005), we computed the infection level as the ratio between the parasite total weight and the eel weight (Wp/W). The relative swim bladder length or Length Ratio Index (LRI), computed as the ratio between the swim bladder length and the eel length (Ls/L), was used for establishing the degree of the mechanical swim bladder damage: the smaller LRI, the higher the damage (Lefebvre et al. 2011).

Gonad investigation

Gonads were histologically examined to assess their developmental stage and identify possible alterations. Small pieces from the cephalic, medial and caudal regions of the gonads were removed from the body after eel dissection, fixed in Bouin's fluid for 24 h and stored in 70 % ethanol. The fixed tissue was dehydrated through a series of graded ethanol solutions and embedded in Paraplast. Cross sections 7-µm thick were mounted on glass slides, stained by Papanicolaou with eosin-hematoxylin solution (Merck, Germany) and examined by a Leitz Aristoplan (Ernst Leitz Wetzlar GmbH, Germany) photomicroscope equipped with a Nikon Digital Sight DS-5 M camera (Nikon Corp., Japan).

The extent of gonad alteration was determined after examining 348 eels: 99 (length: 18.5–67.5 cm) were caught in Caprolace Lake, 100 (length: 25.5–72.5 cm) in Lesina Lagoon and 149 (length: 20.5–56.0 cm) in Tevere River. The scale of gonad alteration was established on the basis of several previous studies on gonad development and differentiation (Colombo et al. 1984; Colombo and Grandi 1995; Colombo and Grandi 1996; Grandi and Colombo 1997; Grandi et al. 2000).

Here follows a detailed description of the classes of Gonad Alteration Level (GAL):

0—no alteration detectable (Fig. 4a, b).
1—slight alterations (some germ cells showing degenerations, easily detected by their pycnotic nucleus and slightly stained cytoplasm) (Fig. 4c).
2—medium (extended degenerations of somatic and germ cells, with appearance of some areas of necrotic and adipose tissues) (Fig. 4d, e).
3—high (extended hypertrophy of the connective tissue, reduced number of germ cells and loss of the normal histological organization) (Fig. 4f).

Statistical analysis of data

One-way ANOVA with Scheffè's test or *t* test were applied to compare morpho-physiological characteristics of eels from the three study sites and/or with different sex and stage. Possible correlations between morpho-physiological characteristics, POP levels (expressed both on w.w. and on lip.) and parasite or gonad alteration indexes were investigated using Pearson (r) test or Spearman (ρ) test if an ordinal variable was present. A principal component analysis (PCA) was performed considering only samples individually analyzed for POP determination as objects and contaminants, eel weight and lipid content as variables. After that data on contamination were $\log(x + 1)$-transformed to obtain a Gaussian distribution, one-way ANOVA with Scheffè's test was applied to compare POP concentrations on lipid basis in eels from the three study sites. All the statistical analysis were performed using STATISTICA (version 8) software. Tests were considered significant for $p < 0.05$.

Results

The samples collected in the three Italian coastal environments exhibited a different composition for size (ANOVA, $p < 0.05$), sex ratio (♂:♀) and age classes (ANOVA, $p < 0.05$) as shown in Table 1. Length was correlated with age in each site (Caprolace Lake: $n = 22$, $r = 0.61$; Lesina Lagoon: $n = 28$, $r = 0.82$; Tevere River: $n = 24$, $r = 0.79$; $p < 0.05$). Caprolace eels were older than Tevere ones, while Lesina eels were the youngest (ANOVA, $p < 0.05$). No significant difference among samples was found for the condition factor (Table 1). K was correlated with age ($n = 28$, $r = 0.63$, $p < 0.05$) and

Table 1 Morphometric and physiological characteristics, POP mean concentrations ± SD (min–max) and number of infected samples by *A. crassus* of eels from Caprolace Lake, Lesina Lagoon and Tevere River divided in three length classes

