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# Spontaneous multicentric myxoma of the dermal nerve sheaths in farmed European eels *Anguilla anguilla*

E. Gjurčević<sup>1</sup>, S. Kužir<sup>2</sup>, A. Sfacteria<sup>3</sup>, K. Drašner<sup>4</sup>, F. Marino<sup>3,\*</sup>

<sup>1</sup>Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia

<sup>2</sup>Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia

<sup>3</sup>Department of Veterinary Sciences, Centro di Ittiopatologia Sperimentale della Sicilia, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy

<sup>4</sup>Fish Farm Poljana, 43283 Kaniška Iva, Croatia

**ABSTRACT:** This report describes a peripheral nerve sheath tumour in 8 European eels *Anguilla anguilla* L. from a fish farm located in Croatia. The newborn tissue appeared as smooth and soft skin nodules without pronounced colour change. Nodules were dome-shaped with a pale crater and were present on different body areas. In general, nodules were located as series of differently sized protrusions extending along the lateral line on both sides of the fish, as well as sensory canals on the head. Cut sections showed a homogeneous, pale white–grey texture. Histologically, the pathological tissue was located in the dermis, occasionally intruding into the hypodermis, and pushing as a space-occupying mass against the underlying muscle tissue without any evident boundaries. The pressure also caused changes in the overlying epidermis, such as atrophy, spongiosis and erosion. In some areas, the epidermis was 1 cell thick and club and goblet cells had completely disappeared. Ultimately, these changes resulted in shallow ulceration. Tumour tissue was characterized by a scant population of spindle or stellate cells, with oval, hyperchromatic nuclei and pale cytoplasm embedded in a copious myxoid matrix. Cells were arranged in fascicles and whorls, extending in a poorly defined manner among the dermal collagen bundles. Occasionally, adipose cells were also detected, mainly in the central portion of the bulges. Myxoid areas appeared rich in metachromatic and alcianophilic mucous ground substance. Reticular fibres and collagenous connective tissue were scarce. Immunohistochemistry (IHC) using antibodies against S-100 and glial fibrillary acidic protein caused a slight positive reaction in neoplastic dendritic cells. High magnification showed the immunostaining to be cytoplasmic in all tumour cells. IHC with anti-calretinin antibody gave only negative results. Macroscopic, histological, histochemical and immunohistochemical findings were consistent with a diagnosis of multicentric myxoma of the dermal nerve sheaths, a tumour not yet reported in fish.

**KEY WORDS:** Myxoma · *Anguilla anguilla* · Peripheral nerve sheath tumour · PNST

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## INTRODUCTION

Peripheral nerve sheath tumours (PNSTs), which are neoplastic growths of the peripheral nerves, have been reported in many freshwater and marine fish species. These tumours affect both wild and farmed teleosts, and they can be differentiated into benign

peripheral nerve sheath tumours (BPNSTs) and malignant peripheral nerve sheath tumours (MPNSTs). Benign forms include schwannoma (neurilemmoma) and neurofibroma, which have been described in many teleost species (Picci 1933, Lucke 1942, Schlumberger 1952, 1957, McArn & Wellings 1967, Duncan & Harkin 1969, Wellings 1969, Mawdesley-Thomas 1972,

\*Corresponding author: fmarino@virgilio.it

Schmale et al. 1983, Masahito et al. 1985, Morrison et al. 1993, Marino et al. 2008, 2010, 2012).

BPNSTs also include myxoma of the dermal nerve sheaths. Myxomas are embryonic tumours of fibroblast origin that produce mucin (Smith et al. 1972, Goldschmidt & Hendrick 2002) regardless of the origin of the fibroblast. Although myxomas such as fibromyxoma and myxosarcoma have been well described in numerous animal species, they have rarely been described in fish (Plehn 1906, McIntosh 1908, Johnstone 1926, Schlumberger & Lucke 1948, Stotk 1958, Honma 1965, Dawe & Harshbarger 1975, Manera & Biavati 1995, Keller et al. 2011).

The present report describes a PNST in 8 farmed European eels *Anguilla anguilla*.

