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REVIEW

# Role of viruses in the induction of skin tumours and tumour-like proliferations of fish

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ABSTRACT: Skin tumours of fish are easily recognizable lesions, some of which have been known for centuries. Causes of fish tumour formation are varied and are often proposed to be multifactorial. In more than half of all cases examined by electron microscopy and virological methods, virus or virus-like particles were found in tumour tissues. So far, oncogenicity has been clearly demonstrated only for herpesviruses isolated from benign tumours. Classification of fish skin tumours, tumour-associated and tumour-inducing viruses, and the possible reasons for seasonal variation in skin tumour prevalence, are reviewed. It is postulated that fundamental principles determine the role of viruses in the induction of skin tumours of fish within a framework of different biological and environmental parameters.

KEY WORDS: Fish viruses · Skin tumours · Oncogenicity · Review

## INTRODUCTION

Due to their distinctive appearance and obvious pathological nature, tumours[1] of fish have been recognized by experts and laymen for centuries. Indeed, one of the first scientific documentations of a diseased fish concerned a *Chaetodon* species with tumour-like growths in the bones (Bell 1793). The author, a physician, wrote, 'The fishermen told me that the tumours were always found in the fish; I therefore conclude them to be natural to it'. Although no present day oncologist would confirm this statement, the nature and etiology of fish tumours are still poorly understood.

Published findings relating to fish tumours are widely scattered in the scientific literature. Comprehensive reviews on their classification have been given by Plehn (1910), Thomas (1931), Schlumberger & Lucké (1948), Mawdesley-Thomas (1975), Dawe & Harshbarger (1975), Budd & Roberts (1978) and Möller & Anders (1986). Harshbarger (1984) reviewed different types of pseudotumours occurring in ectothermic animals, including fish, and Anon. (1984), Couch & Harshbarger (1985) and Mix (1986) gave overviews of associations between cancerous diseases in aquatic animals and anthropogenic pollutants.

The largest registry of tumours in lower animals (RTLA) was established at the Smithsonian Institution in Washington, DC, USA, in 1965 (Harshbarger 1977, Harshbarger et al. 1981). To date, more than 50 basic types of tumours, ranging from benign epidermal papillomas to metastatic melanomas and hepatocellular carcinomas in more than 300 species of fish, have been registered. Among these, tumours of the skin comprise the largest group, accounting for

[1] In the text, except where stated otherwise, the term 'tumour' refers to both 'hyperplasia' and 'neoplasia' (the latter term being synonymous with 'tumour' in current medical literature) taking into account that hyperplasia and neoplasia are distinctly different processes (hyperplasia: increase in normal cell numbers that continues for only as long as the stimulus is present; neoplasia: increase in abnormal or autonomous, i.e. neoplastically transformed, cells which continues even after the stimulus disappears)

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Fig. 1. *Anguilla anguilla*. European eel from the Elbe estuary (Germany) with extensive papillomatous growths on head and jaws (Anders 1988)

roughly 50% of all cases registered (Harshbarger pers. comm.).

Depending upon season and geographical location, certain types of skin tumours may occur at high prevalences in wild fish such as European eel *Anguilla anguilla* (Fig. 1), dab *Limanda limanda* (Fig. 2a), European smelt *Osmerus eperlanus* (Fig. 3a, b), and northern pike *Esox lucius* from NE Atlantic coastal areas (Möller 1979, Dethlefsen 1990, Möller & Anders 1992). While the occurrence of 'carp-pox' lesions in cultured cyprinids has decreased in importance, new tumourous conditions which have appeared in cultured lake trout *Salvelinus namaycush* and Japanese flounder *Paralichthys olivaceus* (Fig. 4c) have contributed to high mortalities among larvae and juvenile specimens.

