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CHECK LIST OF THE PARASITOFAUNA IN ADRIATIC SEA CAGE-REARED FISH

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Along with the fast development of aquaculture in the Mediterranean, a number of emerging parasitic diseases was observed in cage-reared fish, which in confined rearing conditions induced mortality or/and indirect economical losses related to suppressed growth.

The trend of diversification of aquaculture products and the introduction of new fish species in the rearing system, helped the introduction of new parasitic pathogens along with their host in the new environment. The process resulted in the adaptation of parasites and in a switch to resident aquaculture species (sea bass and sea bream) or/and increase of prevalence and abundance of parasites on newly introduced fish species. In both cases, the parasitofauna of reared fish even impoverished in terms of species richness and showed greater population values than in the wild fish population.

Even if the Mediterranean parasitofauna of reared fish was the issue of numerous publications in the past decade, only occasional findings and reports concerned its specificities in the Adriatic Sea.

Key words: Adriatic Sea, parasitofauna, sea bass, sea bream

INTRODUCTION

There are two well established species in the Adriatic aquaculture – sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*), while sharpsnout bream (*Diplodus puntazzo*) and black sea bream (*Pagellus bogaraveo*) comprise only a minor part of the overall production.

The sharpsnout bream population was previously devastated by severe mortalities induced by *Enteromyxum leei* (ex. *Myxidium leei*) (Myxozoa: Myxosporidia), however its market value never reached the level of sea bass and sea bream, hence its reared population is in constant decrease.

In the case of black sea bream, fattened in rearing environment after capture from the wild, although it had a good conversion index, the mesentherial accumulation of adipose tissue made the fish not very appreciated by the consumers.

Northern Atlantic bluefin tuna (*Thunnus thynnus*) is the latest introduced species in Adriatic aquaculture and a commercially valuable fish, while its

pathologies so far seem to be limited on parasitic infections (Mladineo and Tudor 2004), in lesser extent to viral or bacterial diseases (Mladineo 2005, Sugaya *et al.* 2005). Recently an excellent review of pathologies of diverse tuna species has been published, listing mainly pathogens of wild tuna stocks from (sub)tropical waters, mostly of parasitic origin (Munday *et al*. 2003).

Parasites in wild fish populations, with a low prevalence and abundance and minor or negligible pathological effects on their host, tend to proliferate in serious outbreaks of epizootic and spread easily in a host population in confined conditions of a rearing system (Athanassopoulou *et al.,*1999; Company *et al.* 1999). This being optimized by elevated host stock density and by their usually compromised immunity resulting from daily stress.

Most of the parasitosis are very hard to eradicate, especially if the therapy has to be applied in semi- or offshore net pans. The spectrum of chemotherapeutics is very narrow, the most useful compounds being rarely licensed for aquaculture. The key solution lays in good prevention based on optimal husbandry and zootechnical measures, where appropriate stock density, frequent changing of nets and balanced diet play the biggest role.

MATERIAL AND METHODS

Seven fish farms from the east part of the Adriatic sea were monitored from June 2004 to March 2005. The locations of the farms are the following: facility I – Kaldonta bay (Island of Cres), facility II – Vela Luka bay (Island of Šolta), facility III – Peleš bay (near Primošten), facility IV – Island of Žižanj, facility V – Island of Žižanj, facility VI – Island of Maslinovac (peninsula of Pelješac) and facility VII – Island of Tajan (peninsula of Pelješac).

The fish population was sampled over a nine-month period from an off shore net pen, always from the same cage. Fish samples comprised 10-15 sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), sharpsnout bream (*Diplodus puntazzo*) and red sea bream (*Pagellus bogaraveo*), all over one year of age. A total of 673 individuals was examined.

Collected fish were put on ice, brought within hours to the laboratory, where they were autopsied and biometrical measures were recorded.

Fresh smears were taken from the gills, skin and fins, from three different parts of the digestive duct (pyloric area, middle intestine and rectal part), spleen, liver, gonads and kidney. Positive smears were stained by May-Grünwald Giemsa. Myxosporeae were measured and identified according to Lom and Arthur (1989).

Gill monogeneans were counted on the middle third of the first gill arch. Cut gills and fins were placed in Petri dishes, while scrapings of skin and nasal cavities were mounted on slides, all to be examined under a dissecting microscope with a 20x magnification. Monogeneans were detached with dissecting needles, counted and collected in a watch glass. Afterwards, parasites were ruptured between the slide and coverslip under finger pressure and a mixture of 4 % formalin and glycerin (5:1) was added on the edge of a coverslip. After the remaining fixative evaporated the edges were sealed with Du-Noyer sealant.