## MATERIALS AND METHODS

Eight juvenile European eels, 11 to 23 cm in length, were obtained over a 1 yr period from a commercial eel farm in Croatia. All eels collected for this report had multiple, unusual external nodules on different body areas; otherwise, collected eels appeared healthy and in good body condition. In the farm studied, no other fish were affected and no increased mortalities during the survey were observed. The source farm had a standing stock of approximately 700 000 fish, and farming was based on glass eels imported from France. The farm used a recirculation culture system supplied by borehole fresh water. Water temperature in the system was between 22.5 and 23.2°C, and pH was between 5.9 and 6.3.

The collected fish were killed in a separate tank by an overdose of MS 222 or by a blow to the head followed by severing of the spinal cord. Necropsy was performed under a stereoscope. Following necropsy, tissues from afflicted fish were fixed *in toto* in 10% formalin fixative for 24 h and then paraffin-embedded using routine procedures. Tissues were analyzed by histopathology, histochemistry and immunohistochemistry (IHC).

The paraffin blocks were cut into 6 µm thick serial, frontal, horizontal and sagittal sections, mounted on gelatine-coated microscope slides, deparaffinized, dehydrated and processed for routine haematoxylin and eosin, periodic acid-Schiff (PAS), alcian blue-PAS (pH 2.5), Orcein, Van Gieson and Masson's trichrome stainings; slides were also processed for indirect peroxidase IHC. Frozen sections stained with Sudan III were used to visualize fat. For IHC, sections were rinsed in Tris-HCl buffer (0.05 M, pH 7.5) containing 0.1% bovine serum albumin and 0.2% Tri-

ton-X100. Endogenous peroxidase activity and non-specific bindings were blocked, using 3% H<sub>2</sub>O<sub>2</sub> and 2.5% serum albumin respectively. Sections were incubated overnight with rabbit anti-S-100 polyclonal antibody (1:200; Dako); rabbit polyclonal antibody anti-glial fibrillary acidic protein (GFAP), a marker of astrocytes (1:200, code GFAPR40; GeneTex); and anti-calretinin (prediluted; Chemicon). Sections were then washed in the same buffer and incubated for 1 h at room temperature with biotiny-labelled goat anti-rabbit IgG (1:200; BIOSPA). IHC specificity was tested in separate trials by substituting buffer for each primary antibody or for the secondary antibody, and by using specifically preabsorbed antibodies.

## RESULTS AND DISCUSSION

At gross examination, the newborn tissue of European eels (0.8 to 3.2 × 0.2 to 2.3 mm) appeared as smooth and soft skin nodules without pronounced colour change. Nodules were dome-shaped with a pale crater and were present on different body areas. In general, nodules were observed as series of differently sized protrusions extending along the lateral line on both sides of the fish, as well as sensory canals on the head (Fig. 1). Cut sections showed a homogeneous, pale white-grey texture.

Histologically, the pathological tissue was located in the dermis, occasionally intruding into the hypodermis, and pushing as a space-occupying mass against the underlying muscle tissue without any evident boundaries. The pressure also caused changes in the overlying epidermis, such as atrophy, spongiosis and erosion. In some areas, the epidermis was 1 cell thick and club and goblet cells had completely disappeared (Fig. 2). Ultimately, these changes resulted in shallow ulceration.

Tumour tissue was characterized by a scant population of spindle or stellate cells, with oval, hyperchromatic nuclei and pale cytoplasm embedded in a

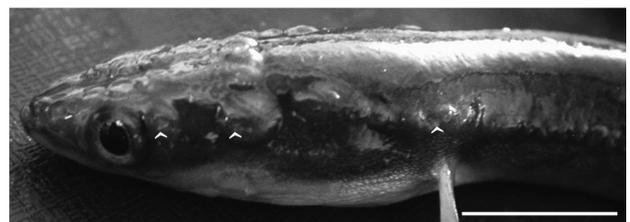


Fig. 1. European eel *Anguilla anguilla* with dome-shaped tumorous nodules along the lateral line and sensory canals on the head. Note the pale craters (arrowheads) on the nodules.

