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ISSN 0792 - 156X

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PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -  
Kibbutz Ein Hamifratz, Mobile Post 25210,  
ISRAEL

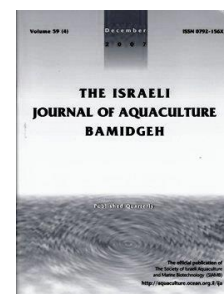
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## Erythrophoromas in Colored Crucian Carp, *Carassius auratus*

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**Key words:** chromatophoromas, colored crucian carp, erythrophores, erythrophoromas, neoplasm

### Abstract

Chromatophoromas, cutaneous pigment cell tumors that originate from skin dermochromatophores of fish, amphibians, and reptiles are rarely reported. The four basic pigment cell types found in poikilothermic vertebrates are melanomas (melanophoromas), iridophoromas, xanthophoroma, and erythrophoromas. In the present study, we diagnosed spontaneous lesions in the skin of a group of ornamental colored crucian carp, *Carassius auratus*, from an artificial ornamental fish pond located in Southeastern China. Necropsy, paraffin section, and hematoxylin-eosin staining methods were used to evaluate the neoplasm. Histological changes were observed and photographed using a Nikon microscope image system. The results showed oval nodular masses observed on the various parts of the body including flank, caudal peduncle, and tail. Histologically, neoplastic stromata were composed of bundles of compact parallel arrays cells. Dendric or spindle-shaped erythrophoroma cells were arranged as sheets or clusters with interlacing connective tissue. Congested capillaries and focal areas of hemorrhage were interspersed through the tissue. The tumor foci were infiltrated with neoplastic cells, inflammation cells, and necrotic tissue cells. Some olive to red intracytoplasmic pigment with polarized light could be seen on many parts of the erythrophoroma tissue. From the gross and histological pathology, we could conclude that the colored crucian carp suffered erythrophoromas. Further study is needed to confirm the etiology of this case.

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### Introduction

Neoplasms of pigment cells in fish, also called chromatophoromas, are normally present in the skin of fish, amphibians, and reptiles. They originate from dermochromatophores and are derived from neural crest. The four basic pigment cell types found in poikilothermic vertebrates are melanophores which contain black or brown pigments (melanin), iridophores which contain colorless pigments (purines), xanthophores which contain yellow pigments (carotenoids), and erythrophores containing red pigments (carotenoids and pteridine). Accordingly, neoplasms of pigment cells in fish are also of four phenotypes, melanomas (melanophoromas), iridophoromas, xanthophoroma and erythrophoromas (Murchelano et al., 1981; Okihiro, 1988). These chromatophoromas are among the most common types in bony fish and seem to be more common in fish than in mammals, including humans. Moreover, there are no mammalian neoplasms corresponding to erythrophores and iridophores, in fish (Masahito et al., 1989). To establish background information on histopathological lesions in ornamental colored crucian carp, *Carassius auratus*, the present report concerns a skin neoplastic lesion, diagnosed as an erythrophoromas, found in 11 ornamental colored crucian carp.

### Materials and Methods

A total of 11 live specimens were collected from an open artificial ornamental fish pond in southeastern China and brought to the laboratory for further investigation. Total numbers of neoplasm were counted on the body surface. Internal organs were examined and the morphology of the masses were photographed and recorded.

All the tissues were selected at the time of necropsy and the following tissue trimmings, neoplasm, gill, brain, eye, kidney, spleen, heart, liver, nares, intestine and body wall (approximately 1cm in diameter), were taken for histopathology and then placed into 10% Neutral Buffered Formalin (1:10 ratio of tissue to fixative). The bone and cartilage of the cranium, eye, body wall, and gills, were decalcified by immersion for 24 hours in a commercially available decalcification fluid. After decalcification, these tissues were trimmed into cassettes, dehydrated in graded ethanol solutions, cleared in xylene, and embedded in paraffin wax. Tissues were cut to 5  $\mu$ m and stained with hematoxylin and eosin (HE). The stained tissues were examined under a microscope, and digital images of histological features were obtained using Nikon microscope image system.

### Results

The infected fish were adult crucian carp and the morbidity of the entire group was approximately 5%. Oval nodular masses were observed on the various parts of the body including flank, caudal peduncle, and tail (Fig. 1). Neoplasms ranged from 0.5-3.9 cm in maximum diameter. They were all orange in color and found only in the red parts of the body protruding outwards, lifting up the epidermis. Ulcerative and congestive surface could be seen on some big neoplasms (Fig. 2).

