Papers and Articles

Papilloma and squamous cell carcinoma in koi carp (*Cyprinus carpio*)

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Over a period of two years four ornamental koi carp (*Cyprinus carpio*) of one variety in a mixed population of 16 were affected with papillomas of the head and body. In one fish there was a transition of these tumours into a squamous cell carcinoma in the region of the head and posterior gill space, with deep invasion of the underlying bone. One of the fish recovered completely after the sloughing of the papillomas. In view of the progressive nature of the lesions, the condition was presumed to be due to an infectious agent, but transmission electron microscopy failed to reveal any virus particles.

PAPILLOMAS are benign neoplasms which originate from epithelial cells. In fish they have been reported in many species such as Atlantic salmon (*Salmo salar*) (Carlisle and Roberts 1977), smelt (*Osmerus eperlanus*) (Anders and Möller 1985), yellow bullheads (*Ictalurus natalis*) and brown bullheads (*Ictalurus nebulosus*) (Harshbarger 1972), black bullheads (*Ictalurus melas*) (Grizzle and others 1981), the European eel (*Anguilla anguilla*) (Delves-Broughton and others 1980) and ornamental carp (*Cyprinus carpio*) (Sano and others 1985a, Hedrick and others 1990).

The appearance of papillomas in fish can vary from flat plaques of epithelial hyperplasia to a raised cauliflower-shaped mass with typical papillary projections of epithelial cells and connective tissue. They may be single or multiple, and may affect many areas of the body surface and fins. They may be colourless or pink, or pigmented if melanocytes are present.

Histologically they are associated with an overgrowth of Malpighian cells with few mucous cells. Lymphocytes are present in large numbers when cell-mediated rejection and sloughing of the tumours occurs (Carlisle and Roberts 1977).

Squamous cell carcinomas are malignant neoplasms of epithelial origin and have been reported less frequently in fish (Fournie and others 1987, Herman 1988, Hanjavanit and others 1990). They can develop from pre-existing papillomas (Roberts and Shepherd 1986) and consist of growths of epidermal cells which invade the dermis and subcutaneous tissues.

This paper describes four cases of papillomatosis and squamous cell carcinoma in a population of ornamental koi (*C carpio*).

Materials and methods

Sixteen koi were kept in a pond situated inside a commercial bank in the City of London. The pond was a circular stainless steel tank with a radius of 2·2 m and approximate water depth of 38 cm, with a single layer of small 10 cm diameter pebbles but no aquatic plants. Most of the light for the fish came from overhead fluorescent lamps, with only a small amount of reflected natural light. The approximate water capacity of the pond was 5500 litres and the water temperature was maintained at about 16 to 20°C. A small illuminated architectural fountain with 50 spray nozzles was the only feature which could be used to aerate the water, but it was rarely used owing to the distracting noise. The pond water was static and there was no mechanical, chemical or bacterial filtration system. A service company drained off 50 to 70 per cent of the water monthly and immediately replaced it with water direct from a mains water supply tap.

The composition of the water was tested periodically during 1989 and 1990 before the changes of water, using commercially available aquarists' test kits and following the recommended protocol. An average pH of 8·7 was measured and there were no significant levels of ammonia, nitrite or nitrate. There were no detectable levels of chlorine or chloramine in the tap water. The level of dissolved oxygen was between 2 and 3 mg/litre.

The pond was established in 1985 with the above management and the fish had grown in size, although there were no records of measurements. At the time of examination the koi ranged in size from 20 to 30 cm (measured from the snout to the base of the tail) and were of mainly mixed breeding, with cream being the predominant body colour. Eight of them were entirely cream-coloured koi, a variety commonly called a single-coloured metallic 'Ogon'. The others had various combinations of red, black and white colouring. All the fish had been purchased at the same time, and no new fish had been introduced. The fish were assumed to be of both sexes but no breeding had occurred. The fish had been fed on various commercial floating pellet foods and had not had any previous health problems.

Case histories

The first case was a 20 cm cream koi which was seen in April 1988 floating on its side for two days, but still alive. It was examined and found to have a massive tumour occupying the right gill space and raising the operculum. The fish was killed with an overdose of tricaine methane sulphonate (MS222; Sandoz). All the other fish appeared healthy at that time.