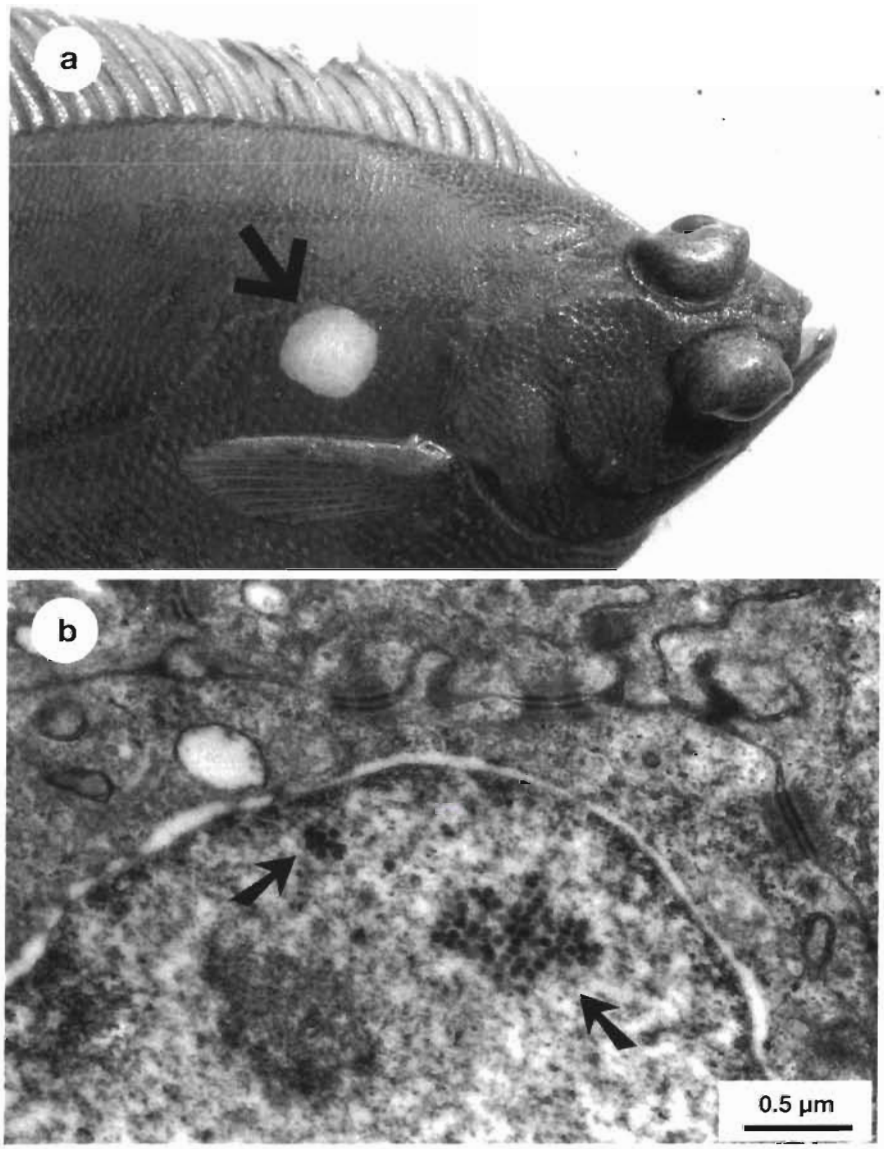



Fig. 2. *Limanda limanda*. Epidermal papillomas in dab from the German Wadden Sea. (a) A typical rounded flat epidermal papilloma on the body surface (Anders 1988); (b) aggregation of adenovirus-like particles (arrows) in the nucleus of a papilloma cell (Anders 1988)

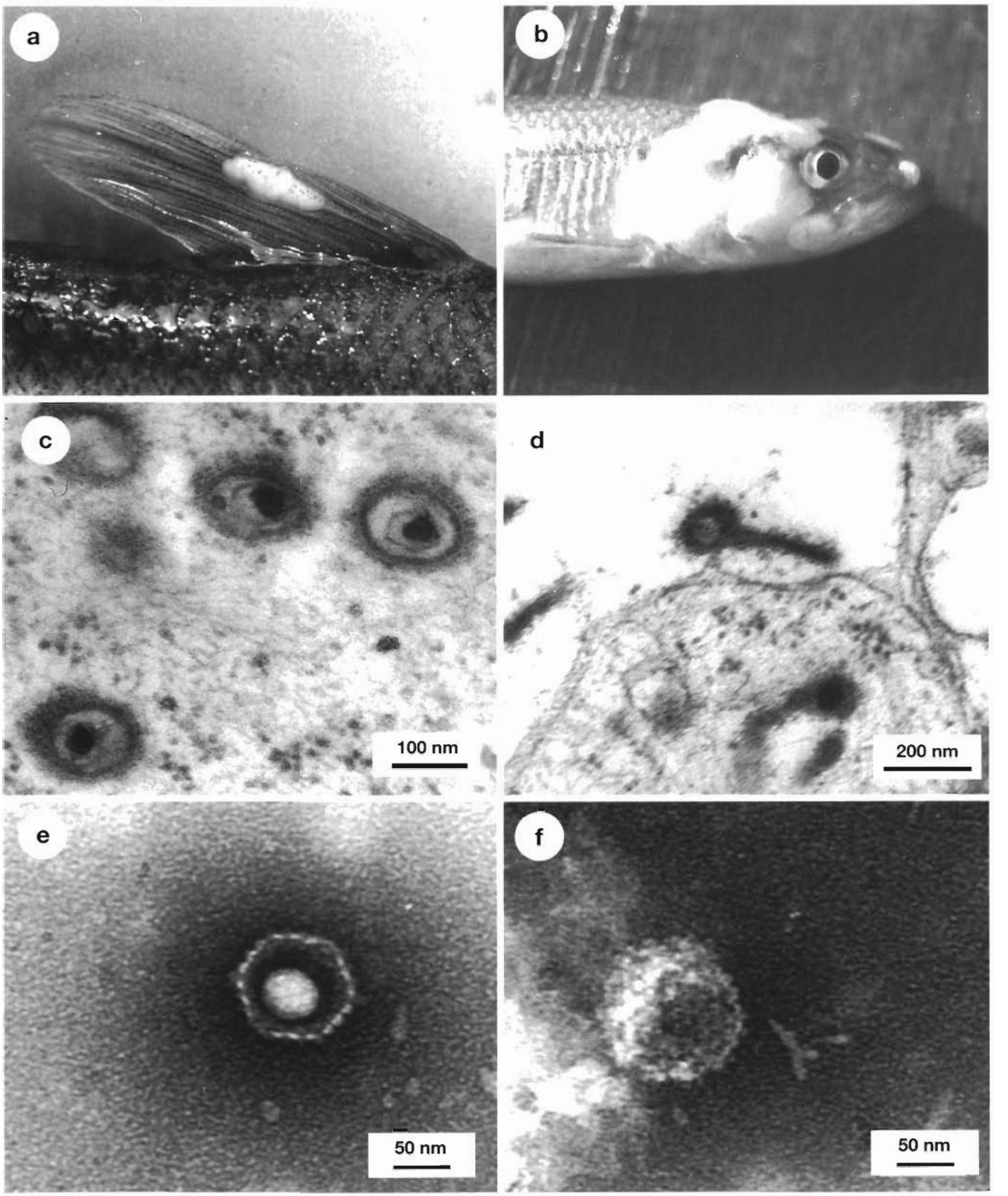



Fig. 3. *Osmerus eperlanus*. Epidermal papillomas in European smelt from the Elbe estuary, Germany. (a) Nodular and (b) flat epidermal hyperplasias/papillomas on fins and body surface; (c) herpesvirus particles in the cytoplasm of a tumour cell; (d) 'tailed' virus particle in cytoplasmic vacuoles of a tumour cell; (e) complete herpesvirus particles after negative staining; (f) non-enveloped particle. (From Anders 1988)