Northern Atlantic bluefin tuna was sampled during the harvest period in January 2004 and July 2005, when mortalities were reported. The facility (facility VIII) is located on the NW part of the Island of Brač. A total of 62 fish were examined.

Visceral organs and gill arches were collected. Fish body surface was inspected for the presence of any changes prior to technical washing of fish trunks. Because of the market value of the carcasses, no incisions of fins, skin or eyes were allowed.

Prior to rearing fish were caught in May 2003, in the waters of the Island of Jabuka, trawled to the farm and reared until January 2004. During this period fish were fed with mixed fish from small trawling boats, combined with frozen imported herrings.

Samples of viscera were individually collected in plastic bags and transported to the laboratory. Blood samples were collected before visceration, from the incision made beneath the pectoral fins, through the heart. Sodium citrate was used as the anticoagulant.

Upon arrival to the laboratory, fresh smears of gills, kidney, spleen, liver, gall bladder, intestinal and stomach mucosa and the endocard from the ventriculus were examined under light microscope. Digenean cysts from gills, cartilaginous parts of gill arch, stomach layers, pyloric caeca, skin and intestine were collected, measured and excysted with fine needles under a stereomicroscope. Individuals were fixed under coverslip pressure, stained in Borax carmine and mounted in Canada balsam. Remaining cysts were collected and fixed in 70 % etanol.

Myxosporidiae and helminthes were collected as described for other cage reared fish.

RESULTS

Seven parasitic species were isolated from sea bass: *Diplectanum aequans, Polysporoplasma sparis, Sphaerospora dicentrarchi, Amyloodinium ocellatum, Cryptocaryon irritans, Ceratomyxa labracis, Ceratothoa oestroides.* Similar parasitofauna was isolated from the sea bream: *Polysporoplasma sparis, Amyloodinium ocellatum, Cryptocaryon irritans, Ceratomyxa sparusaurati, Ceratothoa oestroides, Lamellodiscus elegans, Sparicotyle chrysophrii, Trichodina* sp.

Sharpsnout bream harbored six parasitic species: *Lamellodiscus elegans, Amyloodinium ocellatum, Ceratomyxa diplodae, Polysporoplasma sparis, Sparicotyle chrysophrii, Caligus minimus,* while black sea bream had only three species: *Ceratomyxa diplodae, Hysterothylacium* sp. and *Amyloodinium ocellatum.*

Tuna harbored the most divers parasitic population: *Ceratomyxa* sp., *Celiodidymocystis abdominalis, Didymocystis thunni, Platicystis alalongae, Koellikerioides internogastricus, K. intestinalis, K. apicalis, Anisakis simplex, Oncophora melanocephala, Hepatoxylon trichiuri, Eutetrarhynchus* sp. and *Penella filosa.*

Isolated parasitic forms, gross pathology changes, infection site and values of prevalence and abundance of parasites are shown in Table 1.

Table 1. List of parasites isolated from cage-reared fish in the Adriatic Sea

	Host	Parasitic form	Host status	Infection site	Locality	Prevalence mean (%)	Abundance mean
MASTIGOPHORA							
Amyloodinium ocellatum	sea bass	trophont	no signs	gill	I, II, IV, V, VI, VII	30.84	0.38
	sea bream	trophont	no signs	gill	I, II, IV, V, VI	74.67	1.05
	sharpsnout bream	trophont	no signs	gill	I, IV	73.57	1.13
	black sea bream	trophont	no signs	gill	III	20	0.2
CILIOPHORA							
Cryptocaryon irritans	sea bass	teront	no signs	gill	II	13.33	0.13
	sea bream	teront	no signs	gill	II	60	1.8
Trichodina sp.	sea bream	adult	no signs	gill	V	10	0.1
MYXOZOA							
Ceratomyxa sparusaurati	sea bass	spore, disporoblast	no signs	gall bladder	II	6.66	0.33
	sea bream	spore, disporoblast	no signs	gall bladder	I, IV	14.55	0.48
	sharpsnout bream	spore, disporoblast	no signs	gall bladder	I	30	0.9
	black sea bream	spore, disporoblast	no signs	gall bladder	III	21.55	0.49
Ceratomyxa sp.	tuna	spore, disporoblast	no signs	gall bladder	VIII	23.33	1.9
Sphaerospora dicentrarchi	sea bass	spore, disporoblast	no signs	intestine, gall bladder	I, II, III, IV, V, VI, VII	54.13	1.04
Polysporoplasma sparis	sea bass	spore, disporoblast	no signs	kidney	VI, VII	33.93	0.68
	sea bream	spore, disporoblast	no signs	kidney	I, II, IV, V	42.02	0.76
	sharpsnout bream	spore, disporoblast	no signs	kidney	I	20	0.6
Myxobolus sp.	sharpsnout bream	spore, disporoblast	no signs	intestine	I	10	0.1