A second fish, a 25 cm cream koi, was examined in July 1988 from a distance because there were inadequate trapping facilities. It had extensive raised and roughened (cauliform) non-pigmented masses extending from the dorsal aspect of the head, down to the top lip and round anteroventrally to the right eye. The lesion was anterior to the edge of the operculum and did not invade the gill space. The fish was swimming and eating normally at the time, and no further action was taken. A third fish, a 35 cm cream koi, was examined at the same time and noticed to have darkened areas of epithelium on its head and body. Fourteen months later a localised, raised non-pigmented 1 cm cauliform mass was found on its snout.

In September 1989 both these fish (Figs 1 and 2) were netted, anaesthetised with tricaine methane sulphonate and examined in detail. The staff reported that some of the cauliform masses on the second fish had dropped off during the previous months; however, the masses were similar in size and distribution when compared with photographs taken on the previous visit. There was some progression of the lesion around the right eye but no obvious intraocular damage. Despite the extent of the

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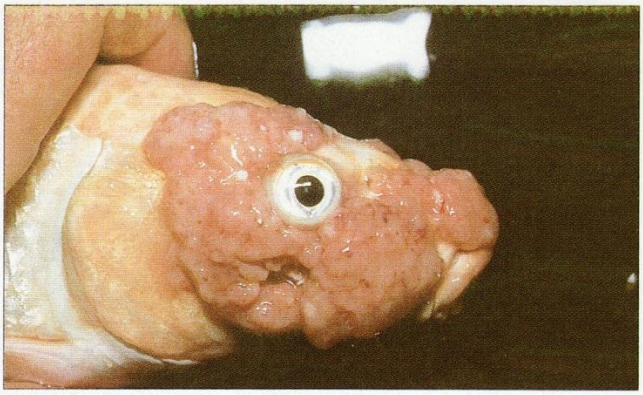

FIG 1: Case 2: lateral view of head with extensive neoplasia (September 1989)

FIG 2: Case 3: anterodorsal view of head with neoplasia on right side of snout (December 1989)

lesions, both fish were still active. There were darker discoloured areas of thickened epidermis on the surface of the fins and flank and ventral abdomen of both fish.

In January 1990, the masses on both fish were covered in a white necrotic layer of tissue. The second fish was anorexic and lifeless and was given 31 mg of sulphadoxine and 6 mg of trimethoprim (Borgal; Hoechst) into the intermuscular space anterior to the dorsal fin. The fish died two days later. At this stage it showed signs of being cannibalised and large areas of the cauliform lesion had been eaten by the other fish. The underlying bone was revealed with distinct areas of necrosis and loss of the structure of the skull where the papillomatous masses had developed.

A similar change developed on the third fish and the whole mass sloughed over a period of two months leaving an intact surface (Fig 3). This fish appeared to recover fully from the disease and was eating and swimming normally in October 1990.

A fourth case, a 25 cm cream koi, was also netted and examined in September 1989. A 2 cm diameter cauliform mass was found on the right side of the head between the eye and the mouth. Two smaller 5 mm diameter masses were found posterior to the first and lying on the operculum. A large tumour occupied about 75 per cent of the left gill space and lifted the operculum (Fig 4). The fish could still swim normally but had lost a lot of bodyweight and was killed.

External examination of the main cauliform mass indicated that it also penetrated into the roof of the mouth. There was extensive thickening of the epithelium over the anterior 25 per cent of the fish which produced a continuous surface, but posteriorly the thickening localised distinctly onto individual scales. This plaque formation was slightly darker than the cream body colour but still allowed identification of the underlying scales. Half of the dorsal aspect of the tail fin was damaged, with thickening of the epithelium.

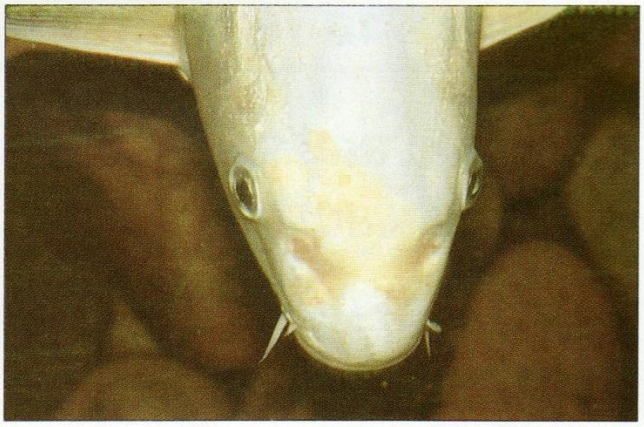

FIG 3: Case 3: anterodorsal view of head after slough of tumour (March 1990)