Length classes	*n*	Length (cm)	Weight (g)	K	Stage	Age (years)	Sex	Lipids (%)	PCBs (ng/g w.w.)
Caprolace Lake									
<30 cm	7	26.11 ± 4.01 (18.5–30.0)	26.91 ± 12.26 (8.5–44.3)	0.14 ± 0.02 (0.12–0.17)	7y	6.00 ± 1.63 (4–9)	2un 4S 1♂	5.51 ± 4.62 (0.51–11.63)	10.53 ± 4.58 (4.70–18.47)
30–40 cm	7	35.91 ± 1.02 (35.0–38.0)	67.55 ± 12.00 (45.6–81.3)	0.14 ± 0.02 (0.11–0.18)	3y 4 s	7.14 ± 1.35 (5–9)	5♂ 2♀	10.77 ± 7.39 (0.33–17.52)	42.91 ± 54.07 (8.93–161.02)
>40 cm	8	50.24 ± 8.05 (40.5–60.0)	202.24 ± 102.20 (91.0–360.3)	0.15 ± 0.02 (0.11–0.19)	5y 3 s	8.38 ± 2.13 (5–11)	8♀	6.30 ± 6.83 (0.49–15.96)	21.56 ± 22.75 (6.71–72.75)
Lesina Lagoon									
<30 cm[a]	5	28.50 ± 1.00 (27.0–29.5)	31.04 ± 3.15 (27.2–35.3)	0.13 ± 0.01 (0.12–0.15)	5y	3.40 ± 1.34 (2–5)	5S	10.90	7.95
30–40 cm[a]	13	34.94 ± 2.83 (30.0–39.0)	65.14 ± 18.22 (39.6–97.6)	0.15 ± 0.02 (0.12–0.18)	13y	3.46 ± 0.78 (2–5)	2S 11♀	10.61	8.16
>40 cm	10	58.83 ± 10.25 (42.0–72.5)	399.24 ± 224.98 (89.0–762.0)	0.17 ± 0.03 (0.12–0.22)	5y 5 s	5.90 ± 1.20 (4–8)	10♀	18.10 ± 5.14 (10.14–24.49)	7.63 ± 3.24 (5.11–15.11)
Tevere River									
<30 cm[a]	9	24.61 ± 2.72 (20.5–29.0)	23.81 ± 9.05 (14.8–42.9)	0.15 ± 0.01 (0.13–0.18)	8y 1 s	4.22 ± 0.67 (3–5)	3un 5S 1♂	10.86	1453.67
30–40 cm[a]	8	34.80 ± 3.40 (30.4–39.0)	75.99 ± 26.17 (39.4–115.3)	0.17 ± 0.02 (0.14–0.22)	4y 4 s	6.00 ± 1.31 (4–8)	1un 4S 3♂	20.80	277.30
>40 cm	7	45.43 ± 5.56 (40.0–56.0)	170.99 ± 66.52 (107.4–312.2)	0.18 ± 0.02 (0.16–0.22)	7 s	6.71 ± 1.70 (5–9)	6♂ 1♀	24.13 ± 7.32 (15.72–37.40)	258.78 ± 146.44 (91.95–476.58)

Length classes	pp′DDE (ng/g w.w.)	pp′DDD (ng/g w.w.)	pp′DDT (ng/g w.w.)	HCB (ng/g w.w.)	α-HCH (ng/g w.w.)	β-HCH (ng/g w.w.)	γ-HCH (ng/g w.w.)	*n* infected
Caprolace Lake								
<30 cm	19.58 ± 15.23 (9.15–53.24)	1.17 ± 0.77 (0.50–2.72)	0.83 ± 0.48 (0.40–1.74)	0.14 ± 0.10 (0.04–0.34)	0.09 ± 0.05 (0.05–0.16)	0.14 ± 0.10 (0.04–0.35)	0.05 ± 0.03 (0.01–0.09)	0
30–40 cm	18.92 ± 9.26 (8.56–32.68)	2.00 ± 1.53 (0.41–4.39)	1.29 ± 0.82 (0.52–2.57)	0.34 ± 0.23 (0.03–0.56)	0.16 ± 0.10 (0.04–0.28)	0.46 ± 0.63 (0.03–1.76)	0.07 ± 0.03 (0.02–0.12)	0
>40 cm	11.04 ± 4.49 (6.67–20.76)	1.00 ± 0.46 (0.42–1.66)	1.43 ± 1.62 (0.24–4.87)	0.21 ± 0.19 (0.04–0.47)	0.08 ± 0.06 (0.04–0.20)	0.20 ± 0.22 (0.02–0.63)	0.05 ± 0.04 (0.01–0.12)	0
Lesina Lagoon								
<30 cm[a]	45.36	4.19	3.10	1.37	0.25	0.85	0.15	1
30–40 cm[a]	105.32	14.98	8.42	1.18	0.34	1.04	0.27	3
>40 cm	89.56 ± 74.84 (45.50–288.05)	7.42 ± 4.82 (3.46–19.50)	4.47 ± 2.48 (2.23–10.95)	1.16 ± 0.45 (0.65–2.07)	0.30 ± 0.15 (0.14–0.67)	0.74 ± 0.29 (0.27–1.25)	0.12 ± 0.08 (0.03–0.25)	3
Tevere River								
<30 cm[a]	245.88	32.76	37.02	3.10	0.37	1.48	0.19	5
30–40 cm[a]	207.16	37.31	22.03	5.41	0.52	2.22	0.16	6
>40 cm	110.89 ± 120.38 (43.94–38.66)	25.96 ± 15.20 (11.22–57.72)	15.53 ± 10.66 (9.07–38.09)	5.84 ± 2.27 (2.90–9.39)	0.92 ± 0.69 (0.35–2.38)	3.94 ± 2.92 (1.29–10.08)	0.25 ± 0.05 (0.19–0.32)	6

n number of samples; *K* Fulton's condition factor; *stage*: *y* yellow, *s* silver; *sex*: *un* undifferentiated, *S* Syrski organ, ♂ male, ♀ female

[a] Samples analysed by pool

lipid content ($n = 10$, $r = 0.63$, $p < 0.05$) in Lesina eels, while K was only correlated with fat percentage ($n = 22$, $r = 0.61$, $p < 0.05$) in Caprolace eels.

Caprolace eels exhibited the highest variation in lipid content (Table 1): yellow eels had lower fat content than silver ones (t test, $p < 0.05$). Both yellow and silver females from Caprolace Lake had lower lipid percentage than Lesina yellow and silver ones, respectively (t test, $p < 0.05$), and silver males in Caprolace Lake had lower lipid content than those in Tevere River (t test, $p < 0.05$).

Despite remarkable inter-site differences, PCBs and pp′DDE provided the main contribution to eel contamination in all the sampling sites, followed by the other DDT homologues and, finally, by HCB and HCHs (Table 1). Positive correlations among all contaminants were observed ($p < 0.05$) with the occasional exception of POPs with concentrations near the detection limit. Eels from Tevere River were the most polluted in each of the three size classes (Table 1), especially those with a length <30 cm.

