

Melanomacrophage centers and diseases occurring in lesser-spotted catsharks, *Scyliorhinus canicula* (L.), from the southern Adriatic Sea - importance for monitoring

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*Melanomacrophages of fish are commonly explored as biomarkers of water pollution and are considered to be sensitive albeit non-specific health indicators in water ecosystems. Sharks as long living marine species are good sentinel species. This study presents morphometric data for splenic and hepatic melanomacrophages (MMC), and observed histopathology in ten lesser-spotted catsharks, *Scyliorhinus canicula* (L.), one of the most abundant shark species in the eastern Adriatic Sea. At necropsy, we collected random tissue samples from liver, brain, gallbladder, pancreas, spleen, kidney, gills, entire digestive system, thyroid gland, rectal gland, entire urogenital (male samples) and genital system (female samples). Collected tissue samples were routinely processed and stained with hematoxylin-eosin, Periodic Acid-Schiff, and Masson Trichrome for microscopic examinations and morphometry. There was a minimal number of histopathological lesions in the examined sharks, but morphometric values reported herein were three folds higher than in previous studies in free-ranging sharks. Studies on larger numbers of sharks are needed to elucidate the biological significance of our finding in the context of population decline of the lesser-spotted catshark.*

Key words: Adriatic, catshark, diseases, melanomacrophage, monitoring

INTRODUCTION

Many fresh and salt water fish species have been investigated as sentinels of water contamination by toxins, heavy metals, microelements, and other anthropogenic pollutants and stress inducing factors (KHAN *et al.*, 1994; HYLLAND *et al.*, 1996; LINDESJÖÖ *et al.*, 1996; STORELLI *et al.*, 2006; GILTRAP *et al.*, 2017; SAYED and YOUNES, 2017). This is especially true for demersal fish due to the pollutant accumulation in the sediment (CRESSON *et al.*, 2016).

Melanomacrophages are commonly explored as biomarkers of water contamination and pollution in various fish species. They are regarded as sensitive and reliable first line bioindicator of water ecosystem health (FOURNIER *et al.*, 2001; LINDESJÖÖ *et al.*, 1996). Long living ocean species like sharks are good candidates for biomonitoring because of their high life expectancies and accumulation of environmental toxins (BORUCINSKA *et al.*, 2009). To date, only several studies investigated MMC in the parenchymatous organs of sharks (PULSFORD *et al.*, 1982; AGIUS & AGBEDE, 1984; BORUCINSKA *et al.*, 2009). It has been postulated that baseline background data of MMC distribution and morphology in every given species of shark should be established in order to assess the relevance of MMC morphometry and distribution to the health status of sharks (BORUCINSKA *et al.*, 2009).

Lesser-spotted catshark, *Scyliorhinus canicula* (L.) (Elasmobranchii: Carcharhiniformes: Scyliorhinidae) inhabits the subtropical waters of the northwest Atlantic, including Mediterranean Sea, usually between depths of 80 and 100 m, although it has been recorded from 10 to 780 m (COMPAGNO, 1984; MYTILNEOU *et al.*, 2005; GAJIĆ & LELO, 2014; GAJIĆ, 2019). Often found in schools segregated by size and sex, at different bottom types such as muddy, sandy, grassy or coralline, populations of lesser-spotted catsharks are widespread across the eastern Adriatic Sea (JUKIĆ-PELADIĆ *et al.*, 2001; KAHRIĆ and GAJIĆ, 2015; GAJIĆ, 2019). However, dramatic decline in abundance has been observed recently in some regions of the Adriatic Sea (GUBILI *et al.*, 2014). In this paper we present baseline data on mor-

phology and distribution of MMC in ten lesser-spotted catsharks from the Adriatic Sea.

