

Research article

## Infiltrative tubular carcinoma in a common clownfish, *Amphiprion ocellaris*

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### Abstract

Neoplasia affecting a variety of tissues has been documented in many fish species. However, there are few reports of neoplasia in the common clownfish, *Amphiprion ocellaris*. An adult female clownfish in a public display aquarium was diagnosed with a locally invasive tubular carcinoma. The tumour was characterised by well-differentiated tubules supported by moderate amounts of pale eosinophilic stroma that infiltrated the skin, skeletal muscle and fascial planes of the vertebrae. This is the first report of a tubular carcinoma in this species.

### Introduction

There have been many documented cases of neoplasms in fish species (Noga 2010). Of these neoplasms, 7% have been gastrointestinal in origin while 12% have been excretory in nature (Harshbarger and Slatick 2001). However, there are few reports of neoplasia in the common clownfish, *Amphiprion ocellaris*. There is a single case series of liposarcoma that affected internal organs, an eye and the central nervous system of several individuals (Sharon et al. 2014). Although tubular carcinomas have been reported in several vertebrate species (Thurman et al. 1995; García et al. 2009), this is the first report of a locally invasive tubular carcinoma in any fish species.

### Methods

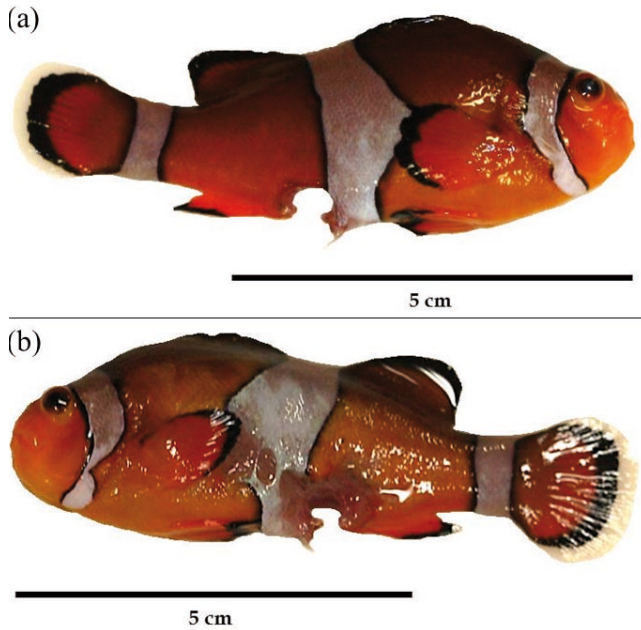
An approximately 6.5-year-old female common clownfish, *Amphiprion ocellaris*, developed abnormal coloration of the skin overlying a suspected mass in the left ventral coelom. This fish was housed in a 2,268 L exhibit that shared water and filtration with a 5,859 L exhibit. The total system volume was 9,261 L and contained other clownfish, anemones (including *Heteractis magnifica* and *Entacmaea quadricolor*) and live coral in direct and indirect contact with this individual. No other clownfish have developed similar lesions. The fish was removed and placed in a 140 L quarantine tank by itself. Over the course of several days, the abnormal coloration progressed

to scale loss and hyperaemia. The fish was treated with a 6-hour enrofloxacin (Professional Compounding Centers of America; London, Ontario) bath at 5 mg/L for five consecutive days.

The fish displayed normal behaviour and appetite. However, four days after the completion of the enrofloxacin baths, the lesion became more severe with marked deep dermal ulceration and exposure of underlying musculature. The decision was made to humanely euthanase the fish with 500 mg/L tricaine methanesulfonate (TMS, Syndel Laboratories Ltd.; Nanaimo, British Columbia) buffered 2:1 with sodium bicarbonate.

A ventral midline incision was made and the entire fish was fixed in 10% neutral buffered formalin. The body was decalcified in an 8% formic acid solution. The head was transected mid-sagittally, the trunk was transected transversely, and the viscera were serially incised and placed in cassettes. All tissues were processed through a graded series of alcohols, by automated processor (Tissue-Tek VIP6, Somagen Diagnostics Inc, Edmonton, Alberta) embedded in paraffin blocks and sectioned at 5 µm. Slides were stained with haematoxylin and eosin (H&E) by an automated stainer (Leica Autostainer XL, Leica, Concord, Ontario), then cover-slipped and reviewed microscopically.

To further define the histiogenesis of the tumour, recuts of the mass were mounted on 0.1% poly-d-lysine (Codon Slides, Fisher Scientific, Edmonton, Alberta) coated slides. Heat-induced epitope retrieval was accomplished in a Tris/EDTA pH 9 buffer and the slides were incubated with mouse anti-

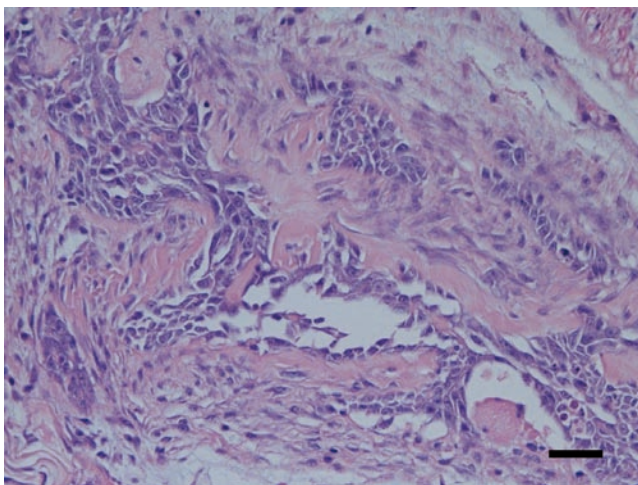


**Figure 1.** The right (a) and left (b) lateral side of the *Amphiprion ocellaris* with significant ulcerative skin lesion determined to be an infiltrative tubular carcinoma.