The first component of the PCA (Fig. 2) accounted for 54.19 % of the variance in the dataset with the highest contributions by most of POPs: pp′DDT (15.85 %), pp′DDD (15.34 %), PCBs (14.74 %), α-HCH (14.67 %), pp′DDE (14.18 %) and γ-HCH (12.74 %), while the second component accounted for 22.13 % of the variance mainly associated with HCB (30.80 %), lipids (30.05 %) and β-HCH (20.73 %).

Tevere eels were a distinct cluster (Fig. 2) characterized by the highest levels of PCBs, pp′DDD, pp′DDT, HCB and β-HCH (ANOVA, $p < 0.05$). Lesina eels were the least polluted (ANOVA, $p < 0.05$), while Caprolace eels were characterized by the greatest heterogeneity and two subgroups could be distinguished: one, partially overlapping with Lesina eels, composed of individuals with higher lipid content and lower POP levels than those constituting the second distinct cluster (Fig. 2). In Caprolace eels, both lipid content and condition factor were negatively correlated with POP levels on lipid basis, with exception of β-HCH ($n = 22$; lipids-PCBs: $r = -0.60$; lipids-DDTs: $r = -0.73$; K-PCBs: $r = -0.65$; K-DDTs: $r = -0.75$; $p < 0.05$).

The PCB congener profile was rather similar in eels from the three study sites; the greatest contribution to PCB contamination was by hexachlorobiphenyls 153 and 138 (>10 %), followed by pentachlorobiphenyl 118 and heptachlorobiphenyls 180 and 187 (>5 %) (Fig. 3). Penta-, hexa- and hepta-Cl PCBs accounted for more than 80 % of the total PCBs, while the sum of 7 indicator PCBs amounts to 74.1 ± 4.7 %, 61.3 ± 5.1 % and 61.6 ± 5.9 % of the total PCBs for Caprolace, Lesina and Tevere eels, respectively.

In all the silver eels analyzed for PCDD and PCDF determination, results were below the detection limit. DL-PCB concentrations, reported in Table 2 as Total TEQ, were the highest in Tevere eels (ANOVA, $p < 0.05$).

Prevalence of *A. crassus* was nearly 70 % in Tevere eels, 25 % in Lesina eels, while no parasites were found in eels from Caprolace Lake (Table 1). Mean intensity was higher in Tevere eels (3.27 ± 2.43; max = 9) than in Lesina eels (2.71 ± 1.89; max = 5), but the difference was not statistically significant (ESM 1; t test, $p = 0.60$).

The analysis of the infected Tevere eels (ESM 1), with the exception of the encysted ones, showed that infection

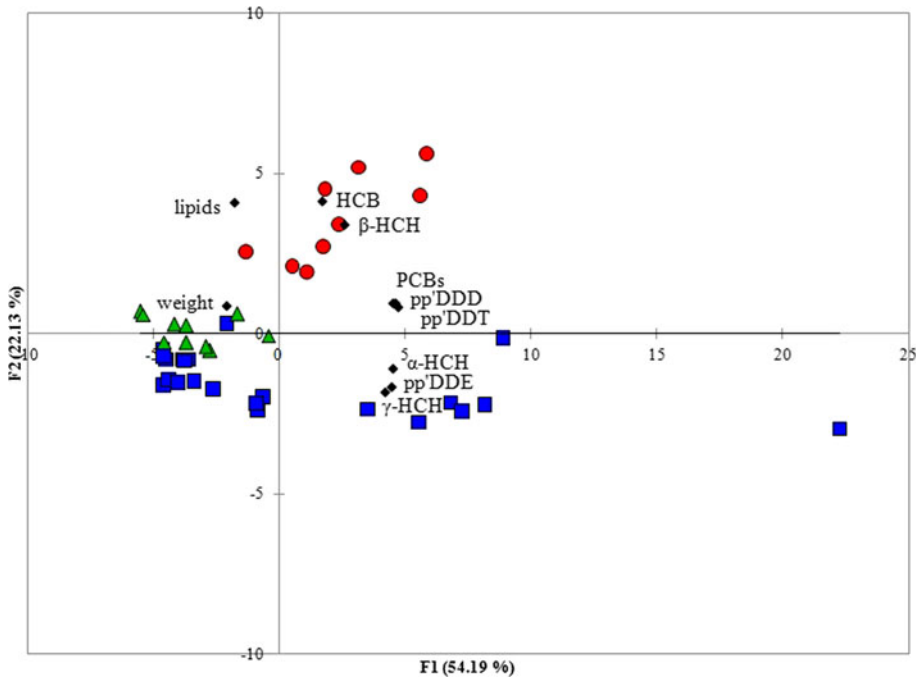

Fig. 2 Principal component analysis biplot for describing analyzed contaminants and grouping eels from the three sampling sites (Caprolace eels: *blue square*, Lesina eels: *green triangle* and Tevere eels: *red circle*). The *horizontal axis* represents the first component, the *vertical axis* represents the second component (Color figure online)