MATERIAL AND METHODS

In the period from January 2017 to January 2019, ten lesser-spotted catsharks were caught as a bycatch by a trawl angler in the area off the island of St. Andrija, eastern Adriatic Sea (approx. latitude: 42.643528 and longitude: 17.963523). Studied samples consisted of six adult females, of which two were fertilized, and four adult males in post-spawning reproductive status. Given site is characterized by muddy bottom with depths range from 70 to 100 meters. Different negative pressures are known in studied region, such as intensively fisheries (trawling and longlines) and its associated habitat loss combined with (micro) plastics, heavy metals and presumably war waste and other pollution. All landed sharks were adult specimens, comprising four males and six females. Immediately after landing, as being a bycatch with reduced post-capture survival rates, all specimens were submerged in 10%-formaldehyde. Prior to the fixation at the field, body cavity was opened in order to avoid decomposition – while entire sharks was placed into formaldehyde. Under the frame of cross country and institutional collaboration the specimens were transported to the Department of Pathology, Faculty of Veterinary Medicine and University of Sarajevo for necropsy and further analyses.

Prior to autopsy weight and length of each specimen were recorded (Table 1). At autopsy, the shark bodies and internal organs were grossly examined, and the random samples of liver, brain, gallbladder, pancreas, spleen, kidney, gills, entire digestive system, thyroid gland, rectal gland, entire urogenital (male samples) and genital system (female samples) were collected for histopathology. The samples were further fixed in 10% neutral buffered formalin for 48 hours, routinely processed, embedded in paraffin, sectioned at 5µm and stained with hematoxylin and eosin (HE), Periodic Acid-Schiff (PAS), and Masson's Trichrome. Stained slides were examined under the light microscope (BX 41, Olym-

Table 1. General data, gross lesions and main microscopic lesions observed in the studied sharks

No	Sample ID	Sex	Life stage	Length (cm)	Weight (g)	Gross pathology	Histopathology
1	4/17	Female	Adult	39	220	NLV ^a	-
2	8/17	Female	Adult, fertile	43	273	NLV	-
3	57/17	Female	Adult, fertile	40	250	NLV	Mild to moderate mononuclear cholangitis
4	67/17	Male	Adult	37	195	NLV	Mild to moderate mononuclear cholangitis
5	110/17	Female	Adult	39	202	NLV	Moderate inflammation and hyaline thrombosis
6	111/17	Female	Adult	41	216	NLV	Necrosis and inflammation of the nidamental gland
7	147/17	Male	Adult	43	233	NLV	Mild to moderate mononuclear cholangitis
8	14/19	Male	Adult	41	213	NLV	-
9	15/19	Female	Adult	45	273	NLV	Moderate mononuclear cholangitis
10	16/19	Male	Adult	41	237	Moderate number of nematode parasites in the coelomic cavity	Moderate mononuclear cholangitis

^a - NLV – No lesions visible

pus, Germany) for the presence of lesions. For the morphometric analysis of MMC 10 random high-power fields (HPF, 400X) were selected on spleen and liver slides of each animal. The number of MMC, average volume of MMC in μm^2 , and percent area of HPF occupied with MMC were measured with imaging software (Cell Image, Olympus, Germany) in 24000 μm^2 area of HPF. The results were expressed as mean and range in parentheses. Difference significance for the number of MMC, average volume of MMC, and percent area of HPF occupied with MMC between liver and spleen tissues were statistically evaluated by t-test.

RESULTS

All examined sharks appeared in good body condition and no gross changes were observed except in one shark with numerous thin, white yellow, up to 2 cm long nematodes (presumably *Anisakis* spp.) in the coelomic cavity (Table 1). Microscopic analysis revealed fat vacuoles in hepatocytes cytoplasm ranging from micro- to macro- vesicular in all examined sharks. As squalene and other lipids accumulate in large fat vacuoles within the hepatocytes which may constitute as much as 80% of the liver (BALDRIDGE, 1987; HOLMGREN and NILSSON, 1999) such changes have not been considered