Histologically, the superficial outer edge of the neoplasm was an epidermis layer. At the edge near normal body skin, the histological structure of the epidermis seemed normal. However, most of the area of the epidermis covering the neoplasm was somewhat thickened and vacuolar and infiltrated by inflamed cells and tumor cells (Fig. 3). Neoplastic stroma were composed of bundles of compact parallel array cells. Dendric or spindle-shaped erythrophoroma cells were arranged as sheets or clusters with interlacing connective tissue. In some part of those compact cells, other cell types formed irregular loose whorls (Fig. 4). Congested capillaries and focal areas of hemorrhages were interspersed through the tissue (Fig. 5). The tumor foci infiltrated neoplastic cells, inflamed cells and necrotic tissue cells. However, in conventional hematoxylin and eosin section, details of the pigment granules were not clear, but some olive to red intracytoplasmic pigment could be seen with polarized light on many parts of the erythrophoroma tissue (Fig. 6). Tumor cell nests were located in those loosened whorls. Multinucleate giant cells, multi-nucleolus cells, and nuclear pleomorphism, however, were frequently seen (Fig. 7). In the internal organs of colored crucian carp with erythrophoroma metastases were not observed.

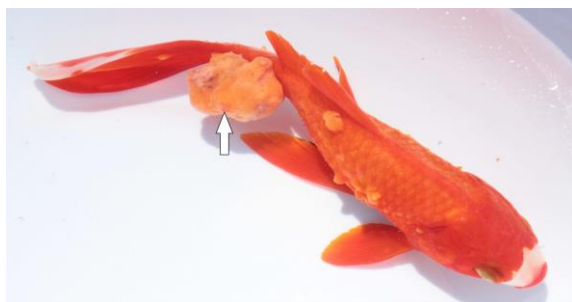


Fig. 1 Oval nodular masses were observed on the various parts of the body including flank, caudal peduncle and tail. Arrow indicates nodular mass.

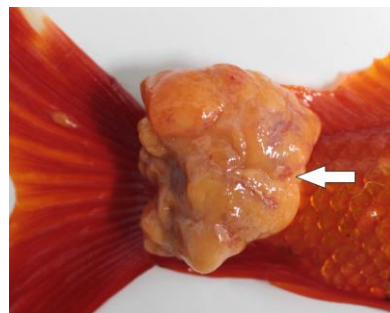


Fig. 2 Neoplasm was oval and the surface was ulcerative and congestive. Arrow indicates amplified nodular mass.

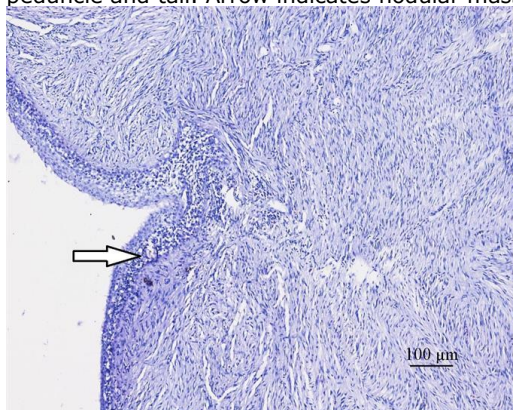


Fig. 3 The covering epidermis of the neoplasm was thickened and vacuolar. HE×100. Arrow indicates covering epidermis.

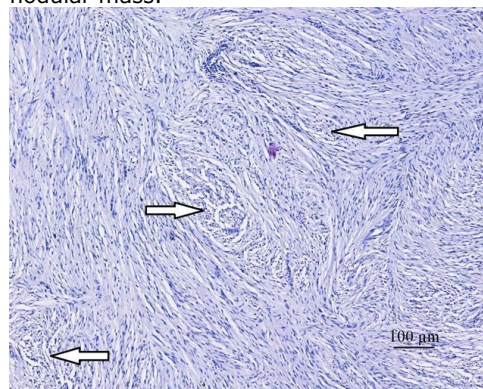


Fig. 4 Neoplastic stroma was composed of bundles of compact parallel arrays of cells. Some cells formed irregular loosened whorls in the neoplastic stroma. HE×100. Arrow indicates irregular whorls.