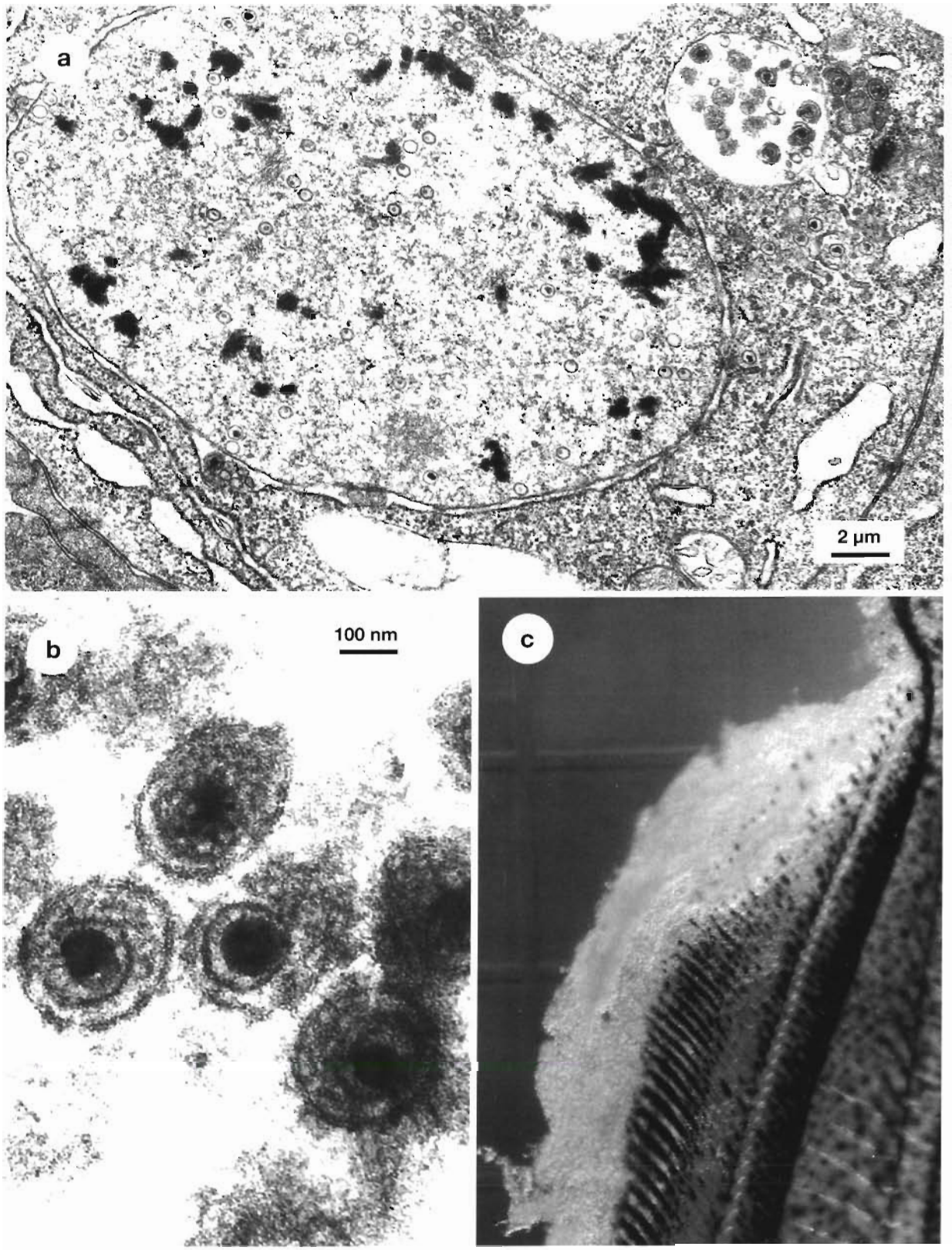



Fig 4 *Paralichthys olivaceus*. Juvenile Japanese flounder from a Japanese hatchery (a) Numerous herpesvirus particles in nucleus, cytoplasm and cytoplasmic vacuoles of a hyperplastic cell, (b) enveloped cytoplasmic virus particles; (c) typical hyperplasia on the fins (Photographs a and c courtesy of Dr T. Nakai, Japan)

The occurrence of tumours with a suspected viral etiology is well documented in a number of vertebrates including mammals, birds, reptiles (Jacobson 1981) and fish. For fish, a viral etiology of papillomas was first suggested by Keysselitz (1908). The present review summarizes and analyses current knowledge on the role of viruses in the pathogenesis of skin tumours of fish.

## CLASSIFICATION OF FISH SKIN TUMOURS

Skin tumours are classified on a histopathological basis according to the origin of the proliferative cells and their degree of malignancy (Budd & Roberts 1978). The lesions originate either from epithelial or mesenchymal cells and comprise benign as well as malignant forms, ranging from epidermal hyperplasias, epidermal papillomas and fibromas to carcinomas and sarcomas. Leukemias which may also affect the skin are not covered in this review.

Among the naturally occurring fish tumours, benign epidermal hyperplasias and papillomas are most frequently observed.

Amebic pseudotumours of several Pacific flatfish species and similar conditions of gills of dab and pseudobranchs of gadids were originally classified as epidermal papillomas until the unique 'X-cells' which were found to be frequently associated with the growths were identified as parasitic amebae (Dawe 1981, Shinkawa & Yamazaki 1987).

## CAUSES OF FORMATION OF FISH SKIN TUMOURS

Causes of fish tumour development are varied and often suspected to be multifactorial.

There is evidence that irritation of the skin due to constant mechanical damage or parasitic encystment may lead to the formation of tumourous growths, mostly epidermal hyperplasias (Nigrelli 1948a, Schlumberger 1953, McQueen et al. 1973, Peters & Watermann 1979). These and other non-viral etiologies such as influences of carcinogenic chemicals and UV radiation are not covered in this review.