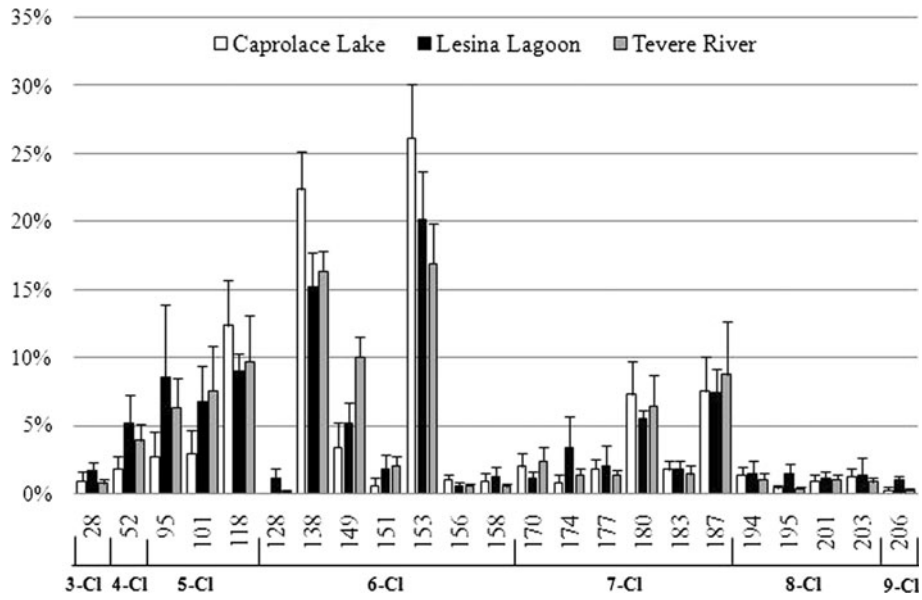

Fig. 3 Mean distribution (%) of PCB homologues (+SD) in the three sampling stations

Table 2 Morphometric and physiological characteristics, DL-PCB TEQ values (pg/g w.w.), calculated using TEF values for human (van den Berg et al. 2006), of three silver eels for each study area

		Caprolace Lake			Lesina Lagoon			Tevere River		
Sample		**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Length (cm)		60.0	35.4	36.0	70.5	72.5	56.0	44.0	41.0	48.5
Weight (g)		360.3	78.4	65.4	762.0	711.2	370.7	144.8	107.4	181.2
Sex		♀	♂	♂	♀	♀	♀	♂	♂	♂
Lipids (%)		15.96	11.50	16.34	24.49	20.81	24.20	26.44	16.01	25.71
Non-*ortho* PCBs	WHO-TEFs 2006									
81	0.0003	0.00349	0.00035	0.00032	0.00012	0.00016	0.00026	0.01347	0.00884	0.00432
77	0.0001	0.00029	0.00002	0.00004	0.00002	0.00015	0.00045	0.00003	0.00006	0.00006
126	0.1	2.00377	0.27189	1.13981	1.40450	1.52455	2.29541	1.71452	2.12983	2.18776
169	0.03	0.01921	0.05745	0.03041	0.01069	0.01955	0.03889	2.35403	1.04097	1.72898
Mono-*ortho* PCBs	WHO-TEFs 2006									
105	0.00003	0.00662	0.00122	0.00554	0.00005	0.00026	0.00049	0.00121	0.00708	0.05171
114	0.00003	0.00014	0.00011	0.00010	0.00009	0.00009	0.00008	0.00005	0.00007	0.00335
118	0.00003	0.07111	0.01661	0.00379	0.00398	0.00317	0.00770	0.20310	0.02139	0.15451
123	0.00003	0.00109	0.00022	0.00006	0.00009	0.00026	0.00101	0.00379	0.00404	0.00260
156	0.00003	0.00531	0.00131	0.00062	0.00044	0.00031	0.00060	0.02614	0.02337	0.02025
157	0.00003	0.00090	0.00042	0.00021	0.00010	0.00002	0.00008	0.00383	0.00389	0.00369
167	0.00003	0.00383	0.00046	0.00036	0.00003	0.00010	0.00042	0.01064	0.00862	0.00903
189	0.00003	0.00061	0.00010	0.00009	0.00001	0.00003	0.00004	0.00771	0.00224	0.00246
TEQs		2.12	0.35	1.18	1.42	1.55	2.35	4.34	3.25	4.17

level was positively correlated to intensity ($n = 15$, $r = 0.57$, $p < 0.05$) and negatively correlated with swim bladder length ($n = 15$, $r = -0.54$, $p < 0.05$). Tevere silver males (Table 3) with the highest parasite abundance and infection level tended to exhibit high PCB and pp′DDT concentrations and low lipid content, but the correlations were not significant ($n = 7$; abundance-PCBs: $r = 0.70$, $p = 0.08$; infection level-PCBs: $r = 0.60$, $p = 0.15$; infection level-pp′DDT: $r = 0.70$, $p = 0.08$; parasite abundance-lipid content: $r = -0.66$, $p = 0.11$).

