

Fishes and their parasites in the water district of Massaciuccoli (Tuscany, Central Italy)

Fabio Macchioni¹, Luca Chelucci¹, Beatrice Torracca¹, Maria Cristina Prati² & Marta Magi¹

¹ Department of Veterinary Science, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy.

² Scuola Normale Superiore di Pisa, Piazza dei Cavalieri 7, 56126 Pisa, Italy.

* Corresponding author at: Ospedale Didattico Veterinario "Mario Modenato", Department of Veterinary Sciences, University of Pisa, Via Livornese [Lato Monte], 56122, San Piero a Grado, Pisa (PI), Italy.
Tel.: +39 050 2210119, Fax: +39 050 2210182, e-mail: fabio.macchioni@unipi.it.

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Keywords

Fishes,
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(Tuscany, Italy),
Opisthorchis felineus,
Parasites.

Summary

This study has been conducted in the district of Massaciuccoli (lake, marsh and reclaimed areas with drainage channels) in Tuscany region (Central Italy). The aim of the research was to detect the presence of parasites in fishes, in particular of *Opisthorchis felineus*, which causes an important zoonosis. Between 2010-2012, the health status of 381 fishes was monitored, morphometric characteristics were determined, and parasites were searched for and identified. Of the 381 examined fishes, 189 were free of parasites while 192 were infected, among them 91 presented multiple infections. *Opisthorchis felineus* was not found in any of the examined fishes.

Indagine parassitologica sui pesci nel distretto di Massaciuccoli (Toscana, centro Italia)

Parole chiave

Distretto di
Massaciuccoli (Toscana),
Opisthorchis felineus,
Parassiti,
Pesci.

Riassunto

Questo studio è stato condotto nel distretto di Massaciuccoli (lago, palude e zone bonificate con canali di drenaggio) (Toscana, centro Italia) con l'obiettivo di rilevare la presenza di parassiti nei pesci, in particolare di *Opisthorchis felineus*, responsabile di un'importante zoonosi. Tra il 2010 e il 2012, è stato monitorato lo stato di salute di 381 pesci, dei quali sono state determinate le caratteristiche morfometriche e sono stati individuati eventuali parassiti. Tra i pesci esaminati, 189 sono risultati privi di parassiti mentre la presenza di parassiti è stata rilevata in 192 animali, tra questi ultimi 91 presentavano infestazioni multiple. *Opisthorchis felineus* non è stato trovato in nessuno dei pesci esaminati.

Introduction

The water district of Massaciuccoli, located in the Tuscany region between the provinces of Lucca and Pisa, Italy, includes the lake, the marsh, and the reclaimed areas with their network of drainage channels (Pedreschi 1956). The lake is an eutrophic coastal basin with a peat substrate to the East near the hills, while to the West the substrate is formed by coastal sandbanks. Its average depth is 2.5 meters, with a variation of 90 cm occurring between the Summer season and the Winter; the average pH is 8.2 and the average temperature ranges from 7°C in Winter to 22°C in Summer (Spandre and Meriggi 1972).

In recent years the fish fauna of the water district of Massaciuccoli has undergone a gradual

change which caused a reduction of the species evolutionarily originated in the basin, and a progressive increase of introduced species. Of the 22 known species, 11 are autochthonous [*Petromyzon marinus*, *Anguilla anguilla*, *Tinca tinca* (almost disappeared), *Esox lucius*, *Atherina boyeri*, *Dicentrarchus labrax*, *Liza ramada*, *Mugil cephalus*, *Salaria pavo*, *Salaria fluviatilis*, *Gobius niger*]; 11 species are non-native (*Rutilus erythrophthalmus*, *Scardinius erythrophthalmus*, *Carassius auratus*, *Cyprinus carpio*, *Pseudorasbora parva*, *Alburnus alburnus*, *Silurus glanis*, *Ictalurus melas*, *Gambusia holbrooki*, *Lepomis gibbosus*, *Micropterus salmoides*). They have been introduced either from other Italian water districts or from Northern Europe, North America, and East Asia (Alessio *et al.* 1992, Chelucci 2005). Among the molluscs, Gasteropoda

(Ancyliidae, Lymnaeidae, *Physa fontanilis*) and Bivalvia (*Sinanodonta woodiana*, allochthonous) are common. A Louisiana's crayfish (*Procambarus clarkii*) has appeared after escaping from a badly built breeding installation.

The purpose of this study was to detect the parasites of the fishes, and the eventual presence of *Opisthorchis felineus* (Rivolta 1884) metacercariae, in this water district, an area never investigated before.