Scale bar = 1 cm

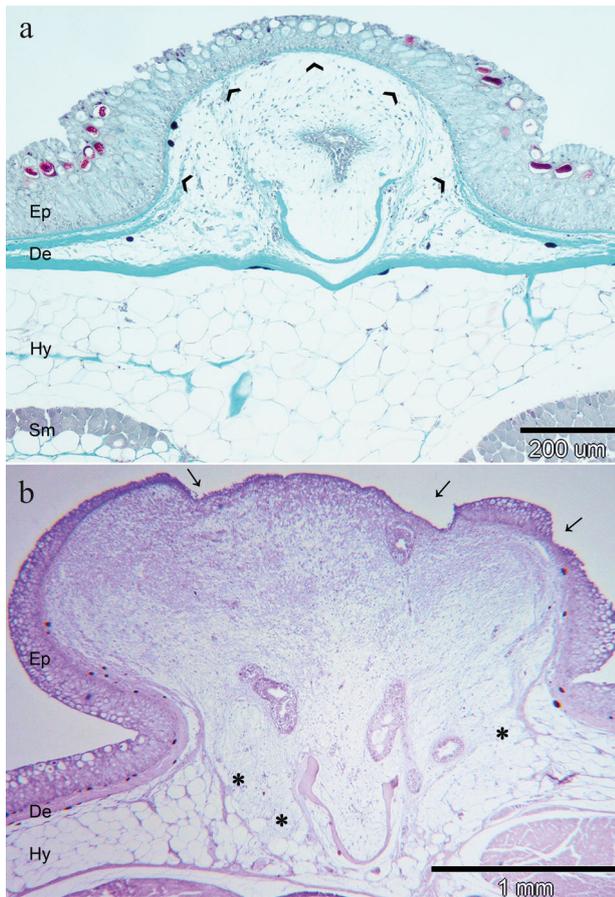


Fig. 2. (a) Histological section of an early myxoma lesion in European eel *Anguilla anguilla* showing cores of pathological tissue extending towards the epidermal tissue (arrowheads). Absence of normal sensory canal architecture is evident (Masson's trichrome). (b) Advanced lesion showing that pathological tissue has penetrated the hypodermis (\*) and the epidermis has been eroded (haematoxylin and eosin). Note injuries to the epidermis (arrows). Ep: epidermis; De: dermis; Hy: hypodermis; Sm: skeletal muscle

copious myxoid matrix. Cells were arranged in fascicles and whorls extending in a poorly defined manner among the dermal collagen bundles. Occasionally, adipose cells were also detected, mainly in the central portion of the bulges. Myxoid areas appeared rich in metachromatic and alcianophilic mucous ground substance. Reticular fibres and collagenous connective tissue were scarce. The loose stroma contained voluminous stellate, reticular cells. These myxoid cells displayed an abundant, granular, PAS-positive cytoplasm and voluminous, pale nuclei round to oval in shape. No mitotic figures were seen.

Tumours showed a low degree of vascularization, and neither haemorrhage nor necrosis was observed. Rare nerve fibres were observed close to the tumours. All nodules showed similar features.

IHC using antibodies against S-100 and GFAP caused a slight positive reaction in neoplastic dendritic cells (Fig. 3). High magnification showed the immunostaining to be cytoplasmic in all tumour cells. IHC with anti-calretinin antibody gave only negative results.

## CONCLUSIONS

Macroscopic, histological, histochemical and immunohistochemical findings in European eels were consistent with a diagnosis of multicentric myxoma of the dermal nerve sheaths, a tumour never reported to date in fish. Schwannoma and neurofibroma are the most common PNSTs in higher as well as in lower vertebrates such as teleostean fish. Although schwannoma develop from Schwann cells and neurofibroma from fibroblast cells of peripheral nerves, distinguishing these 2 BPNSTs was difficult before the development of more sophisticated diagnostic techniques based on IHC (Marino et al. 2007, 2008, 2012) and ultrastructure (Duncan & Harkin 1969). Myxoma can be diagnosed only on the basis of tumour histopathology, because the loose tissue does not give clear and reliable IHC results. The histological findings of the cases in this study, such as the presence of stellate, reticular, PAS-positive cells lying in a metachromatic, alcianophilic mucoïd matrix, are consistent with myxoma (Sirtori 1954, Pulley & Stannard 1990, Manera & Biavati 1995). In fact, the tumours described here resemble those reported in the carangid fish, the yellowtail amberjack *Seriola lalandi* (Keller et al. 2011), as well as in domestic animals and humans.

