

The first finding of Hassall's corpuscles in the thymi of cultured sturgeons

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ABSTRACT: Histological examination of thymi from juvenile *Acipenser ruthenus* and *Acipenser brevirostrum* after haematoxylin and eosin staining revealed the presence of Hassall's corpuscles, in a scattered distribution within the thymic medulla in two of seven, and one of nine examined specimens, respectively. Morphologically, the Hassall's corpuscles were of the juvenile and premature type and no degenerative changes were seen.

Keywords: *Acipenser ruthenus*; *Acipenser brevirostrum*; histology; thymus

The thymus in fish is a paired lymphoepithelial organ lying on the upper inside edge of the operculum, just beneath the cuboidal or flat epithelium covering the branchial cavity (Roberts and Ellis 2012). Embryologically, the thymus is derived from the third pharyngeal pouch. Thymi in sturgeons persist until the fish reach sexual maturity. Later, these undergo age-related atrophy (Fange 1986).

Histologically, the thymus is lobulated and organised in the outer cortex enclosing the central medulla (Petrie-Hanson and Peterman 2005). A diversity of different cell types is found within the thymus. The cortex is predominantly composed of closely packed lymphocytes. The medulla is composed of sparsely organised lymphocytes, macrophages, reticular cells and Hassall's corpuscles that have a disperse distribution (Fange 1986; Petrie-Hanson and Peterman 2005; Peterman and Petrie-Hanson 2006).

Hassall's corpuscles are components of the non-lymphocytic microenvironment of the thymic medulla. Histologically, the corpuscles are rounded or spherical epithelial structures varying in size. They are composed of eosinophilic reticuloepithelial cells, often concentrically arranged. The central part of the corpuscle may undergo the process of degeneration. Regressive changes including hyalin-

isation, calcification and cyst formation can also be observed. The number of Hassall's corpuscles may vary according to the age, the course of disease or the presence of stress (Furukawa 2012). Mammalian Hassall's corpuscles may be divided into four types according to their morphology: juvenile, premature, mature and senescent (advanced) type (Asghar et al. 2012). The juvenile type represents the early developmental stage of Hassall's corpuscles, in which one or two reticuloepithelial cells are arranged in a cluster. The premature stage is represented by small groups of hypertrophic cells showing early processes of keratinisation, but without the evident tendency to concentric arrangement, or a flattened appearance. In the mature type, the reticuloepithelial cells are flattened, and concentrically arranged around the mixture of keratin and degenerated lymphocytes and macrophages. In the advanced stage, Hassall's corpuscles demonstrate varying degrees of material deposition both in the centre as well as on the periphery of the corpuscle. The shape of the corpuscle seems to be distorted, and in some cases the tendency of corpuscles to fuse is evident. Regressive changes including cystic space formation, and pronounced hyalinisation or calcification are often present (Asghar et al. 2012).

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Table 1. Length, weight and age characteristics of histologically examined fish

Species	Total length (mm) range/median	Total weight (g) range/median	Age (months) range/median	No. of specimens
<i>Acipenser ruthenus</i>	136–480/180	9–180/28	6–19/14.5	7
<i>Acipenser brevirostrum</i>	100–140/120	3–12/6.5	2.5–11/6.25	9

MATERIAL AND METHODS

The study material comprised seven juvenile functionally diploid (2n) specimens of *Acipenser ruthenus* and nine juvenile functionally hexaploid (6n) specimens of *Acipenser brevirostrum* originating from the hatchery of the Faculty of Fisheries and Protection of Waters, University of South Bohemia, Czech Republic. Length and weight characteristics of the examined fish are given in Table 1. The study was carried out in accordance with the Czech Law No. 246/1992 regarding animal welfare. Protocols underwent an ethical review process at the University of South Bohemia animal care committee (PP3/FROV/2012; in Czech). Fish were sacrificed with all efforts made to minimise suffering. Clove oil in the amount of 30–40 mg/l was used for anaesthesia; subsequently, a double dose was used for euthanasia. Whole specimens were fixed in 10% neutral-buffered formalin. After fixation, specimens were cut vertically, and in three cases horizontally as well. The tissue was embedded in paraffin, conventionally processed and stained with haematoxylin and eosin (H-E), Giemsa and periodic acid-Schiff staining. Alcian blue staining was done in one case, and mucin staining was performed in four cases. Obtained slides were inspected under an

Olympus BHS microscope, recorded with a 3CCD Sony DXC-9100P colour camera and processed in Olympus MicroImage v. 4.0 software.