Materials and methods

In the period from July 2010 to June 2012, 381 fishes were caught in the water district of Massaciuccoli (113 in the lake, 228 in the marsh, and 40 in the adjoining drainage ditches). The sampling sites were chosen according to the presence of fish and wildlife species. Fishes were caught in all watercourses, with the exception of the channels in the hills, which are mostly devoid of fish fauna except of eels. Commercial fishing gear (fixed networks, mobile networks, and 'bertovelli') and sport devices (fishing rods) were used for the captures. The fishes were transported in a portable refrigerator and analyzed at the Department of Veterinary Science, University of Pisa.

The following morphometric parameters of the fishes were determined: total length from the apex of the mouth to the apex of the caudal fin, height from the ventral fin to the highest point of the back and weight (accuracy: ± 1 mm and ± 1 g respectively). The condition factor K of each subject was evaluated. This parameter takes into account morphological changes over time and is correlated to the nutritional status and the wellbeing of the fish. K is calculated according to Beckmann's formula (Beckmann 1948), it is equal to 1 for a perfectly isometric grown fish and is bigger than 1 for well fed fishes. If P is the weight in grams and L is the length in centimeters one has:

$$[1] K = (P / L^3) * 100$$

The weight P and the length L are related by an equation of the type: $P = \beta * L^\alpha$

The exponent α varies generally from 2 to 4, it is equal to 3 in the case of isometric growth and bigger than 3 for stocky subjects.

Skin, eyes, gills, digestive system, liver, bladder, and kidneys of each fish were examined for the presence of ecto and endoparasites. Diagnostic procedures were based on external observation of the skin and subsequent examination of various specimens of the fish under a stereomicroscope (magnification 8X - 35X) and an optical microscope (10X, 40X). The parasites found were classified according to keys (Kabata 1988, Kennedy 1974, Lom and Dykova 1992, Moravec 1994, Yamaguti 1963).

The research of *O. felineus* was conducted by submitting epiaxial and hypoaxial muscles of the fish to a compression technique and a digestion technique with pepsin and hydrochloric acid.

For the statistical analysis, the prevalence and its 95% confidence interval were calculated. Linear regression was carried out in order to calculate the parameters alpha and beta in equation [1] (after taking logarithms). Significance of the tests was reached for P values less than 0.05.

Results

The captured fishes are classified in Table I. In Table II the parasites found in the various fish species are listed along with their location on the fish. Of the 381 fishes examined, 189 were free of parasites, 192 were infected by parasites. Of the latter category, 91 fishes presented multiple infections. Table II shows that ectoparasites (in 172 fishes) are more common than endoparasites (in 60 fishes). Italian bleak (*Alburnus alburnus albidus*) was found for the first time in the Massaciuccoli district. The 4 specimens

Table I. Classification of the 381 fishes captured between 2010-2012 in the water district Massaciuccoli (Tuscany, Central Italy).

Order	Family	Species	Common name	Italian name	Origin	N fishes
Cypriniformes	Cyprinidae	<i>Alburnus alburnus albidus</i> (Costa, 1838)	Italian bleak	Alborella	Non-native	4
		<i>Carassius auratus</i> (Linneus, 1758)	Goldfish	Carassio	Non-native	103
		<i>Cyprinus carpio</i> (Linneus, 1758)	Carp	Carpa	Non-native	147
		<i>Pseudorasbora parva</i> (Temminck & Schlegel, 1842)	Stone moroko	Pseudorasbra	Non-native	13
		<i>Scardinius erythrophthalmus</i> (Linneus, 1758)	Rudd	Scardola	Non-native	32
		<i>Rutilus erythrophthalmus</i> (Zerunin, 1982)	Triotto	Triotto	Non-native	20
Mugiliformes	Mugilidae	<i>Liza ramada</i> (Risso, 1826)	Grey mullet	Muggine	Native	7
Perciformes	Centrarchidae	<i>Lepomis gibbosus</i> (Linneus, 1758)	Pumpkinseed	Persico sole	Non-native	15
		<i>Micropterus salmoides</i> (Lacépède, 1802)	Black bass	Persico trota	Non-native	9
Siluriformes	Ictaluridae	<i>Ictalurus melas</i> (Rafinesque, 1820)	Black catfish	Pesce gatto	Non-native	31

Table II. The parasites of the 381 fishes captured in Massaciuccoli district, Tuscany (Central Italy) between 2010–2012, the table shows their prevalence and its 95% confidence interval.