Due to the frequent epidemic occurrence of fish tumours, an infectious viral etiology has been suggested by many scientists, even though they could not always demonstrate visible virus particles. In these cases, evidence was usually based on the exclusion of other potential causative factors. From a detailed study of the scientific literature, it is concluded that viruses play an important role in the induction of skin tumours of fish. In about 50% of all cases where electronmicroscopical and virological methods have been applied, viruses or virus-like particles were identified in tumour tissue. In benign tumours, such as epidermal hyperplasias, papillomas and fibromas, these were mostly herpesviruses, and less frequently adeno-, retro-, rhabdo-, birna- or picornaviruses (Table 1). In malignant forms like sarcomas and lymphosarcomas, there was evidence for the involvement of retroviruses only (Table 2). Typically, the tumours carried just one virus type. In rare cases, different skin tumour types associated with different viruses occurred in the same specimen (Yamamoto et al. 1985b).

The significance of these viruses and virus-like particles for tumour induction is mostly speculative. So far, only for herpesviruses isolated from benign tumours of masu salmon *Oncorhynchus masou* and Japanese Asagi carp *Cyprinus carpio* has oncogenicity been clearly demonstrated (Table 3). Although in certain other cases tumour formation could be induced experimentally by inoculation of cell-free filtrates and/or live tumour cells (Tables 3 & 4), attempts to isolate viruses in cell culture were unsuccessful.

Four categories of viruses or virus-like particles have been identified which are listed in descending order according to their degree of elucidation as causative agents. Each virus is listed only once.

### Viruses of proven oncogenicity

For 2 herpesviruses, pathogenicity and oncogenicity have been clearly verified by successful isolation of the causative virus in cell culture and fulfillment of River's postulates. So far, nothing is known about possible oncogenes of these viruses.

***Oncorhynchus masou* virus (OMV).** This agent is a salmonid herpesvirus first isolated from ovarian fluids of masu salmon in Hokkaido, Japan, in 1978 (Fig. 5b) (Kimura et al. 1981a). The virus has since been isolated frequently from mature masu salmon from Hokkaido hatcheries. OMV is pathogenic for the salmonid species masu salmon *Oncorhynchus masou*, chum salmon *O. keta*, kokanee salmon *O. nerka*, coho salmon *O. kisutch* and rainbow trout *O. mykiss*.

Affected fish become anorexic and may show exophthalmia or petechiation on the body surface. Cumulative mortality reached 80 to 100% in masu, chum and kokanee salmon (1 mo old), and 29 to 39% in coho salmon and rainbow trout (Kimura et al. 1983). Beginning at about 4 mo post-infection and persisting for at least 1 yr, 12 to 100% of surviving chum, coho and masu salmon and rainbow trout developed epithelial tumours around the mouth (Kimura et al. 1981b, Yoshimizu et al. 1987). The tumours were characterized as epidermal papillomas (Fig. 5a). Several layers of squamous epithelial cells in a papillomatous array

Table 1. Naturally occurring benign fish skin tumours associated with viruses or virus-like particles (VLP). P: epidermal papilloma; H: epidermal hyperplasia; M: melanoma; F: fibroma; 1 to 5: currently accepted tumour names (1 = 'cauliflower' tumour/stomatopapilloma, 2 = 'spawning papilloma(tosis)', 3 = walleye diffuse epidermal hyperplasia, 4 = walleye discrete epidermal hyperplasia, 5 = walleye dermal 'sarcoma'); T: transmission electron microscopy of tumour tissue; I: isolation from tumour tissue; E: successful experimental infection; R: reisolation; Pr: activation of virus by tumour promoter. *Evidence for reverse transcriptase activity; • proven oncogenicity; syn.: synonym

| Host species | Tumour type | Associated virus or VLP | Proof by | Source |
|---|---|---|---|---|
| *Anguilla anguilla* | P1 | Rhabdovirus | I | Ahne et al. (1987) |
| | H | Herpesvirus | T | Bekesi et al. (1986) |
| *Oncorhynchus masou* | P | Herpesvirus (OMV)• | I, E, R | Kimura et al. (1981a, b), Yoshimizu et al. (1987) |
| | | (YTV)• (syn.) | I, E, R | Sano et al. (1983) |
| | P | Rhabdovirus (IHNV) | I | Yoshimizu et al. (1989) |
| | P | Birnavirus (IPNV) | I | Yoshimizu et al. (1989) |
| *Salmo salar* | H, P | Retro-VLP | T | Carlisle (1977) |
| | P | Herpes-VLP | T | Shchelkunov et al. (1992) |
| *Salvelinus namaycush* | H(?) | Herpesvirus | T, E | McAllister & Herman (1989), Bradley et al. (1989) |
| *Osmerus eperlanus* | H2 | Herpesvirus | T | Anders & Möller (1985), Anders (1989), Lee & Whitfield (1992) |
| | H | Retro-VLP | T | Anders (1989) |
| | H | Picorna-VLP | I | Ahne et al. (1990) |
| *Esox lucius* | H | Herpesvirus | T | Yamamoto et al. (1984) |
| Europe | H | Retro-VLP | T | Winqvist et al. (1968) |
| North America | H | Retro-VLP | T | Yamamoto et al. (1984), Sonstegard (1976) |
| *Cyprinus carpio* | | | | |
| Asagi carp (Japan) | P | Herpesvirus (CHV)• | T, I, E, R | Sano et al. (1985a, b, 1990) |
| Common carp (Europe) | P | Herpesvirus | T | Schubert (1964, 1966) |
| *Leuciscus idus* | H, P | Herpesvirus | T | McAllister et al. (1985) |
| *Catostomus commersoni* | P | Retro-VLP* | T | Sonstegard (1973, 1977) |
| *Xiphophorus* hybrids | M | Papova-VLP | T, Pr | Kollinger et al. (1979) |
| *Ictalurus nebulosus* | P | VLP | T | Edwards & Samsonoff (1977) |
| *Silurus glanis* | H, P | Herpesvirus | T | Bekesi et al. (1981) |
| *Gadus morhua* | H | Adeno-VLP | T | Jensen & Bloch (1980) |
| *Merlangius merlangus* | H | VLP | T | Anders (1988) |
| *Stizostedion vitreum* | H3 | Herpesvirus | T, I | Kelly et al. (1980, 1983), Yamamoto et al. (1985b) |
| | H4 | Retro-VLP | T | Walker (1969a), Yamamoto et al. (1985a, b) |
| | F5 | Retrovirus (foamy virus group) | T, R | Yamamoto et al. (1976) |
| *Sparus aurata* | P | VLP | T | Gutierrez et al. (1977) |
| *Agonus cataphractus* | F | Retro-VLP (lentivirus group) | T | Anders et al. (1991) |
| *Limanda limanda* | H, P | Adeno-VLP | T | Bloch et al. (1986) |
| | H | Birnavirus | I | Olesen et al. (1988) |
| *Pseudopleuronectes americanus* | H, P | Papova(?)-VLP | T | Emerson et al. (1985) |
| *Paralichthys olivaceus* | H | Herpesvirus | T, E | Iida et al. (1989), Masumura et al. (1989), Kimura & Yoshimizu (1991b) |