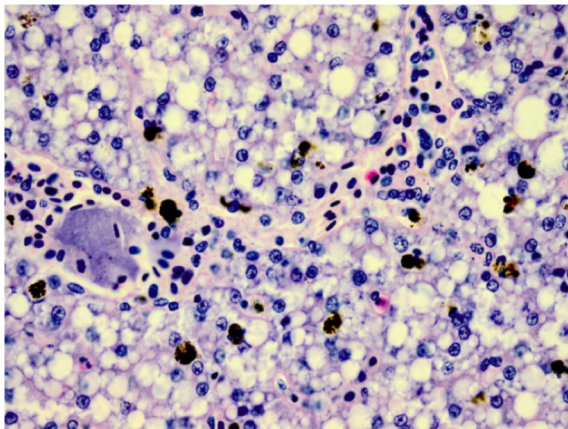


Fig. 1. Numerous MMC packed with golden brown granular material in the liver parenchyma of the lesser-spotted catshark. Note normal vacuolar degeneration of the hepatocytes. Hematoxylin and eosin, 40X

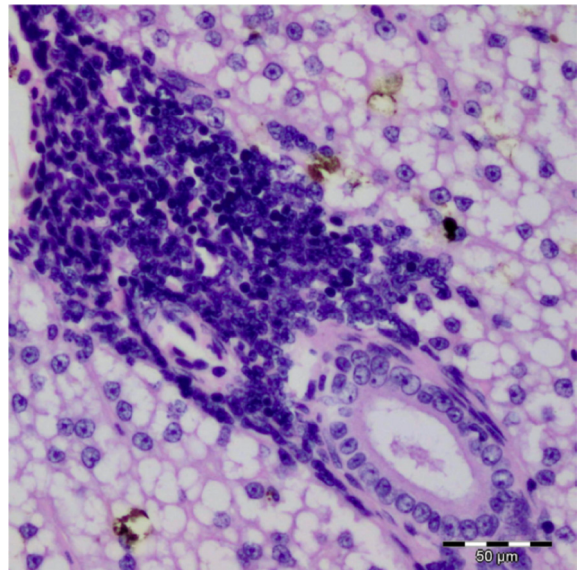


Fig. 2. Focal periductal aggregate of mononuclear cells in the liver of a lesser-spotted catshark. Hematoxylin and eosin, X20

as pathological. In addition, in the liver of five sharks, multifocal irregular aggregates of moderate numbers of lymphocytes, macrophages and plasma cells were observed (Fig. 2). Several mononuclear cells with eosinophilic cytoplasm were scattered among the aggregates. In some aggregates, single or several groups of MMC were present. Multifocal, in the parenchyma there were small aggregates of lymphocytes and macrophages often surrounding the central core of hyper-eosinophilic material. Histopathology also demonstrated lesions in other organs in two sharks (Table 1), the brain and nidamental gland notably. Specifically, one shark had perivascular, minimal lymphocytic aggregates within meninges, and in another animal nidamental gland had moderate necrosis with mononuclear inflammation. Melanomacrophage centres composed of one to several loosely arranged cells were scattered throughout the liver and spleen parenchyma. The cells were laden with granular yellow to dark brown to black material (Fig. 1).

Morphometric values of MMC in the liver and spleen are presented in Table 2. The average number of MMC per HPF - high power field (8.41 (4.4-13.7) for liver and 10.76 (1.8-17.5) for spleen) and average percent areas of HPF occupied by MMC (4.37 (1.7-8.3) for liver and 2.89 (0.61-8.03) for spleen) were not statistically different between spleen and liver. However, the average surface area of single MMC was larger in liver compared to spleen ($p < 0,05$). Considerable variations in the number of MMC per HPF through the studied samples were observed.