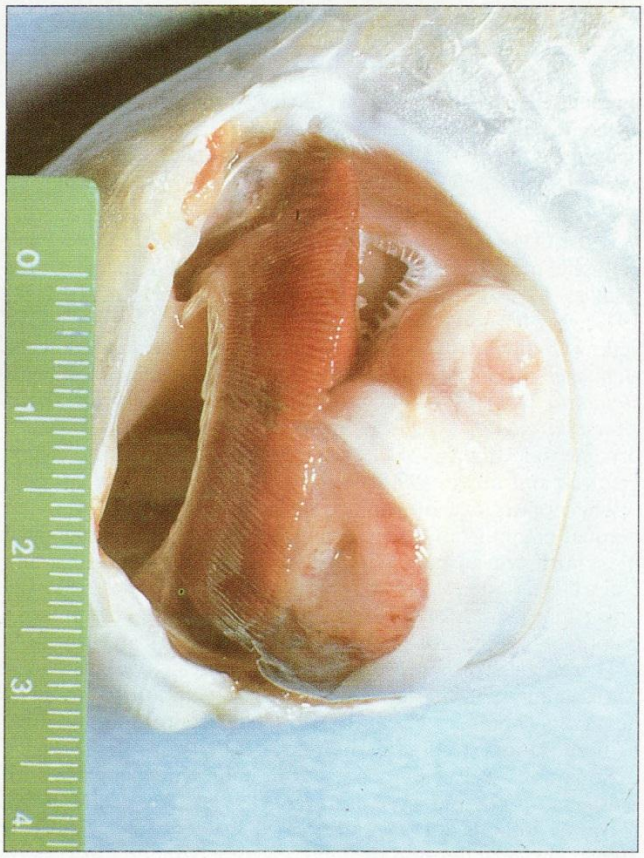

FIG 4: Case 4: left side of gill space with neoplasia (small scale in mm, September 1989)

Results

In the first fish a gross post mortem examination revealed that the neoplasm involved about 90 per cent of the posterior surface of the gill space and appeared to extend to the cervical spine and region of the pharyngeal teeth. There was no involvement of the gills or rakers and there were no external skin lesions.

In December 1989, fresh samples of the masses were taken from the second and third cases and fixed in 2 per cent buffered glutaraldehyde. They were sent to the Royal College of Surgeons of England for examination by transmission electron microscopy, but no viral particles or inclusions were identified.

A histological examination of the second fish revealed necrotic areas with large numbers of bacteria which were considered to be the cause of the lesions observed after the sloughing of the tumours. Wet smears of the body mucus revealed some *Gyrodactylus* species of skin flukes and the pond was treated with trichlorphon (Dipterex 80; Bayer). Subsequent investiga-

tion of skin scrapings from several fish failed to reveal any external trematodes.

In the fourth case the mass in the left gill space measured 35 × 20 mm and was ulcerated. It appeared to originate from deep within the branchial area of the body but was localised with no apparent effect on the gill structures. There was an excess of body mucus but no protozoa or monogenetic flukes were found on a direct wet smear. A snip preparation of some gill filaments revealed the presence of *Dactylogyrus* species of gill flukes. Internal examination revealed a large mass of tapeworms, *Bothriocephalus* species, occupying the proximal intestine.

The head was fixed in 10 per cent formol saline and sent for examination to Penrith Veterinary Investigation Centre, and later to Weymouth Fish Diseases Laboratory. Haematoxylin and eosin stained sections were prepared and sent to Mr John E. Cooper at the Royal College of Surgeons of England, London and Dr John C. Harshbarger at the Registry of Tumors in Lower Animals, Smithsonian Institution, Washington DC for further definitive opinions. (These slides have been deposited at the Registry as Accession RTLA 5053.)

Histopathological examination confirmed that the masses were neoplasms and initially they were considered to be epidermal papillomas. Further sections revealed the invasive nature of the neoplasm and it was considered to be a low grade 'squamous cell' carcinoma (Figs 5 to 9).

Histologically the tissue ranged from thick solid plaques to papillary masses consisting of arrays of epidermal pegs separated by fibrovascular papillae. Many of the pegs contained areas of squamous metaplasia characterised by flattened swirling epidermal cells, including some epidermal 'pearls' without a keratin centre. The pegs were sharply bordered by a basal layer except at a few sites where its integrity had been breached by amoeboid cells invading the connective tissue. Some pegs extended deeply with extensions into niches in the underlying bone. Several poorly formed denticles were present in the portion of the tumour near tooth-bearing surfaces. Mitotic figures were abundant. Inclusions suggestive of viral infection were not apparent. A diagnosis of squamous carcinoma of oral, branchial and tegumental origin was made by Dr Harshbarger.