Table 2. Naturally occurring malignant fish skin tumours associated with viruses or virus-like particles (VLP). S: sarcoma; L: lymphosarcoma; T: transmission electron microscopy of tumour tissue; Rt: evidence of reverse transcriptase activity

| Host species | Tumour type | Associated virus or VLP | Proof by | Source |
|---|---|---|---|---|
| *Esox lucius* | S | Retro-VLP | T | Winqvist et al. (1973) |
| | L | Retrovirus | Rt | Papas et al. (1976) |
| *Stizostedion vitreum* | S | Retro-VLP | T, Rt | Yamamoto et al. (1976) |

Table 3. Experimentally induced benign skin tumours in fish. P: epidermal papilloma; H: epidermal hyperplasia; F: fibroma ('walleye dermal sarcoma'). • Presumable oncogenicity, indicated by means of cell-free filtrates

| Host species | Tumour type | Oncogenic virus | Source |
|---|---|---|---|
| *Oncorhynchus keta* | P | Herpesvirus (OMV & YTV) | Kimura et al. (1981a, b, 1983), Sano et al. (1983), Yoshimizu et al. (1987) |
| *Oncorhynchus kisutch* | P | Herpesvirus (OMV) | Yoshimizu et al. (1987) |
| *Oncorhynchus masou* | P | Herpesvirus (OMV & YTV) | Sano et al. (1983), Yoshimizu et al. (1987) |
| *Oncorhynchus mykiss* | P | Herpesvirus (OMV) | Yoshimizu et al. (1987) |
| *Salvelinus namaycush* | H | Herpesvirus• | McAllister & Herman (1989) |
| *Cyprinus carpio* | P | Herpesvirus | Sano et al. (1985a, b, 1990) |
| *Stizostedion vitreum* | F | Retrovirus | Martineau et al. (1990), Bowser et al. (1990) |
| *Paralichthys olivaceus* | H | Herpesvirus• | Iida et al. (1989), Masumura et al. (1989) |

Table 4. Experimentally induced malignant skin tumours in fish. L: lymphosarcoma; S: sarcoma; Sc: squamous cell carcinoma; N: neurofibroma. [a]Inoculation of cell-free filtrates, [b]inoculation of homogenized tumour tissue, [c]inoculation of live tumour cells; •evidence for visible retrovirus in tumour tissue by electron microscopy

| Host species | Tumour type | Source |
|---|---|---|
| *Esox masquinongy* | L[a] | Sonstegard (1976) |
| *Esox lucius* | L[a, c] | Sonstegard (1976), Mulcahy & O'Leary (1970) |
| | S[c, •] | Ljungberg (1976) |
| *Scardinius erythrophthalmus* | Sc[c] | Hanjavanit & Mulcahy (1989) |
| *Pomacentrus partitus* | N[b] | Schmale & Hensley (1988) |

were supported by a fine connective tissue stroma. Abundant mitotic figures suggested a highly proliferative nature. Tumours appearing on the caudal fin, gill cover, body surface, corneas of the eye and kidney showed characteristics similar to those of the mouth (Yoshimizu et al. 1987). Electron microscopy revealed that the tumour cells had a typical neoplastic feature of variability in nuclear size and loose intercellular connections. However, OMV particles have never been found in either the nuclei or cytoplasm of tumour cells (Kimura et al. 1981b, Yoshimizu et al. 1987).

In 1981, Sano and co-workers (1983) isolated a virus from immature masu salmon bearing spontaneous papillomas on the jaws. They named the virus YTV (Yamame tumour virus). Comparative virological studies on both OMV and YTV revealed that they were closely related. OMV and YTV strains isolated from masu salmon were even the same (Hedrick et al. 1987, Eaton et al. 1991). Therefore, it can be concluded that YTV is a synonym for OMV due to its later description. Recently, OMV was isolated from tumour tissue of pen-cultured coho salmon and from coho salmon cultured in freshwater as well as in seawater (Kimura & Yoshimizu 1991b).

**Cyprinid herpesvirus (CHV).** Schubert (1964) first visualized this virus by electron microscopy. It is associated with the earliest known skin tumour of fish, the so-called 'carp-pox' papilloma of common carp *Cyprinus carpio*. After several unsuccessful attempts to isolate the virus in cell culture by European virologists, it was recovered from naturally occurring papillomas of Japanese fancy carps (Sano et al. 1985a, b). The isolate was virulent for carp fry following exposure by immersion. Cumulative mortality in 2 wk old common carp was 85.7% and in 4 wk old fancy carp 20%. The virus was reisolated from all moribund fish and all survivors. At 5 to 6 mo post-infection, 83% of surviving common and fancy carp fry developed epidermal papillomas on fins, body surface, mandibles and the sites of inoculation. Experimental infection of adult specimens led to 13% tumour incidence in mirror carp and 10% in fancy carp. In 50% of the cases, CHV was successfully reisolated from tumourous fry (Sano et al. 1985a, b).