DISCUSSION

Data on MMC distribution in elasmobranch species and their relevance to adverse conditions of the environment are scarce (PULSFORD *et al.*, 1982; AGIUS and AGBEDE, 1984; BORUCINSKA *et al.*,

Table 2. The morphometric values of MMC in the liver and spleen of lesser-spotted catsharks

Organ (n=10)	Mean number of MMC in HPF	Mean volume of one MMC in μm^2	Mean % HPF occupied with MMC
Liver	8.41 (4.4-13.7)	123.68 (91.98-152.66)	4.37 (1.7-8.3)
Spleen	10.76 (1.8-17.5)	64.21 (43.42-107.79)	2.89 (0.61-8.03)

2003; BORUCINSKA *et al.*, 2009). To date, morphometry, distribution and pigment content of MMC were only assessed in three species of sharks (BORUCINSKA *et al.*, 2009). Given the fact that studied samples arrived from the boat already fixed in formalin, and provided measures by fisherman was inaccurate – thus it was not possible to calculate CF. Further studies will include CF/MMC relationships in order to support given relations to the MMC presence and body conditions. The results of the present study are the first data on MMC in lesser-spotted catsharks, and the first data on MMC distribution in fish from the Adriatic Sea. The morphology of MMC observed here is similar to those previously reported in other shark species (BORUCINSKA *et al.*, 2009). Furthermore, the number of MMC per HPF (Table 2) fit in the range of previously observed numbers in apparently healthy sharks (BORUCINSKA *et al.*, 2009).

The morphometric values of MMC documented here were three folds higher than those recorded in three other shark species. The values are in particular high in the liver where large MMC were observed. Observed difference might be explained by different areas used for counting and acquisition of morphometric values. In the present study we used a field of $24000 \mu\text{m}^2$ (Cell image, Olympus, Germany) of original HPF. This is smaller area compared to one used by BORUCINSKA *et al.* (2009) and hence might have resulted in higher values. Moreover, higher values could be the result of the differences in the biologic behaviour, reproductive cycle, habitat and feeding of various shark species. The lesser-spotted catsharks investigated here, in contrast to the blue shark (*Prionace glauca*), the shortfin mako (*Isurus oxyrinchus*), and the thresher (*Alopias vulpinus*) examined by BORUCINSKA *et al.* (2009), inhabits the bottom of the sea where pollutants accumulation might be higher. However, compared to data for other fish species where the positive correlation of MMC with sediment contamination and hypoxia has been proved for MMC density greater than $40/\text{mm}^2$ (FOURNIER *et al.*, 2001), values recorded here are far beyond this limit. The high morphometric values observed here could

also be the response to pathologies of various causes. Based on isolated cases, the number of MMC in diseased sharks varied between 4 and 62 per HPF (BORUCINSKA *et al.*, 2003; BORUCINSKA & ADAMS, 2013; ADAMS *et al.*, 2015). In one shark examined here with moderate multifocal mononuclear cholangitis (Table 1) we counted equally low numbers of liver MMC with similar morphometric values as in average recorded in studied specimens. This may suggest that all pathologies do not induce the same response of MMC to the injury.

To date, a very limited data on pathology of free-living elasmobranch species exist. Most of the studies are single reports of lesions affecting different organs (BORUCINSKA *et al.*, 2003; BORUCINSKA & ADAMS, 2013). Similarly, data on pathology of elasmobranch species in captivity are scarce too. Recently, a retrospective study reviewed various pathologies observed in more than 1500 specimens of at least 60 elasmobranch species from captivity (GARNER, 2013). Beside the MMC data presented above, we observed mild to moderate peribiliary aggregates of mixed populations of mononuclear cells. Based on histopathology alone we classified these cells as lymphocytes, plasmacytes and macrophages.