A few small pieces of fresh tissue taken from the large cauliform mass before fixation, failed to reveal any virus by transmission electron microscopy.

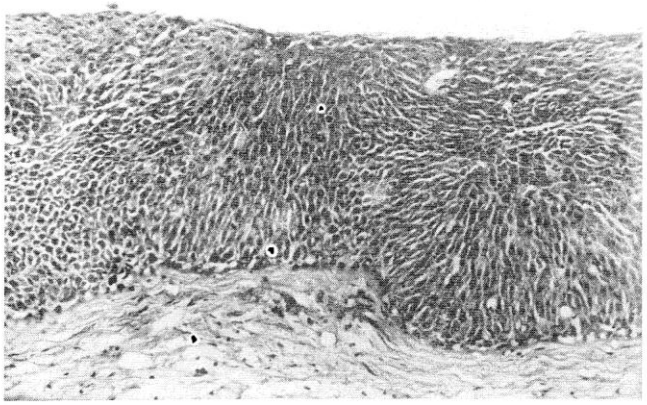

FIG 7: Case 4: area of tumour showing spindle cell pattern. Haematoxylin and eosin × 100

FIG 5: Case 4: low power view of histological section of neoplasm in Fig 4 showing transition between normal epithelium (arrow) and tumour mass which is invading deeply into the underlying tissue. Haematoxylin and eosin × 40

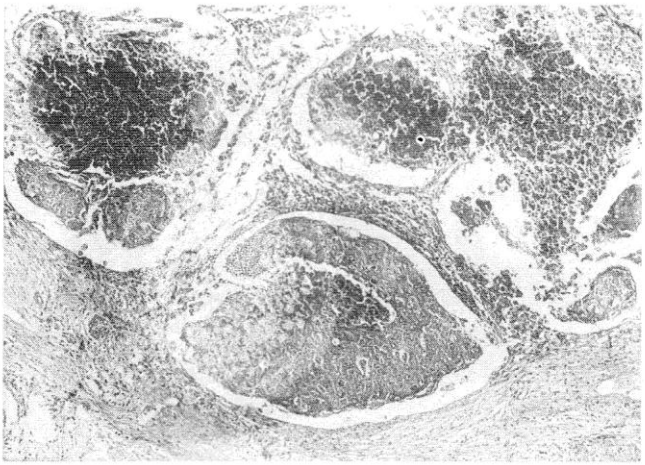

FIG 8: Case 4: area of tumour showing central necrosis separated by bands of reactive fibrous tissue. Haematoxylin and eosin × 100

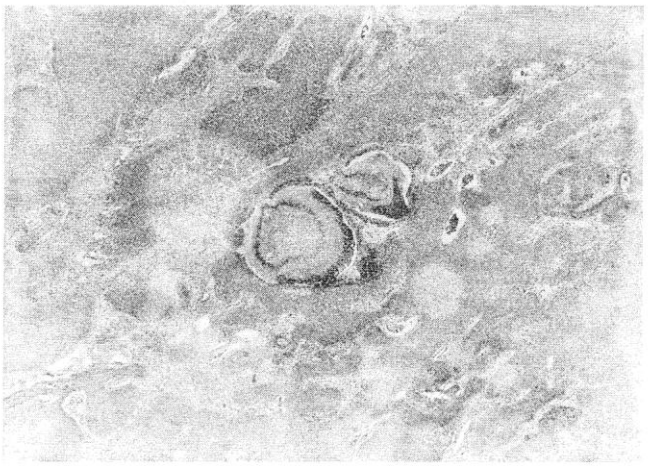

FIG 6: Case 4: low power view of tumour cell cords surrounding denticles. Haematoxylin and eosin × 40