## Non-isolated viruses associated with successful experimental transmission

There is strong evidence for potential oncogenicity of 2 additional herpesviruses, and possibly more than 2 retroviruses, which have not yet been isolated in cell culture but which have been associated with successful experimental transmissions of infections.

Between 1985 and 1987, several outbreaks of a disease in larval and juvenile Japanese flounder *Paralichthys olivaceus* were recorded in Japanese hatcheries (Iida et al. 1989). The disease occurred in 10 to 30 d old fish which were reared at water temperatures of 18 to 20°C. Within a few weeks, mortalities reached 80 to 90%. Affected specimens had opaque fins and a

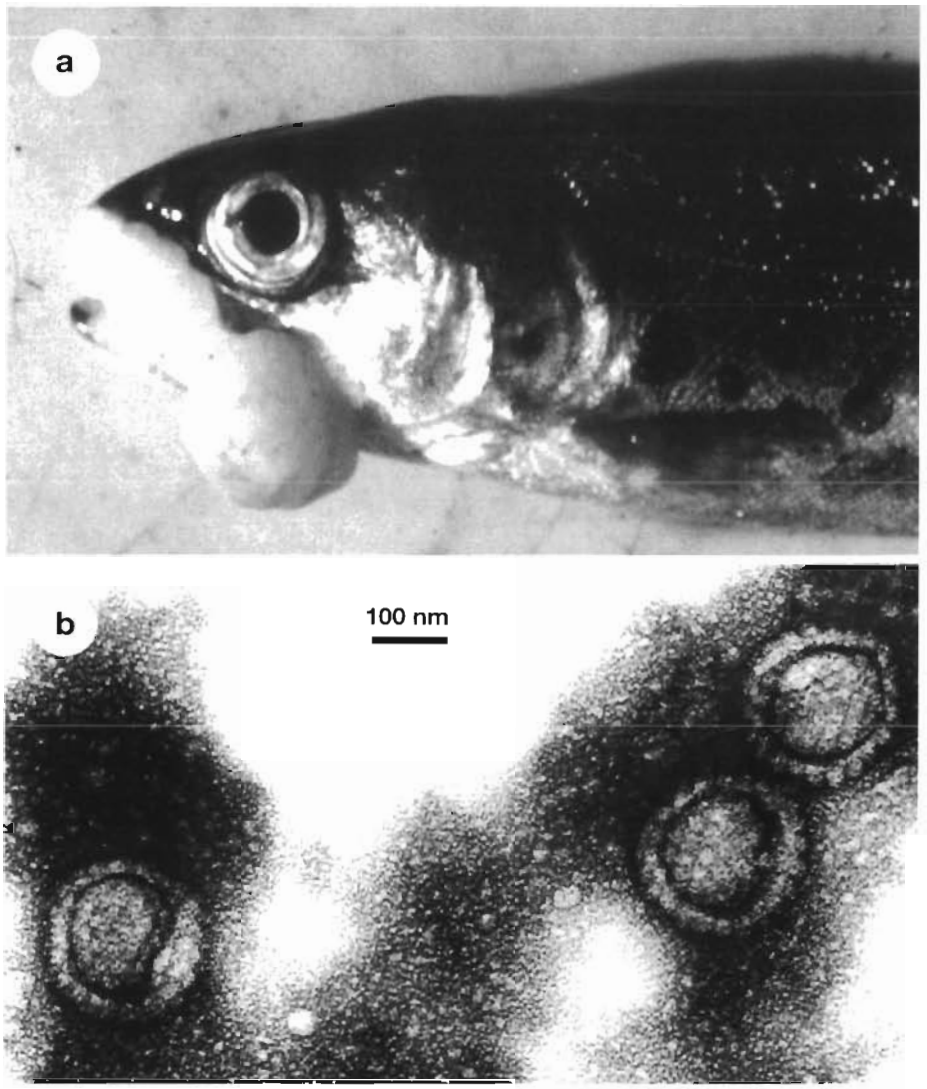



Fig. 5. *Oncorhynchus masou*. Papillomas in Japanese masu salmon. (a) Typical epidermal papillomas on the jaws; (b) isolated herpesvirus particles after negative staining (Kimura & Yoshimizu 1991a)

layer of proliferated epithelial cells on their fins and body surface (Fig. 4c). Electron microscopy revealed numerous hexagonal virus-like particles in both the nucleus and cytoplasm of the tumour cells (Fig. 4a, b). The condition was transmitted to healthy larval flounder by exposure to a 0.45 µm filtrate of tumour tissue homogenate. The morphological features (as demonstrated by electron microscopy) and sensitivity of the agent to ether and pH 3 indicated that it was a herpesvirus (Iida et al. 1989) but attempted isolation using 33 fish cell lines was not successful (Kimura & Yoshimizu 1991a).

Bradley et al. (1989) and McAllister & Herman (1989) reported on epizootic mortalities among hatchery-reared fingerling and yearling lake trout *Salvelinus namaycush*. Epithelial hyperplasias on jaws, the body surface or inside the mouth were consistently found associated with the disease. In both cases, a putative herpesvirus was observed in tumour tissue by electron microscopy. Experimental transmission of the infection was successful by cohabitation with diseased fish, by exposure to water from tanks holding diseased fish, and by bath challenge using cell-free filtrates of epidermal hyperplastic tissue.

Martineau et al. (1990) and Bowser et al. (1990) successfully transmitted the so-called 'dermal sarcoma' of walleye *Stizostedion vitreum*, which is considered benign.

It is notable that successful experimental induction of benign tumours was mostly associated with herpesviruses, whereas formation of malignant forms seems to be restricted to the involvement of retroviruses of the subfamily Oncovirinae.

It has not yet been possible to isolate a tumour-associated retrovirus in cell culture. However, in several cases experimental induction of malignant skin

tumours using cell-free filtrates or live tumour cells was successful, thus suggesting a viral etiology (Table 4). Furthermore, several research groups are circumventing problems with cell culture by taking a molecular approach.