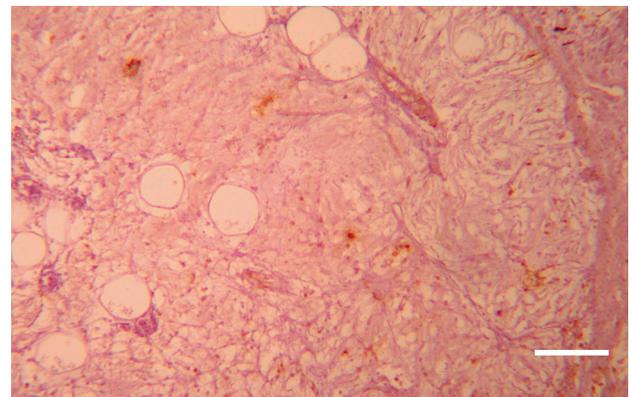


Fig. 3. Histological section of European eel *Anguilla anguilla* at high magnification showing the tumour cellularity characterized by loosely arranged stellate or spindle cells interspersed in a mixoid matrix. Only a few scattered cells show positive staining when tested with anti-gial fibrillary acidic protein (immunohistochemistry). Scale bar = 50 µm

The location, close to or around the lateral line, and the histological features of the nerve sheath neoplasm described here, such as loosely arranged cells enclosed in a myxomatous stroma sometimes resembling Antoni type B tissue, suggest that this tumour originated from the peripheral nerves related to the lateral line system. Such an origin is also supported by the fact that tumour cells stained positive for S-100 and GFAP, although the scarce cellularity of the tumour does not permit an easy detection of positive tumour cells.

Most studies seem to attribute PNSTs in fish to genetic causes, although toxins (Gardner & Yevich 1988) and viruses (Schmale et al. 2002) have also been suggested. Recently, a temperature-sensitive mutant fish line offering a vertebrate tp53-inducible system that can be regulated *in vivo*, by simply controlling the water temperature, has been developed (Berghmans et al. 2005). Thus, it is possible that changes in water temperature, especially during early developmental stages, may play a role in inducing PNSTs, which may have triggered tumour transformation in the fish in the present study, assuming that they were genetically predisposed.