RESULTS

Thymi were found in sixteen specimens, and were examined histologically. The thymi were lobulated and were composed of the outer cortex and the inner medulla. In two of the seven specimens of *Acipenser ruthenus* (one 6-month-old and one 14-month-old sterlet), and one of nine specimens of *Acipenser brevirostrum* (8.5-month-old), Hassall's corpuscles were found, dispersed within the thymic medulla. Hassall's corpuscles were round or oval in shape and eosinophilic in H-E staining (Figures 1 and 2). Their size, i.e. diameter of round corpuscles and the maximum and minimum diameters of the oval ones was 36 µm, and 20 µm × 78 µm, respectively. The cytoplasm was slightly granular. According to the mammalian criteria for morphological assessment, only the juvenile and the premature type of Hassall's corpuscles were observed. They consisted of small clusters of reticuloepithelial cells without signs of keratinisation and flattening, or clusters of hypertrophic reticuloepithelial cells with early

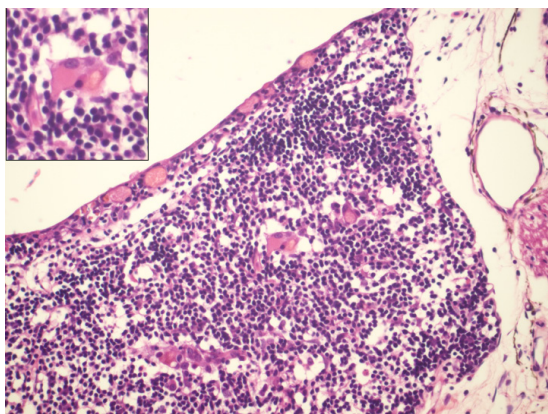


Figure 1. Histological section of the thymus of the juvenile sterlet, *Acipenser ruthenus* after staining with haematoxylin-eosin, under × 400 magnification. Hassall's corpuscle is presented as an inset in the figure

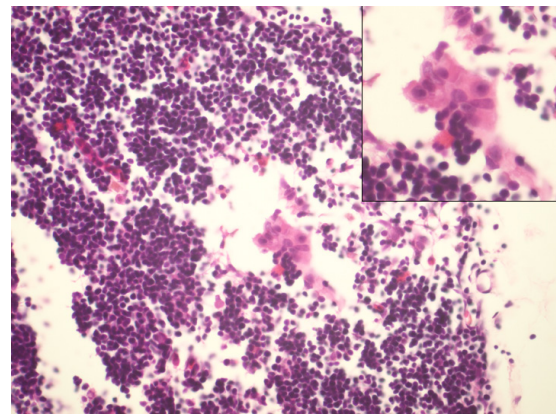


Figure 2. Histological section of the thymus of juvenile *Acipenser brevirostrum* after staining with haematoxylin-eosin, under × 400 magnification. Hassall's corpuscle is presented as an inset in the figure

stages of keratinisation. Mucin staining was negative in the premature stage of Hassall's corpuscles. No tendency of corpuscles to fuse was found; the Hassall's corpuscles did not exhibit any degenerative changes.

DISCUSSION

Bearing the name of the British physician Arthur Hill Hassall, who first described these structures in 1849 in the thymi of children (Hassall 1849), Hassall's corpuscles are commonly found in the thymic medulla in different animals. They were described in lungfish (Mohammad et al. 2007), in teleosts (Bowden et al. 2005) and in mammals (Furukawa et al. 2012).

Among acipenseriform fish, they were reported only in American paddlefish *Polyodon spathula* (Walbaum 1792) (Petrie-Hanson and Peterman 2005). Histological examination of the lymphoid tissue was performed on the specimens at the age of 18 months post-hatch. Whorls of epithelial cells and early Hassall's corpuscles were noted within *Polyodon* thymi (Petrie-Hanson and Peterman 2005).

Sturgeon lymphoid organs were described by Fange (1986). His study included 20 specimens of the Pacific white sturgeon (*Acipenser transmontanus*), with body weights ranging from 60.8 to 33 200 g. Some of the specimens were caught in the wild. Hatchery-reared young hybrid sturgeons (*Huso huso* × *Acipenser ruthenus*), were also included in the study. Additionally, thymi were found and examined in three specimens of *Acipenser transmontanus* (body weights varying between 179 and 33 200 g, ages varying between half a year and two and a half years, one specimen was caught in the wild and was of unknown age) and Hassall's corpuscles were not found. Thymic atrophy related to age was noted in one specimen.

Our observation in juvenile *Acipenser ruthenus* and *Acipenser brevirostrum* seems to be the first description of Hassall's corpuscles in sturgeons. Juvenile and premature stages of Hassall's corpuscles were found. We propose to extend the histological and immunohistochemical examinations of these thymi.

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