### Viruses visualized by electron microscopy

This group includes herpesvirus, adenovirus, papovavirus and retrovirus members as well as unidentified particles of a doubtful viral nature.

**Herpesviruses.** To date, a total of 22 apparently distinct viruses, including OMV and CHV, have been described from cartilaginous and bony fish (Hedrick & Sano 1989). Twelve were associated with epidermal tumours including OMV and CHV, 1 with spawning papillomatosis of smelt (Fig. 3c to f), 3 with so-called 'giant cell hyperplasias' (McArn et al. 1978, McCain et al. 1979, Yamamoto et al. 1984, Leibovitz & Lebouitz 1985), and 1 with giant cell formation within a non-altered epidermis (Buchanan & Madeley 1978). The other 5 comprise the 2 salmonid herpesviruses *Herpesvirus salmonis* (Wolf & Taylor 1975, Hedrick et al. 1986) and NeVTA (Nerka Virus in Towada Lake Akita and Aomori Prefecture; Sano 1976), a herpesvirus of European and Japanese eels (Sano et al. 1988), channel catfish virus (Fijan et al. 1970), and a recently described herpesvirus from white sturgeon (Hedrick et al. 1991). Little is known about the phylogenetic relationships of fish herpesviruses except for the non-tumour-associated salmonid virus species. Close serological and genetic relationships were determined for 2 North American isolates of *H. salmonis* and 3 Japanese isolates (NeVTA and OMV), but the 2 groups differed significantly from each other (Hedrick et al. 1987, Eaton et al. 1991, Guo et al. 1991).

**Adenoviruses.** Adenovirus-like particles were described in Baltic cod *Gadus morhua* by Jensen & Bloch (1980) and North Sea dab *Limanda limanda* by Bloch et al. (1986). In both cases, virus-like particles were associated with epidermal hyperplasias and papillomas (Fig. 2b). Neither agent could be isolated in fish cell cultures. Needham (cited by Carlisle 1975) found virus-like particles 40 to 70 nm in diameter in the nuclei of salmon papilloma cells by electron microscopy. These particles morphologically resembled an adenovirus.

**Papovaviruses.** Known to be responsible for the formation of mammalian warts (Almeida et al. 1962, Amtmann & Sauer 1982), naturally occurring papovavirus-associated tumours in fish have not been observed so far. However, there is evidence for production of papovavirus-like particles in melanomas of *Xiphophorus* hybrids following treatment of tumour-bearing fish with the tumour promoter 5-bromodeoxyuridine (BrUdr) (Kollinger et al. 1979).

**Retroviruses.** Seventeen retroviruses and retrovirus-like particles have been found in fish to date. Ten were associated with skin tumours, 1 (doubtful case) with a granuloma (Moser et al. 1986), 1 with a swimbladder fibrosarcoma in Atlantic salmon *Salmo salar* (Duncan 1978), and 1 within the capsular material of a lymphocystis cell (Walker 1985). Four spontaneously productive C-type retroviruses infecting 4 cell lines derived from 3 species of tropical fish represent the first *in vitro* cultured retroviruses of fish (Frerichs et al. 1991). It is not known whether these 4 isolates are related to any tumourous condition of fish. In 3 additional cases, the finding of tumour-associated virus-like particles remains doubtful due to insufficient ultrastructural evidence (Edwards & Samsonoff 1977, Gutierrez et al. 1977, Emerson et al. 1985).

In 2 of the virus-associated skin tumours examined histologically, retrovirus particles were classified as members of the foamy virus and the lentivirus groups; the other retroviruses could not be assigned to any of the other 5 genera of the Retroviridae family as defined recently by Francki et al. (1991). The first case is a possible member of the lentivirus group (Fig. 6a, b), representing the first report of a tumour-associated lentivirus (Anders et al. 1991, Anders & Möller 1992). The other case is a possible member of the foamy virus group which is associated with the so-called 'walleye dermal sarcoma' (Martineau et al. 1991a, b). Reverse transcriptase activity in tissue extracts could be shown for 3 skin-tumour-associated oncoviruses from *Esox lucius* (Papas et al. 1976), *Catostomus commersoni* (Sonstegard 1977), and *Stizostedion vitreum* (Martineau et al. 1991b). The latter authors found strong evidence for the involvement of a unique exogenous retrovirus which was found predominantly unintegrated in tumour cells in walleye dermal sarcoma (WDS).

### Viruses isolated from but not visualized in tumour tissue

All tumour-associated rhabdovirus, birnavirus and picornavirus-like particles fall within this group.

The 2 best-studied conditions are the so-called 'cauliflower tumour' of European eel and papillomas of Japanese masu salmon. In the case of eel, *Rhabdovirus anguilla*, an infectious pancreatic necrosis (IPN)-like virus and an as yet unidentified agent were isolated from tumour tissue (Ahne & Thomsen 1985, Ahne et al. 1987). Using a co-culture method, Yoshimizu and coworkers (1989) isolated OMV herpesvirus, infectious haematopoietic necrosis (IHN) rhabdovirus and IPN