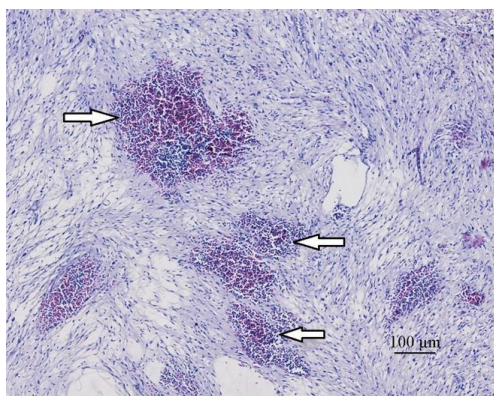


Fig. 5 Congested capillaries and focal areas of hemorrhage were interspersed through the tissue. HE×100. Arrow indicates focal hemorrhage.

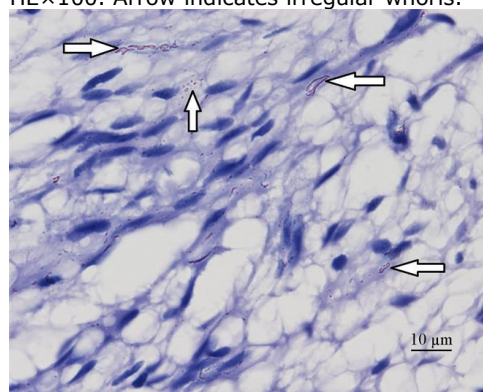


Fig. 6 some olive to red intracytoplasmic pigment with polarized light could be seen on many parts of the erythrophoroma tissue. HE×1000. Arrow indicates intracytoplasmic pigments.

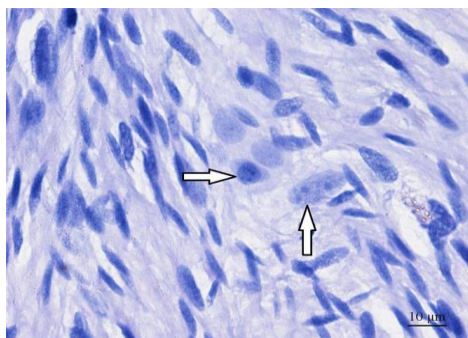


Fig. 7 Varying-sized cells found in tumorous tissue. Enlarged neoplastic cells with three to four nucleoli were frequent. HE×1000. Arrow indicates multiple nucleolus.



### Discussion

Although pigment analyses were not conducted, the morphologic characteristics of the tumorous mass and tumor cells and the olive to red intracytoplasmic pigment with polarized light suggested the diagnosis of erythrophoroma. The neoplastic stroma composed of bundles of compact parallel arrays cells was improperly diagnosed as fibroma in which the cells are also spindle-shaped with long narrow nuclei and long cytoplasmic processes. However, this case was diagnosed as erythrophoroma according to the evidence of tumor cell nests and red intracytoplasmic pigment. From some reports mitoses are rarely seen in fibroma (Kreyberg 1937; Grizzle 1983; Constantino et al., 1999). However, they were easily seen in the present case.

Generally speaking, the etiology of neoplasm in fish is obscure. Certain viruses, chemicals, ultraviolet radiation, heredity, and environmental pollution are believed to be the main carcinogenic factors of fish. Retroviruses cause epizootic cancer in fish, including walleye dermal sarcoma (caused by walleye dermal sarcoma virus, WDSV), northern pike lymphosarcoma (caused by pike lymphosarcoma retrovirus) and Atlantic salmon swimbladder fibrosarcoma (Ferguson 2006). Furthermore, some neoplasms of fish are associated with heredity. Melanomas in platyfish/swordtail hybrids, for example, have strong genetic determination (Masahito et al., 1989). It is also widely believed that tumor outbreaks are associated with contaminants. Some man-made carcinogens, such as polycyclic hydrocarbons (PAH's) have been found to be carcinogenic (Reynaud et al., 2006). Furthermore, fish with PAHs such as B(a)P or sediment extracts containing carcinogenic PAHs have developed skin and liver neoplasms (Baumann, 1998). The correlation between environmental chemicals, such as N-nitroso-N-methylurea, and neoplasia of the pigmented cells has also been suggested in some species (Kazianis et al., 2001). The present specimen was collected from an open ornamental fish pond. Morbidity reached about 5% which is unusual in artificial food fish culture. The water source of the pond originated from a nearby river flowing from downtown. The culture time was long enough to allow for tumor formation and this could be the possible cause of the erythrophoroma. Further study of some chemicals and heavy metals to which the fish may have been exposed is needed to confirm the etiology of the neoplasm.

### Acknowledgments

We would like to thank David Groman and Melanie Buote from the University of Prince Edward Island for their contribution in the diagnosis of this case.

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