The histological analysis showed that eels collected in Tevere River had mainly undifferentiated gonads (35.3 %) and Syrski organs (46.0 %). Among eels with sexually differentiated gonads, males were largely prevalent over

Table 3 Male silver eels from Tevere River analysed individually for POP determination: number (n) and weight of parasites (Wp), swim bladder length (Ls), damage (Ls/L) and infection level (Wp/W), Gonad Alteration Level (GAL) and PCB, pp′DDE, pp′DDD and pp′DDT concentrations on lipid basis

Sample	*n* parasites	Wp (g)	Ls (cm)	Damage	Infection * 10^4	GAL	Lipids (%)	PCBs (ng/g lip.)	pp′DDE (ng/g lip.)	pp′DDD (ng/g lip.)	pp′DDT (ng/g lip.)
1	2	0.0006	4.96	0.11	0.04	1	26.44	1301.52	321.14	107.20	39.73
2	9	0.4134	3.49	0.09	44.65	3	17.26	1761.69	454.89	154.80	113.57
3	7	0.0282	3.97	0.10	2.96	2	18.32	1266.56	297.36	65.82	40.27
4	4	0.1625	4.99	0.12	15.13	1	16.01	1011.48	274.35	70.09	56.64
5	2	0.0046	6.01	0.13	0.29	0	24.23	1177.23	336.98	93.36	47.94
6	1	0.0308	3.98	0.10	2.32	3	37.40	1281.22	1024.16	154.85	102.41
7	0	0	3.99	0.10	0	2	23.40	392.41	218.26	70.30	41.78
8	Encysted	–	7.00	0.14	–	2	25.70	1373.02	286.18	103.88	78.42

females (92.8 %); nevertheless the initial stages of differentiation exhibited female features. Lesina and Caprolace eels were mainly females (75.8 and 49.5 %, respectively), in agreement with the comparison between the average length and weight of eels belonging to the over 40 cm size class from the three study sites (Table 1; ANOVA, $p < 0.05$).

In eels from Tevere River, 19 out of 53 undifferentiated gonads showed GAL 1. Among the 69 Syrski organs, 26 had GAL 1 (Fig. 4c), 13 GAL 2 (Fig. 4d) and 9 GAL 3. Among the 25 testes, 7 showed GAL 1, 6 GAL 2 (Fig. 4e) and 5 GAL 3 (Fig. 4f). No alterations were detected in the two ovaries found. In eels from Lesina Lagoon, 4 out of 23 Syrski organs showed GAL 1. No alterations were detected in the 76 ovaries and in the only undifferentiated gonad found. In eels from Caprolace Lake, 3 out of 23 Syrski organs showed GAL 1. Among the 25 testes, 2 had GAL 1. No alterations were detected in the 49 ovaries and 2 undifferentiated gonads found.

Concerning the eels sampled for POP determination, among 24 Tevere individuals (Table 1), one of the four undifferentiated gonads showed GAL 1. More severe alterations were found in all Syrski organs: 2 eels with GAL 1 (Fig. 4c), 4 with GAL 2 (Fig. 4d) and 3 with GAL 3. Among testes, one was normal and all others showed alterations: 3 with GAL 1, 3 with GAL 2 (Fig. 4e) and 3 with GAL 3 (Fig. 4f). The only ovary found was normal. Among the 28 eels from Lesina Lagoon (Table 1), only one (a Syrski organ) showed GAL 1. Among the 22 eels from Caprolace Lake (Table 1), only one (a testis) showed GAL 1.

Considering infected Tevere eels (ESM 1), with the exception of the encysted ones, a significant positive correlation between GAL and parasite weight ($n = 15$, $\rho = 0.62$, $p < 0.05$) and a negative correlation between GAL and Ls/L ratio ($n = 15$, $\rho = -0.58$, $p < 0.05$) were found. Considering Tevere male silver eels in Table 3, correlations between GAL and POP concentrations were not significant, the highest ρ value and the lowest p value being that between GAL and pp′DDT ($n = 8$, $\rho = 0.60$, $p = 0.14$).

Discussion

Lipid content

Our analysis showed that eels caught in winter in Caprolace Lake were older and thinner than those of the same sex and size from Tevere River and Lesina Lagoon. Significant geographical variations in fat content are common in eels and have been described earlier: Belpaire et al. (2009) recently reported considerable variation between sites both in Belgium and in The Netherlands, with minimum values of the mean lipid content per site similar to those found in Caprolace Lake. Eels from other Italian environments such as Orbetello and Santa Giusta lagoons (Mariottini et al. 2006) and Garigliano River (Ferrante et al. 2010) showed fat percentages similar to those reported for Lesina and Tevere eels. The low growth rate and condition of Caprolace eels may be explained by the extremely low productivity and the comparatively limited resources in the winter season of these salinized waters (Edeline and Elie 2004; Cairns et al. 2009). Other causes should be excluded, as parasite infection was absent and pollution was not particularly high in this group.

Food paucity in Caprolace Lake adversely affected yellow eels substantially more than silver ones: lipid content was $\leq$2 % in half of the yellow specimens while exceeded 10 % in all silver muscle tissues, even though it was always below 20 % for all specimens and below 15 % for two silver females. Although lipid content in starving fish might be underestimated (Bettinetti et al. 2011), the difference between yellow and silver eels is evident and in