MONOGENEA							
Diplectanum aequans	sea bass	adult, eggs	gill necrosis	gill	I, II, III, IV, V, VI, VII	62.03	2.07
Lamellodiscus elegans	sea bream	adult, eggs	no signs	gill	I, II, III, IV, V	40.06	0.97
	sharpsnout bream	adult, eggs	no signs	gill	I, III, IV	97.62	29.01
Sparicotyle chrisophrii	sea bream	adult, eggs	no signs	gill	I, II, III, V	33.9	0.46
	sharpsnout bream	adult, eggs	no signs	gill	I, IV, V	32.21	1.09
DIGENEA							
Coeliodidymocystis abdominalis	tuna	encysted adult	no signs	among pyloric caeca	VIII	63.16	3.79
Didymocistis thunni	tuna	encysted adult	desquamation	gill	VIII	73.68	13.26
Platicystis alalongae	tuna	encysted adult	no signs	skin	VIII	21.05	0.95
Koellikerioides internogastricus	tuna	encysted adult	inflamation	stomach layers	VIII	21.05	0.32
K. apicalis	tuna	encysted adult	no signs	cartilage	VIII	73.68	6.79
K. intestinalis	tuna	encysted adult	infalammation	intestinal mucosa	VIII	57.89	12.47
NEMATODA							
Hysterothylacium aduncum	black sea bream	third stage larvae	no signs	submesentherially	I	12.5	0.11
Anisakis simplex	tuna	third stage larvae	no signs	submesentherially	VIII	11.5	0.15
Oncophora melanocephala	tuna	adult	haemorrhagies	pyloric caeca mucosa	VIII	57.89	1.74
CESTODA							
Eutetrarhinchus sp.	tuna	blastocistic larvae	no signs	visceral cavity	VIII	24.73	0.08
Hepatoxylon trichiuri	tuna	plerocercoid	haemorrhagies	stomach mucosa	VIII	12.4	0.12
COPEPODA							
Penella filosa	tuna	adult, juvenile	ulceration	skin	VIII	12	0.41
Caligus minimus	sharpsnout bream	adult	no signs	gill	I	8.57	0.09
ISOPODA							
Ceratothoa oestroides	sea bass	adult	emaciation	buccal cavity	II, IV	8.89	0.15
	sea bream	adult	emaciation	buccal cavity	II	30.24	0.41

DISCUSSION

A number of detailed overviews of parasites in rearing conditions and feral fish are already published (Christofilogiannis 1992, Rodgers and Furones 1998, Le Breton 1999, Scholz 1999, Munday *et al.* 2003), however reared-fish parasitofauna of certain regions and areas generally differ greatly, and a complete overview of data from the Adriatic Sea was missing.

The first review of diseases of Adriatic sea bass in rearing conditions was reported by Šarušić (1990). The author isolated only four pathogens; *Trichodina* sp., *Amyloodinium ocellatum, Diplectanum aequans* and *Caligus minimus*. Among these species, the author emphasized *A. ocellatum* as the most pathogenic especially for larval sea bass where mortalities reached up to 90 %. Paperna and Baudin Laurencin (1979) reported similar composition of parasitofauna in Franch fishing facilities, with the exception of *Colponema* sp. from sea bass gills which were never reported in the Adriatic sea. Interesting is their finding of *Enteromyxum* sp. in sea bass, usually isolated in sparids and rare in the Adriatic. In the sea bream, they isolated only *Trichodina, A. ocellatum* and *Colponema* sp., thus reporting a much simpler parasite composition than in Adriatic farms. Nevertheless, in the sea bream from Spain kept in different culture systems, a much greater number of species was isolated (Alvarez-Pellitero *et al.* 1995), differing from the Adriatic situation only in the presence of the coccidian (*Eimeria* sp.) and one myxosporidian species (*Leptotheca* sp.).