Cytokeratin Pan (clones AE1/AE3: 1:200 dilution) antibodies and detection was by an HRP-labelled polymer detection reagent (EnVision+ System – HRP Labelled Polymer, Dako Canada Inc., Mississauga, ON). Appropriate controls were incorporated in the staining process.

## Results

There was a deep perforating wound with a loss of 6.5 mm x 4.0 mm of the body wall on the ventral aspect of the fish, between the anal fin and the vent, where the musculature was absent. The left aspect of the lesion was more severe with deep dermal ulceration extending from the margin of the perforation and exposure of underlying muscle. There was deep dermal ulceration caudal



**Figure 2.** Hematoxylin and eosin stain of the tubular carcinoma identified in an *Amphiprion ocellaris*. Marker bar is 10  $\mu$ m.

to the left pectoral fin (Figure 1). The edges of the lesions were hyperaemic. The fish was otherwise in good body condition.

Histologic examination revealed that the ulcerative skin lesion noted grossly was a poorly demarcated carcinoma that infiltrated the skin, skeletal muscle and fascial planes of the vertebrae (Figure 2). The neoplasm mostly infiltrated the left side of the fish, from the ventral ulcer dorsally to midway between the lateral line and the dorsum. On that side of the fish, the tumour formed moderately well differentiated tubules supported by moderate amounts of pale eosinophilic stroma. Tumour cells infiltrated around, and sometimes within, skeletal myofibres. On the other side of the fish, there was a thin solid mass of tumour cells that infiltrated the hypodermis dorsally to the level of the lateral line. Some foci of tumour cells had three mitotic figures per high power field.

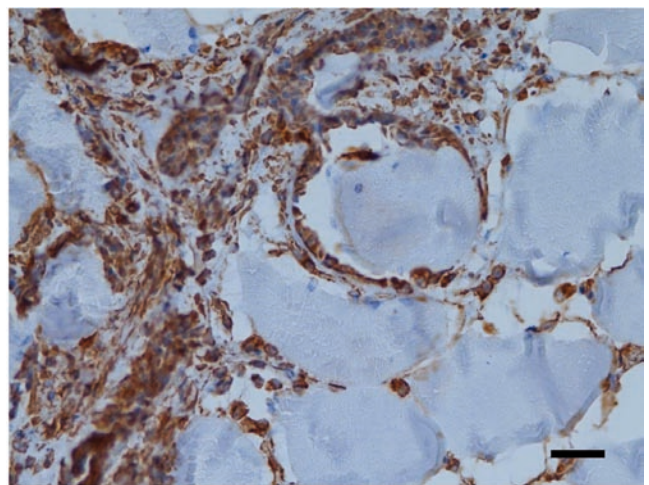
There was also an abundance of pigmented macrophage aggregates observed in this fish. Approximately 4% of splenic volume, 1% of head kidney, and less than 1% each for trunk kidney and liver were macrophage aggregates.

There were small amounts of hepatocellular glycogen and mesenteric adipose tissue. There was an abundant amount of ingesta within the gastrointestinal tract and the ovary was composed entirely of immature primary follicles. These patterns in measures of physiologic condition were consistent with a relatively healthy fish prior to euthanasia.

Immunohistochemistry of the mass disclosed prominent cytoplasmic staining of the neoplastic cells that tracked along and expanded epi and endomyrial planes (Figure 3). There was sparse cytoplasmic staining of intralésional macrophages and renal epithelia with no apparent chromagen in renal sinusoidal histiocytes.

## Discussion

The origin of the locally invasive tubular carcinoma in this individual was probably renal, although the primary differential of intestinal, cutaneous or some other origin cannot completely be ruled out. In German shepherd dogs, renal carcinomas can be associated with large ulcerative skin lesions (Moore and Ogilvie 2006). The extent of infiltration, significant skin ulceration, overall normal appearance of the gastrointestinal tract and IHC cyokeratin staining were most consistent with a renal tubular carcinoma. Epithelioid macrophages associated with granulomatous inflammation in fish have acquired hemidesmosomes and tonofilaments, as well as cyokeratin staining by biotin-streptavidin immunoperoxidase



**Figure 3.** Immunohistochemistry for cytokeratin revealed strong cytoplasmic staining between myofibers in the *Amphiprion ocellaris* with a tubular carcinoma. Marker bar is 10  $\mu$ m.

(Noga et al. 1989). In this fish, the histologic features of the mass are distinct from chronic inflammation.

It has been hypothesised that environmental factors may contribute to a variety of neoplasms in fish, but no significant correlation has been identified (Mix 1985). This fish was housed in a community tank where no other individuals were affected, suggesting that environmental factors may not be the primary factor involved.

Pigmented macrophage aggregates are normal structures commonly found within hematopoietic tissues such as spleen, kidney and liver (Agius and Roberts 2003). The volume of these structures has been reported to increase with age, inflammation, toxin exposure and environmental stress (Agius and Roberts 2003). The increased amount of macrophage aggregates within the tissues of this individual may represent evidence of chronic stimulation, secondary to the invasive tumour.

In conclusion, this previously unreported neoplasm should be considered in the differential diagnosis for ulcerative skin lesions that are non-responsive to antimicrobial therapy in otherwise healthy clownfish.

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