#### LITERATURE CITED

- Berghmans S, Murphey RD, Wienholds E, Neuberg D and others (2005) tp53 mutant zebrafish develop malignant peripheral nerve sheath tumors. *Proc Natl Acad Sci USA* 102:407–412
- Dawe CJ, Harshbarger JC (1975) Neoplasm in feral fishes: their significance to cancer research. In: Ribelin WE, Migaki G (eds) *The pathology of fishes*. The University of Wisconsin Press, Madison, WI, p 871–894
- Duncan TE, Harkin JC (1969) Electron microscopic studies of goldfish tumours previously termed neurofibromas and schwannomas. *Am J Pathol* 55:191–202
- Gardner R, Yevich PP (1988) Comparative histopathological effects of chemically contaminated sediment on marine organisms. 4th Int Symp on responses of marine organisms to pollutants, April 1987, Woods Hole, MA. *Mar Environ Res* 24:311–316
- Goldschmidt MH, Hendrick MJ (2002) Tumors of the skin and soft tissues. In: Meuten DJ (ed) *Tumors in domestic animals*, 4th edn. Blackwell Publishing, Ames, IA, p 91–92
- Honma DJY (1965) A case of the myxoma developed in the head of the salmonoid fish, the ayu *Plecoglossus altivelis* Temminck et Schlegel. *Bull Jpn Soc Sci Fish* 31:192–197
- Johnstone J (1926) Malignant and other tumours in marine fishes. *Proceedings and Transactions of the Liverpool Biological Society* 40:75–98
- Keller M, Han S, Snekvik K (2011) Severe anisakiasis and cutaneous myxoma in a California yellowtail, *Seriola lalandi* Valenciennes. *J Fish Dis* 34:635–639
- Lucke B (1942) Tumours of the nerve sheaths in fish of the snapper family (Lutianidae). *Arch Pathol (Chic)* 34:133–150
- Manera M, Biavati S (1995) A fibromyxoma in a tench, *Tinca tinca* L. *J Fish Dis* 18:627–630
- Marino F, Macrì D, Lanteri G, Manganaro M, Monaco S, Germana A (2010) Neurofibroma in a mullet (*Mugil cephalus*). Histochemical and immunohistochemical study. *J Aquat Anim Health* 22:92–94
- Marino F, Germanà A, Bambir S, Helgason S, De Vico G, Macrì B (2007) Calretinin and S-100 expression in goldfish schwannoma. *J Fish Dis* 30:251–253
- Marino F, Germanà A, Panebianco A (2008) A case of schwannoma in farmed sea bream *Sparus aurata*. *Dis Aquat Org* 82:249–252
- Marino F, Lanteri G, Rapisarda G, Perillo A, Macrì B (2012) Spontaneous schwannoma in zebrafish, *Danio rerio* (Hamilton). *J Fish Dis* 35:239–242
- Masahito P, Ishikawa T, Yanagisawa A, Sugano H, Ikeda K (1985) Neurogenic tumors in coho salmon (*Oncorhynchus kisutch*) reared in well water in Japan. *J Natl Cancer Inst* 75:779–790
- Mawdesley-Thomas LE (1972) *Diseases of fish*. Academic Press, New York, NY
- McArn G, Wellings SR (1967) Neurofibroma in a teleost fish *Lumpenus sagitta*. *J Fish Res Board Can* 24:2007–2009
- McIntosh WC (1908) On a tumour in a plaice. *Ann Mag Nat Hist* 1:373–387
- Morrison CM, Harshbarger JC, McGladdery SE (1993) Schwannoma in rainbow smelt. *J Aquat Anim Health* 5:317–323
- Picci L (1933) Di un non comune tumore di un pesce (Neurinoma). [On an uncommon tumour in a fish (Neurinoma)]. *Sperimentale* 86:128–130
- Plehn M (1906) Ueber Geschwülste bei Kaltblütern. *Z Krebsforsch* 4:525–564
- Pulley LT, Stannard AA (1990) Tumors of the skin and soft tissues. In: Moulton JE (eds) *Tumors in domestic animals*. University of California Press, Berkeley, CA, p 23–87
- Schlumberger HG (1952) Nerve sheath tumors in an isolated goldfish population. *Cancer Res* 12:890–899
- Schlumberger HG (1957) Tumors characteristic for certain animal species. A review. *Cancer Res* 17:823–832
- Schlumberger HG, Lucke B (1948) Tumours of fishes, amphibians, and reptiles. *Cancer Res* 11:651–712
- Schmale MC, Henseley G, Udey LR (1983) Neurofibromatosis, von Recklinghausen's diseases, multiple schwannomas, malignant schwannomas. Multiple schwannomas in the bicolor damselfish, *Pomacentrus partitus* (Pisces, Pomacentridae). *Am J Pathol* 112:238–241
- Schmale MC, Gibbs PDL, Campbell CE (2002) A virus-like agent associated with neurofibromatosis in damselfish. *Dis Aquat Org* 49:107–115
- Sirtori C (1954) Mixoma. In: *Enciclopedia medica italiana*. Sansoni Edizioni Scientifiche S.P.A, Florence, p 1276–1277
- Smith HA, Jones TCJ, Hunt RD (1972) Neoplasia. In: *Veterinary pathology*. Lea & Eebiger, Philadelphia, PA, p 190–298
- Stolk A (1958) Tumours of fishes. XX. Myxoma of the skin in characid *Phenacogrammus interruptus* (Boulenger). *Proc Koninklijke Nederlandse Akademie van Wetenschappen* 61(C):101–106
- Wellings SR (1969) Neoplasia and primitive vertebrate phylogeny: echinoderms, prevertebrates, and fishes—a review. *Natl Cancer Inst Monogr* 31:59–128