Parasites	Organ/tissue	Goldfish	Carp	Grey mullet	Pumpkin-seed	Black bass	Black catfish	Stone moroko	Rudd	Triotto	Prevalence %	95% C.I.
Protozoa												
<i>Amoeba</i> sp	Gills		1								0.26	0-0.78
<i>Trichodina</i> sp	Skin, gills	10	³⁶		3	4	13	2	2		18.37	14.48-22.26
<i>Trichodinella</i> sp	Skin, gills	14	30	1	3	2	2	3	1	1	14.96	11.38-18.54
<i>Apiosoma cylindriciformis</i>	Gills	2	5								1.84	0.49-3.19
<i>Ichthyophthirius multifiliis</i>	Skin	4									1.05	0.03-2.07
<i>Myxobolus cerebralis</i>	Kidney		3								0.79	0-1.67
<i>Tetrahymena corlissi</i>	Skin, gills		2							1	0.79	0-1.67
<i>Ichthyobodo necator</i>	Skin, gills								1		0.26	0-0.78
Platyhelminthes												
<i>Dactylogirus vastator</i>	Skin, gills	5	45	2	4		6		6	1	18.11	14.24-21.98
<i>Gyrodactylus elegans</i>	Skin, gills	8	9				2				4.99	2.80-7.17
<i>Diplozoon paradoxum</i>	Gills		9						6		3.94	1.98-5.89
<i>Diplostomum spathaceum</i>	Crystalline	1	3								1.05	0.03-2.07
Caryophyllidae	Intestine		34					1			9.19	6.29-12.09
<i>Proteocephalus</i> sp	Intestine					9					2.36	0.84-3.89
<i>Botriocephalus</i> sp	Intestine	1	2								0.79	0-1.67
Trematoda	Liver, spleen					5					1.31	0.17-2.46
Nemathelminthes												
Capillaridae	Intestine	1	1					2		1	1.31	0.17-2.46
Acanthocephala												
<i>Acanthocephalus lucii</i>	Intestine				1					3	1.05	0.03-2.07
Annelida												
<i>Piscicola geometra</i>	Skin		1						1		0.52	0-1.25
Crustacea												
<i>Lernaea cyprinacea</i>	Skin	3	4		1		2	1	1	2	3.67	1.78-5.56
<i>Argulus foliaceus</i>	Skin	1	2								0.79	0-1.67
<i>Ceratothoa</i> sp	Skin		1								0.26	0-0.78

Table III. Seasonality of the 381 captures and fish sex, divided into male, female and indeterminate (Massaciuccoli district, Tuscany, Central Italy).

Species	Spring			Summer			Autumn			Winter			Total		
	M	I	F	M	I	F	M	I	F	M	I	F	M	I	F
Italian bleak				2		2							2		2
Goldfish	1		20	8		33	6		25			10	15		88
Carp	12		12	26	4	27	21	3	19	10		13	69	7	71
Grey mullet	3		1	1			2						6		1
Pumpkinseed	4	2	1	3		5							7	2	6
Black bass	1		2	2		2	1		1				4		5
Black catfish	7	2	7	2		2	1	6	4				10	8	13
Stone moroko	2	2	4	1	1	3							3	3	7
Rudd	4		3	5		9	6	1	3	1			16	1	15
Triotto			1	6		12					1		6		14

M = male; I = Indeterminate sex; F = Female.

Table IV. Morphometric characteristics of the 381 fishes captured between 2010–2012 in the Massaciuccoli district (Tuscany, Central Italy).