In the Adriatic Sea bass, the monogenean *D. aequans* was the most abundant parasite, followed by *S. dicentrarchi,* while the lowest occurence was by *Ceratomyxa* sp. This data is in accordance with findings in the wild sea bass which, however should show much greater parasitofaunal richness (Santos, 1996).

After the trend of sharpsnout bream introduction in the Adriatic aquaculture during the past decade, the production was overcame by an opposite effect, and only two farms maintain this fish in the rearing system up to date. The reason for the current low production was the devastation of most stocks by *Enteromyxum leei* (Myxozoa) infection which afterwards was never again isolated, as well as the low appreciation and value of sharpsnout bream on the Croatian market. Although the major production of sharpsnout bream is conducted mainly in Greece, only two myxosporidia (*Enteromyxum leei*, *Ceratomyxa* sp.), a coccidia (*Eimeria* sp.), and two monogeneans (*Lamellodiscus* sp., *Sparicotyle* sp.) were reported as regular parasitofauna (Company *et al.* 1999). In the Adriatic sharpsnout bream sample, monogenean *L. elegans* was the most prevalent parasite, and because of its extreme values, the total parasite load of this fish exceeded others. The lowest appeared to be *C. minimus*, which is usually found rarely in healthy Adriatic reared fish.

In Adriatic conditions however, there is evidence of host switch and exchange of parasites between sparid hosts (sea bream, sharpsnout bream and black sea bream) (Mladineo and Maršić-Lučić 2005), thus the majority of parasites present in sea bream were isolated from sharpsnout bream, only showing different prevalence/ abundance values. These species are usually maintained in

polyculture, so the passage of the parasites is facilitated by the high stock density of different species.

In Adriatic Sea bream the most prevalent was *A. ocellatum*, the most abundant *C. irritans*, while the lowest prevalence had *Ceratomyxa* sp.

Black sea bream parasitofauna comprised major parasites like the two sampled sparids; *A. ocellatum* and *C. sparusaurati.* The only exception was *H. aduncum* ubiquitous in wild populations, understandable because the host was caught in the wild and subsequently fattened in cages.

Sampled tuna reveled a vast presence of members of Didymozoidae family, recently reported in the Adriatic Sea, along with the newly isolated myxosporidian (Mladineo, *in preparation*). *Didymocystis* spp. had the highest prevalence/abundance values, in certain cases leading to gross pathological changes when isolated from the stomach, intestine and gills. A potential threat for the farming of tuna fish are the camallanid *Oncophora melanocephala* and copepod *Penella filosa*. The former induce deep ulcerations with hemorrhages in the pyloric caeca mucosa, while the later burrows the skin surface, leaving deep and unsightly necrotic ulcerations in the epidermis, able to drastically drop the trunk quality.

In conclusion, Adriatic cage-reared sea bass parasitofauna is similar to the one reported previously, while sparid parasitofauna differs by the lower diversity of myxosporidians and the absence of the coccidian species.

Tuna parasitofauna is unique in its diversity of digenean species characteristic for wild populations where the food web makes possible to enhance and accumulate a wide number of different intermediate hosts, otherwise impossible to isolate from hatchery-reared fish. The research of tuna diseases is still a young and developing field, and for the moment no appropriate studies in the Mediterranean were carried on to make a plausible comparison with the situation in the Adriatic Sea.

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PARAZITI RIBA JADRANSKOG MORA UZGAJANIH U KAVEZIMA

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SADRŽAJ

Zajedno sa snažnim razvojem akvakulture u Mediteranu, uočene su brojne nove parazitske bolesti u kavezno uzgojene ribe, koje u ograničenim uzgojnim uvjetima induciraju mortalitete ili/ i indirektne ekonomske gubitke vezane za smanjen prirast.

Trend diverzifikacije proizvoda akvakulture i uvođenje novih vrsta riba u uzgojne sustave, pomogao je uspiješnoj indukciji novih parazitskih patogena zajedno s njihovim domadarom u novi okoliš. Proces je rezultirao adaptacijom parazita i prelaskom na rezidentne riblje vrste u akvakulturi (lubin, komarča) ili/ i porastom prevalencije i abundancije parazita na novouvedenim vrstama riba. U oba slučaja, parazitofauna uzgojene ribe iako osiromašena s gledišta bogatstva vrsta, pokazala je veće populacijske vrijednosti nego u populacijama divlje ribe.

Iako je parazitofauna mediteranske uzgojene ribe bila objektom brojnih publikacija prethodne dekade, samo su se povremena istraživanja i izvještaji odnosili na njenu specifičnost u Jadranskom moru.