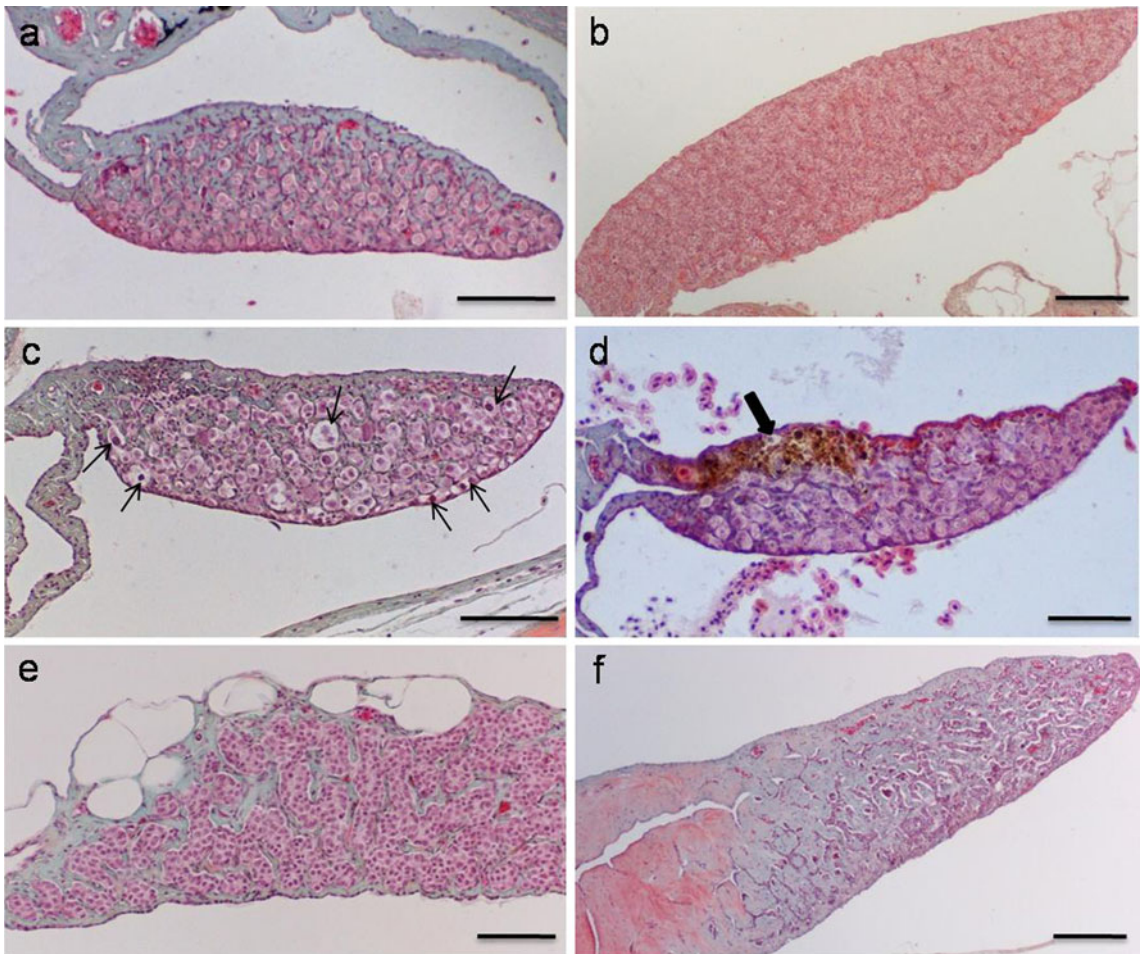

Fig. 4 Cross sections of gonads from eels of Tevere River without structural alterations (**a** and **b**) and with alterations (**c**, **d**, **e** and **f**). **a** Syrski organ from a 27.8 cm yellow eel showing normal histological structure (*scale bar* 100 μm). **b** Testis from a 46.5 cm silver eel showing normal histological structure (*scale bar* 500 μm). **c** Syrski organ from a 29.2 cm yellow eel showing Gonad Alteration Level (GAL) 1 (some degenerations of germ cells) (*thin arrows*) (*scale bar* 100 μm). **d** Syrski organ from a 25.8 cm yellow eel showing GAL 2 (a large region of necrotic tissue) (*thick arrows*) (*scale bar* 50 μm). **e** Testis from a 42.3 cm silver eel showing GAL 2 (some areas of adipose tissue, *top* of the figure) (*scale bar* 200 μm). **f** Testis from a 44.3 cm silver eel showing GAL 3 (extended hypertrophy of the connective tissue, reduced number of germ cells and loss of testis histological organization) (*scale bar* 500 μm)

agreement with the fact that yellow eels are still growing while silver eels are developing or conserving lipid reserves for migration (Durif et al. 2005). Boetius and Boetius (1980) argued that lipid storage needs to be at least 20 % of the body weight to support the energetic costs of migration and gamete production for this species. Van den Thillart et al. (2004) argued that energy reserves may be insufficient to reach the Sargassum Sea when fat level drops below 15 % of the body weight. If this is the case, Caprolace silver eels might have minimal chances of successfully migrating to the spawning area.

Parasite infection

Palstra et al. (2007) observed that significant swim bladder damage resulted from heavy infection, i.e. when more than 5 parasites were present. This also seems to be true in the present study, as parasite infection was associated with a reduction of swim bladder length. As swim bladder damages may significantly impair eel swimming ability (Palstra et al. 2007; Sjoberg et al. 2009), it is likely that heavily infected eels from Tevere River are unable to reach the spawning ground to contribute to recruitment.

The relation between parasite abundance and lipid content was negative but not significant. This is in accordance with other published studies that failed to verify a relationship between infection level and yellow eel condition (Kelly et al. 2000; Machut and Limburg 2008). Nonetheless, it is known that nematodes are capable of inducing stress in eels and increasing cortisol plasma levels (Sures et al. 2001). This should theoretically lead to an increase in metabolism, potentially affecting energy

accumulation (Robinet and Feunteun 2002). In the present case, the lack of significance in the relationship between parasite abundance and lipid content may reflect the actual absence of effects by parasitic load on eel condition or it may be just a consequence of the limited sample size (i.e. only 7 specimens) used to test this relationship.