Species	N fishes	Mean width	Mean length	Mean weight 95% CI	K	Alpha	Beta
Black catfish	31	3.07 (2.72:3.42)	16.37 (14.76:17.99)	63.16 (43.09:83.23)	1.14 (1.08:1.20)	3.02 (2.84:3.21)	-4.54 (-5.06:-4.03)
Common rudd	32	5.00 (4.42:5.58)	18.73 (16.89:20.58)	106.03 (67.01:145.05)	1.19 (1.13:1.25)	3.38 (3.27:3.50)	-5.55 (-5.88:-5.22)
Goldfish	103	10.09 (9.70:10.47)	28.73 (27.90:29.56)	479.56 (437.62:521.51)	1.92 (1.81:2.03)	3 (2.65:3.34)	-3.98 (-5.14:-2.82)
Carp	147	9.86 (9.51:10.22)	34.28 (33.03:35.52)	701.75 (629.38:774.12)	1.59 (1.50:1.67)	2.81 (2.63:2.99)	-3.52 (-4.14:-2.89)
Stone moroko	13	1.57 (1.43:1.71)	7.65 (7.08:8.21)	3.77 (2.66:4.88)	0.79 (0.67:0.90)	-0.03 (-0.17:0.11)	2.04 (1.92:2.16)
Triotto	20	3.6 (3.02:4.18)	12.97 (11.65:14.28)	31.05 (21.08:41.02)	1.15 (1.07:1.24)	3.59 (3.34:3.84)	-5.97 (-6.61:-5.32)
Pumpkinseed	15	4.14 (3.59:4.69)	10.88 (9.80:11.96)	25.13 (17.49:32.77)	1.64 (1.40:1.88)	4.25 (3.49:5.01)	-7.11 (-8.92:-5.31)
Black bass	9	5.64 (4.65:6.64)	21.71 (17.64:25.79)	167.78 (62.49:273.07)	1.28 (1.19:1.36)	3.23 (2.93:3.52)	-5.05 (-5.96:-4.15)
Grey mullet	7	5.01 (4.48:6.81)	18.30 (21.48:35.52)	245.52 (134.12:385.60)	1.04 (0.68:1.41)	2.58 (0.93:4.24)	-3.29 (-8.77:2.19)
Italian bleak	4	2.38 (2.05:2.70)	11.6 (10.34:12.86)	13 (6.75:19.25)	0.79 (0.63:0.94)	4.13 (-0.95:9.21)	-7.63 (-20.07:4.81)

K = condition factor; Alpha and beta coefficients in the equation relating the weight of the fish to its length.

captured had no parasites; *O. felineus* was not found in any of the examined fishes.

In Table III the fishes are classified according to the period of the capture and to their gender, as females, males, and specimens with immature gonads. Among goldfishes (*Carassius auratus*) more females than males were caught, in agreement with the reproduction of this species by gynaogenesis. The eggs are activated by male gametes of species, much as it happens in crucian (*Carassius carassius*) and carp (*Cyprinus carpio*), and develop embryos with maternal genes, which grow into female adults.

The morphological characteristics of the fishes are displayed in Table IV, together with their 95% confidence intervals. Table IV also shows the values of the parameters α and β in the equation [1] relating weight and length of the fishes and their 95% confidence intervals, as estimated by linear regression techniques.

Discussion

According to the data provided by the Azienda regionale per la protezione ambientale della Toscana (2014), the dark cloudy waters of the district of Massaciuccoli are classified as eutrophic, due to high concentrations of nitrogen and phosphorus derived from intensive farming and the lack of depurating devices or their poor functioning. The mean seasonal temperatures have risen by 1–2 degrees since the middle of the past

century. Non-native fishes dominate because they are more resistant (Chubb 1997, Galli 2000, Ghittino et al. 2004). From their mean K factor, it appears that these species are well nourished, although a general worsening is observed comparing the results of this study to those of a previous study (Alessio et al. 1992).

The actual bad conditions of the water district may result in a high susceptibility of the fishes to parasite infection (Galli 2000). The non-native fish species have probably introduced into the water their most common parasites, such as *Caryophyllaeus* spp. for the carp and *Proteocephalus* spp. for the catfish (Gozlan et al. 2010).

This parasitological investigation has revealed the presence of many ectoparasites, most probably because the fishes live in infected and not very mobile eutrophic waters, with poor water exchange (Galli 2000). One factor that exposes the fishes to parasite infection can be the increasing of the water average temperature. Furthermore, the gregarious behaviour of the fishes can be a vehicle for transmission of parasites.

Acanthocephalus lucii was found only in the drainage ditches in the Northern part of the basin, infesting the intestines of percids, such as pumpkinseed (*Lepomis gibbosus*). *Diplozoon paradoxum* showed a typical pattern, being present in the gills mainly from Spring to Summer, it showed a marked preference for some fish species, such as carp and rudd. *Opisthorchis felineus* was not found during this study,

although its presence has been reported several times in the province of Pisa (Italy) (Macchioni *et al.* 2012, Macchioni 1963) and in other parts of Italy (De Liberato *et al.* 2010).

The fishes of the Massaciuccoli basin, especially carps, goldfishes and catfishes are often used for aquaculture and for sportive fishing, therefore their parasites can contaminate other ponds. Sometimes these fishes are eaten in spite of prohibitions so that more attention to parasitological fauna of the fishes in this basin should be increased.

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