We could not establish the exact cause of these lesions. Despite being absent from the observed lesions, migratory parasitic stages should be regarded as a possible etiology, at least in the case where numerous intracoelomic nematodes (*Anisakis spp.*) were present (Table 1). However, peribiliary infiltrates observed here were mostly larger, aggregate like, without any structural segmentation. It is possible that these lesions represent toxicopathic injury associated with environmental hepatotoxins. Such toxins can be further associated with certain anthropogenic pressures such as pollution and war waste, but also with several natural causes as naturally higher concentrations of heavy metals and algal blooms. Heavy metals are among the most common contaminants of marine ecosystems (De BOECK *et al.*, 2010). The concentrations of heavy metals in the eastern Adriatic Sea and sediments are mostly below the threshold levels, but anthropogenic activities may cause

local variations (VUKADIN *et al.*, 1994; POPADIĆ *et al.*, 2013). For instance, the highest values of some heavy metals such as cadmium, copper, zinc and lead in the eastern Adriatic Sea have been recorded in the vicinity of our study area (Dubrovnik area) (BOGNER *et al.*, 2005). However, as being far less studied in elasmobranch tissues than in other marine fish, data on metal accumulations and their effects merit further monitoring and investigations. An experimental study on lesser-spotted catsharks confirmed accumulation of heavy metals in different tissues, and affinity of some metals to certain tissues, the liver for example in the case of Cu (De BOECK *et al.*, 2010). Also, it is shown that some radioactive elements accumulate at faster rate in lesser-spotted catshark than in turbot (*Psetta maxima*) (JEFFREE *et al.*, 2006).

Apart from five specimens with mononuclear cholangitis and mild histopathologic lesions observed in other specimens (Table 1) we regarded the sharks examined here as healthy. Hence, as suggested by BORUCINSKA *et al.* (2009), the data on MMC from this study may be used as pilot of baseline for lesser-spotted catsharks. Still, the limited number of lesser-spotted catsharks examined here urges further studies on larger sample in order to make the

background data in this shark species more reliable. Further larger studies should be undertaken to shed light on the effects of different pollutants and aetiologies on overall health of sharks in the eastern Adriatic Sea.

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Melanomakrofagni centri i oboljenja morske mačke bljedice *Scyliorhinus canicula* (L.), iz južnog Jadrana – značaj za budući monitoring

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SAŽETAK

Melanomakrofagni centri različitih taksa ribolikih životinja nerijetko se koriste kao biomarkeri u ekološkim studijama, te se smatraju pouzdanim pokazateljima stanja u ispitivanju onečišćenja. Morski psi, kao objektivno dugoživuće marinske vrste, predstavljaju idealne modele za ovakve studije. U ovom radu autori su prezentirali detaljne morfometrijske podatke za melanomakrofagne centre (MMC) opservirane u jetri i slezeni, kao i uočene histopatološke promjene na deset jedinki morske mačke bljedice, *Scyliorhinus canicula* (L.) - kao jedne od najzastupljenijih prečnousta istočnog Jadranskog mora. Tijekom obdukcije, uzorkovani su svi organi koji si potom rutinski histološki bojani (hematoksilin-eozin, Periodic Acid-Schiff i Masson's Trichtome) s ciljem detaljnijih mikroskopskih opservacija. Uočena morfologija, kao i brojnost, MMC ispitivanih jedinki odgovara literaturnim podacima za druge vrste prečnousta. Međutim, dokumentirane morfometrijske vrijednosti bile su tri puta veće u odnosu na prethodne studije. Prosječni broj MMC po vidnom polju (HPF) i prosječan postotak područja okupiranog od strne MMC u jetri i slezeni nisu pokazali statistički značajnu razliku. Ipak, prosječna površina jednog jetrenog MMC bila je veća u odnosu na one u slezeni ($p < 0,05$). Opisani podaci o MMC značajno doprinose još uvijek dosta ograničenim podacima o distribuciji, morfologiji i morfometrija MMC kod landovine, te možemo reći da predstavljaju polaznu vrijednost za sljedeće studije. Daljnje, multidisciplinarnе, studije neophodne su kako bi se ostvario širi uvid u učinke i povezanost MMC s različitim patološkim stanjima i razumijevanje negativnog utjecaja pritisaka koji vladaju u staništu na zdravstveno stanje mačke bljedice, ali i drugih vrsta, u Jadranskom moru.

Ključne riječi: Jadran, mačka bljedica, bolesti, melanomakrofag, monitoring