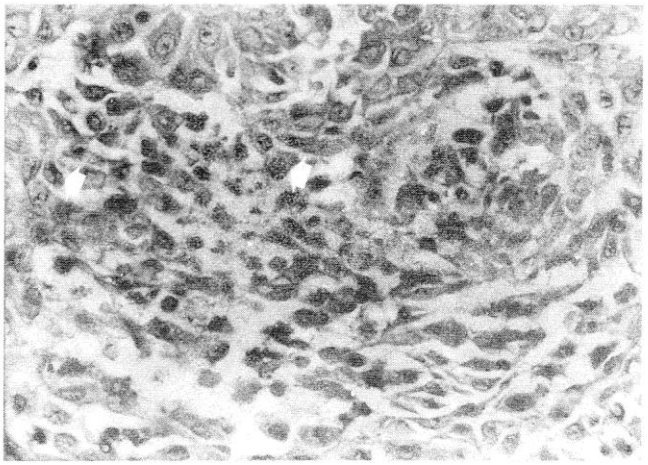

FIG 9: Case 4: high power view showing carcinoma cells, many undergoing necrosis. Evidence of mitotic activity is present (arrows). Haematoxylin and eosin × 400

Discussion

The prevalence of papillomas in specific fish populations may be very high. As many as 59 per cent of wild white suckers (*Catostomus commersoni*) have been reported in one survey (Smith and others 1989). A recent study of neoplasms in bony fish described several epizootics of epidermal papillomas in North America (Harshbarger and Clark 1990). Although there were only 16 koi in the pond four of the eight Ogon variety developed lesions. There is no obvious reason why this variety should be more susceptible because all the different varieties have been developed from the common carp (*C carpio*) but it may be that genetic influences are a factor. The classification of the varieties of koi is based purely on descriptive terms of scale formation, colour and pattern. The intensity of the pigmented colours may be enhanced by certain nutritional components and by the amount of natural lighting. It was not possible to determine whether the lack of natural light may have resulted in a weakening of the colours in these fish, or whether it had any other physiological effect which may have contributed to the higher prevalence in one variety.

The fish were estimated to be between five and seven years old, based on their size when purchased. Some surveys show that skin papillomas are more prevalent at certain ages in different species. In dab (*Limanda limanda*) in the eastern North Sea, papillomas start to develop between two and three years old (Mellergaard and Nielsen 1987) and in white suckers at five to six years old (Smith and others 1989).

Various aetiologies for epidermal papilloma have been proposed, but owing to the complex environment of the fish it is likely that a number of interrelated factors are responsible for the disease. In a few species of fish, the inoculation of healthy fish with cultures of affected tissues has indicated that specific viruses cause papillomas. In Japan, a herpesvirus was isolated from papilloma tissue from an ornamental asagi carp (*C carpio*) and designated *Herpesvirus cyprini* (Sano and others 1985a). This neoplasm was a milky-white, raised wart of 'carp pox', a well recognised viral disease with the histological features which now classify it as 'papillosum cyprini'. The prevalence of this specific disease may reach 30 per cent in experimentally inoculated fish (Sano and others 1985b) but none of these typical 'molten candle-wax' lesions was seen on any of the fish in this series. This virus was recently found by electron microscopy in koi carp with epidermal hyperplasia in northern California (Hedrick and others 1990). It was suggested (J. C. Harshbarger, personal communication) that an infectious agent was most likely for this type of lesion because there is evidence that epidermal neoplasms that initially appear as a plaque-like hyperplasia, have a viral aetiology (Sonstegard and Sonstegard 1978, Bloch and others 1986). The failure to reveal virus by electron microscopy does not exclude such an aetiology, bearing in mind that only single samples from three fish were examined. Electron microscopy has only rarely revealed the presence of virus or virus-like particles in spontaneous tumours, such as those in adult smelt (Anders and Möller 1985). Virus may also have been present at an early stage and have disappeared.

In previous reports (Wellings 1969) the presence of monogenetic trematodes of the *Gyrodactylus* species was noted. It was not possible to determine whether these viviparous parasites could carry oncogenic viruses or whether they could initiate an effect by direct irritation of the skin. This skin fluke was seen on the carcase of the third case and the confined pond environment would aid the spread of the parasite by direct contact.

Environmental factors have been implicated in the development of papillomas because of the intimate contact fish have with their surroundings. In one survey, 73 per cent of a population of black bullhead fish exposed to chlorinated waste water effluent developed oral papillomas (Grizzle and others 1984). The prevalence decreased to 23 per cent when the residual chlorine in the effluent was decreased from a maximum level of 3·1 mg/litre to 1·2 mg/litre. Carcinogenic chemicals were not identified in the waste water but chlorination was implicated as a contributing factor. There were no known chemicals used near the pond in this case, and the Thames Water Authority stated that the maximum level of chlorine in the water supply leaving the reservoir was 0·35 mg/litre and that the level was usually less than 0·2 mg/litre by the time it reached the consumer.