Our study provided evidence of a positive correlation between parasite weight and swim bladder damage and gonad alteration level. This result raises the possibility that parasite induced stress negatively affects gonad development. However, we cannot rule out that the observed correlations might be spurious, driven by a third variable, such as POPs and/or other contaminants, directly causing gonad abnormalities and indirectly affecting an increase of parasite infection. Our results showed that PCBs and pp′DDT were correlated with parasite abundance and infection level, but these relationships were just above the 5 % significance level, possibly as a consequence of the limited sample size.

The absence of *A. crassus* in Caprolace eels is probably explained by the high salinity of the lake that makes this water body an unsuitable environment for parasite development and transmission (De Charleroy et al. 1989; Kirk et al. 2000).

POP contamination

The high variability of eel contamination levels observed in the present study probably reflects both between-site differences in land use and protection levels and within-site small-scale habitat heterogeneity (Bettinetti et al. 2011). We found a negative correlation between POP concentrations on lipid basis and lipid content in the 22 Caprolace eels. The overall amount of contaminants in these eels did not depend on the lipid content. This is somewhat surprising, since, in general, specimens with higher fat percentage may be expected to exhibit also higher accumulation of lipophilic chemicals. It is possible that POPs concentrate in Caprolace eels while lipids are metabolized in starving specimens during the winter period. This mechanism would be similar to that described by Larsson et al. (1990) for fasting eels during migration to the spawning ground.

In this study pp′DDE is the dominant chlorinated pesticide in eels. Although it is well recognized that this compound is a powerful anti-androgen (Kelce et al. 1995) affecting development, reproduction and sexual behaviour by causing feminization (Zhang and Hu 2008; Bayley et al. 2002), little is still known about its effects on eels. A study on Baltic herring (*Clupea harengus*) found that ovarian pp′DDE levels above 18 ng/g w.w. are significantly associated with reduced hatching viability (Matthiessen 2003). As interspecific differences clearly exist (Carlson et al. 2000; Foster et al. 2001), pp′DDE effects on eels cannot be simply extrapolated from studies on other species.

HCB and HCH isomers occurred at low concentrations in all the samples and are unlikely to cause adverse effects on eels. β-HCH concentrations in Tevere eels were surprisingly higher than those of lindane (γ-HCH): in fact, β-HCH production and use were banned since 2001 in Italy while lindane is still presently used. Our conjecture is that the higher β-HCH concentrations in Tevere eels may be due to a point pollution source: high levels of β-HCH were found in 2005 in the milk of cows farmed in the same region (Sacco River valley, Latium; ASL 2008) and more recently in the water withdrawn from some wells in the same area. Thus, β-HCH contamination could be the consequence of improperly managed wastes produced by a local chemical industry and accidentally discharged in the environment during the second half of the nineteen century.

The bioaccumulation pattern of PCBs in eels observed in this study is consistent with others previously reported both for eels and for other fish or aquatic organisms (Bordajandi et al. 2003; Mariottini et al. 2006; Storelli et al. 2007) and it is generally correlated with the degree of chlorination, the stereochemistry and the lipophilicity of the different congeners (Fox et al. 1994).

Some PCBs may reduce the spawning success of *Anguilla anguilla* by interfering with the ovarian development (Robinet and Feunteun 2002; Corsi et al. 2005). Levels above 120 ng/g w.w. of PCBs in ovaries of Baltic flounder (*Platichthys flesus*) and Baltic herring (*Clupea harengus*) were correlated with impaired egg development and fry survival (Matthiessen 2003). It is thus remarkable that 86 % of Tevere eels exceeded this threshold in muscle tissues: according to investigations by Svobodová et al. (2003) on common carp and by Palstra et al. (2006) on the European eel, PCB concentration in eel ovary should be similar or even higher than in muscle. The impact of PCB contamination on reproductive success of Tevere eels could be significant.

Palstra et al. (2006) observed that DL-PCBs caused disrupting effects on development and survival of eel embryos below 4 pg TEQ/g gonad, which is the concentration found in the muscle of the three analyzed Tevere eels. Negative consequences of DL-PCBs on reproduction should be expected for the most polluted individuals of Tevere stock, as DL-PCBs accumulated in muscle fats are also incorporated in oocytes of the mature eels. In a recent study (Miniero et al. 2011), TEQ values of 11.8, 17.5 and 21.3 were measured in three different pools of eels collected in the urban tract (Rome) of Tevere River, several times higher than those found in this study. In addition, long term adverse effects caused by DL-PCBs cannot be excluded for Caprolace and Lesina eels with TEQ levels

higher than 1 pg TEQ/g w.w., the lowest value at which Palstra et al. (2006) documented reproduction impairment.

Although high POP levels may cause changes in gonad histology and alterations of fish tissue structure (Louiz et al. 2009), in Tevere eels the relations between pollutant concentrations and GAL were not significant, probably because of the complexity of the contaminant mixture in the natural environments and/or to the small sample size.

Besides reproductive failure, POPs—endocrine disruptors in particular—might cause negative effects on the immune system and may be associated with an increased sensitivity to infectious diseases and parasites (Robinet and Feunteun 2002; Lawrence and Elliot 2003). In field studies, increased infection and disease incidence in benthic fish from various coastal areas has been associated with PCB contamination (Vethaak and Reinhalt 1992). In the case of eel, Sures and Knopf (2004) showed that a dose of 100 ng/g w.w. of PCB 126 suppress antibody response, thus increasing vulnerability to infection by *A. crassus*. Machut and Limburg (2008) found elevated infection rates when urbanized lands exceeded 15 % of the tributary catchment area, as in the case of the lower Tevere River, and suggested that urbanization may increase eel susceptibility to infection by increasing stressors. The relation between PCB concentration and parasite abundance in Tevere eels was positive but not significant, probably because of the limited sample size. Even though we did not determine POP levels in swim bladder nematodes, there was no evidence that the highest infected eels were the least polluted and, on the basis of this result, we believe that bioaccumulation of the investigated contaminants did not occur in the parasites.

Risk for eel predators and human consumption

The risk for eel consumers, such as otters, fish-eating birds and humans, should not be ruled out due to the potential for POP biomagnification. The Eurasian otter (*Lutra lutra*) has declined significantly since 1970 in Western and Central Europe, and it is believed that this decline partially occurred as a consequence of the increasing contamination of the aquatic ecosystems (Kruuk and Conroy 1996). 86 % of Tevere eels and one Caprolace specimen exceeded the less protective PCB concentration of 145 ng/g w.w. (Boscher et al. 2010) considered critical for otter survival, while all Tevere and 18 % of Caprolace samples exceeded the more protective value of 25 ng/g w.w. (Boscher et al. 2010) for long-term otter conservation. According to the Great Lakes Water Quality Agreement (GLWQA 1987) PCB and DDT concentrations in fish tissues should not exceed 100 ng/g w.w. and 1,000 ng/g w.w., respectively, for the protection of fish-eating birds and other vertebrates. In this study DDTs never exceeded the proposed limit, while PCBs should constitute a risk for eel predators.

The European eel is a valuable and popular gastronomic delicacy in several Italian regions from North to South. Therefore, high contamination levels of eels may significantly contribute to increased human health risk (Belpaire and Goemans 2007; Maes et al. 2008). Our analysis showed that 36 % of eels from Tevere River and 20 % of those collected in Lesina Lagoon (all specimens less than 40 cm long) exceeded the sanitary limits of DDTs for human consumption in Italy (50 ng/g w.w. DDTs for fish with lipid content <5 %; 100 ng/g w.w. for lipid content 5–20 %; 150 ng/g w.w. for lipid content 20–40 %; OM 1990), while none exceeded the Italian sanitary limits for HCB and HCHs (DM 1998). Only the pool of smallest eels from Tevere River exceeded the limit of 300 ng/g w.w. recently set up for the six indicator non DL-PCBs by the European Union (EU 2011). As DL-PCBs, none of the nine analyzed silver eels exceeded 10 pg TEQ/g w.w. (EU 2011) even if the estimated TEQs for the most polluted specimens should exceed the sanitary limit since the ratio between total PCBs and TEQs is usually constant within the same ecosystem. This observation is supported by a recent study in which the maximum allowed concentration was exceeded by eels collected in the urban (Rome) tract of Tevere River (Miniero et al. 2011), whose human consumption is considered dangerous.

Conclusions

Our analysis confirmed that *A. anguilla* is highly sensitive to water quality degradation as previously observed in other surveys carried out in Northern and Central Europe. Tevere eels were highly parasitized and contaminated and displayed the highest incidence and severities of reproductive organ abnormalities. At Caprolace Lake, eels were older than in Tevere River, exhibited low lipid content, low levels of contaminants, no reproductive organ abnormalities and no infections. The poor condition and older age of Caprolace eels were consistent with lack of fishing pressure, winter collection and residence in oligotrophic salinized waters where *A. crassus* life cycle is not supported; moderate contamination was not associated with any apparent gonad abnormality. The contamination level on lipid basis was inversely correlated with lipid content, a possible indication that starving eels may concentrate pollutants while consuming their fat storage. At Lesina Lagoon, eels were younger than in Tevere River and in better condition than in Caprolace Lake. They were also minimally polluted, moderately parasitized and did not exhibit gonad abnormalities. Fishing pressure may explain younger age distribution and young aged eels might partially exhibit low contamination. Yet, condition and lack of reproductive organ abnormalities suggest relatively good health.

Tevere eels exhibited a poor health status: their high contamination and infection levels have the potential to significantly affect eel reproductive ability by causing severe damage to gonads and embryos and by reducing the chance of successful migration to the spawning area. Even though only marginally contaminated and without infection, Caprolace eels also exhibited critical health conditions because of their poor nutritional status and the lack of sufficient lipid reserves to meet the energy requirement for the transoceanic migration. On the contrary, Lesina eels could be considered in good health on the basis of their minimal contamination and infection levels. Unfortunately, these eels might also be unable to fully develop their reproductive potential as overfishing in this lagoon dramatically reduces the abundance of the future spawners.

This study thus confirms that a multiplicity of stressors may impair, independently or jointly, the reproductive ability of the European eel. Our analysis did not demonstrate a significant interaction between the intensity of infection and the contamination level in Tevere eels. Further investigation is necessary to assess whether the joint effect of these multiple stressors is simply additive or could be compensatory or synergistic. Our study clearly shows that any conservation strategy for the recovery of *A. anguilla* should be devised so as to preserve both spawner quality and abundance of this threatened species.

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Conflict of interest The authors declare that they have no conflict of interest.

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