In the common dab seasonal variations related to oxygen depletion led to an increase of some diseases, including epidermal papilloma (Dethlefsen 1987, Mellergaard and Nielsen 1987). The solubility of oxygen in water decreases with an increase in temperature. The lowest oxygen concentration required for normal life is different for each species and depends on a number of physical factors such as body size, age, activity level and general condition. In farmed carp, the minimum desirable oxygen level for growth is 6 mg of dissolved oxygen/litre (Michaels 1988). Although fish may adapt to lower levels of oxygen, such low levels can adversely affect growth, reproduction and normal function and make the fish more susceptible to disease. The relatively high water temperature of 16 to 20°C and the low oxygen concentrations of 2 to 3 mg/litre may have been contributing factors to physiological stress and the development of these neoplasms in the koi.

Mechanical stimuli may cause papillomatous tumours of the lower jaw to develop in many species of fish after long periods of captivity (Peters and Watermann 1979). In these cases, the tumours have been found only on the ventral surfaces of the body, and on the operculum and jaw which had mechanical contact with the walls of their containers. However, it is doubtful whether trauma produces true neoplasms or merely stimulates reparative hyperplasia of the epidermis with seemingly invasive tongues of epithelial cells (Rosai 1981). Although the koi in the present series were fed floating food, their naturally inquisitive and scavenging nature may have exposed them to some trauma from the surfaces of the pond.

In Atlantic salmon the host's response to an epidermal papilloma is a massive infiltration with lymphocytes (Carlisle and Roberts 1977) followed by ulceration and rapid epithelialisation. This process occurs in all affected salmon in their first year, usually when they become smolts between July and December. There is no similar major physiological change in carp, and the two cases in this survey in which sloughing was observed occurred after they were sampled for electron microscopy. This physical trauma may have initiated the rejection of the tumours. Similarly, no significant ulceration was observed after sloughing, even where large areas were affected by hyperplasia or tumours. This behaviour is more typical of the desquamation of all the lesions of 'carp pox' which leaves a smooth and non-haemorrhagic surface (Sano and others 1985b). The regression and disappearance of epidermal papillomas affecting white suckers has also been reported by Smith and Zajdlik (1987).

In a review of the literature, Mawdesley-Thomas (1972) stated that reports of squamous cell carcinoma are less common than reports of papillomas. Notable features of these well differentiated tumours are slow growth, spread by direct invasion and the absence of metastases. They are found mainly on the lips and oral mucosa but may occur anywhere on the body (Roberts 1989) and in some cases develop from papillomas. Harshbarger (1972) described the histological features of papillomas in brown bullhead catfish, in which there is a transition at the bases of the papillae and invasion of the underlying dermis, but no metastases. Similar changes and invasion into the local dermis were identified in a small number of white suckers (Smith and others 1989) and in a gulf menhaden (*Brevoortia patronus* Goode) (Fournie and others 1987). In the present series only the tumours from the fourth case were examined histologically, but the similar gross appearance of the tumour in the first case and its deep invasion of the posterior gill space suggests that it was also a squamous cell carcinoma, although it did not have any visible papillomas.

Epizootics of squamous cell carcinomas have been recorded in a population of rainbow smelt (*Osmerus mordax*) (Herman 1988) with a highly invasive tumour affecting the head and mouth. Electron microscopy failed to reveal evidence of a virus and the cause was not determined. In rudd (*Scardinius erythrophthalmus*) a sample of fish were affected in a lake in an

area where there was a high mortality from human oesophageal carcinoma (Doyle and others 1987). Preliminary investigations suggested that nitrosamines due to environmental pollution were the likely carcinogenic agents.

Based on the histopathological findings, it is suspected that the tumours in this case were multicentric in origin rather than metastatic from a primary lesion because none of the lesions was highly invasive. Fish do not have well developed lymph nodes like those of mammals (Roberts 1989) and, probably because of the lower body temperature, cancers metastasise less readily (J. C. Harshbarger, personal communication).

It has not been possible to ascertain the true cause of these tumours, although a viral agent and, or, environmental factors appear to be most probable. The high mortality and small numbers of affected fish, coupled with their high individual value, precluded transmission studies or attempts to isolate a virus. However, it is hoped that the investigations may prove useful and relevant to keepers of ornamental fish.

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