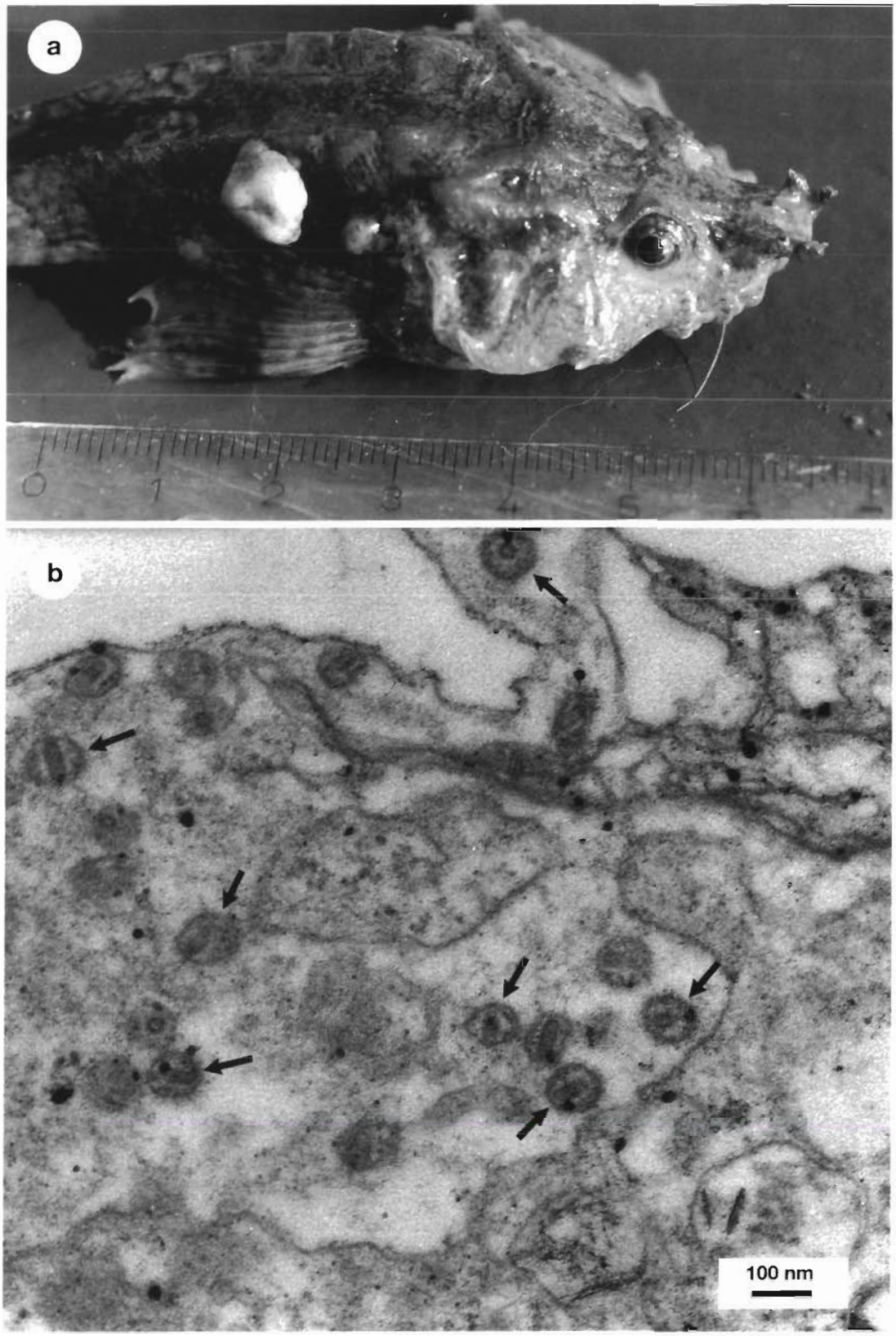



Fig. 6. *Agonus cataphractus*. Hooknose from the German Wadden Sea. (a) Two conspicuous fibroma-like tumours on the body surface; (b) numerous lentivirus-like particles (arrows) in the cytoplasm of a tumour cell

birnavirus (serotype VR 299) from papilloma tissue of a naturally infected masu salmon. The recent isolation of a picorna-like virus from epidermal hyperplasias of European smelt is the first report of an association of this type of virus with fish tumours (Ahne et al. 1990). Additionally, a virus belonging to serogroup II of aquatic birnaviruses has been isolated from one of 64 dab specimens carrying epidermal hyperplasias (Olesen et al. 1988).

Experimental infection trials were unsuccessful with all isolated rhabdo- and birnaviruses. No experimental infection studies were undertaken with the picornavirus. The role of these viruses in the development of tumours therefore remains unclear. Their presence in tumour tissue is most likely to be coincidental.

## SEASONAL VARIATION IN TUMOUR PREVALENCE

There is a marked seasonality in the incidence of several frequently occurring skin tumours of fish (Table 5). The highest infection rates are particularly evident during spawning periods (many species), smoltification (Atlantic salmon *Salmo salar*, lake trout *Salvelinus namaycush*), onset of differentiation of the gonads (European eel *Anguilla anguilla*) and metamorphosis (Japanese flounder *Paralichthys olivaceus*). In all cases, these periods of maximum tumour prevalence correspond with those life stages of the affected fish where profound endocrinological changes occur in the animal, presumably affecting both condition of the skin and replication of associated viruses.

## PROPOSED INTERACTIONS BETWEEN THE SKIN, TUMOUR VIRUSES, BIOLOGICAL AND ENVIRONMENTAL FACTORS

There is strong evidence that fundamental principles govern the development of skin tumours of fish within a framework of environmental parameters described in the preceeding sections of this review. Analysis of present knowledge has led to a hypothesis which is supported by extrapolation of evidence from work with different groups of fish. The hypothesis is illustrated in Fig. 7 and stated below.

First, it is considered that the formation of most, if not all, fish epidermal tumours is induced by viruses of the Herpes-, Adeno-, Papova- and Retroviridae families. It should be noted that in human oncology, representatives of the same virus families are also held responsible for induction of skin tumours (zur Hausen 1980).

Table 5. Times of the year and lifestages of host fish with maximum prevalences of skin tumours of fish. P: epidermal papilloma; H: epidermal hyperplasia; S: sarcoma; L: lymphoma; Sp: spring; Su: late summer; W: winter; –: no seasonality observed; NR: not reported; La: larvae; J: juvenile specimens; A: adult specimens, increasing prevalences were found with increasing age/length of host fish. *Virus- or VLP-associated cases

| Host species | Tumour type | Season | Life stage | Mortality | Source |
|---|---|---|---|---|---|
| *Anguilla anguilla* | P* | Su | J | – | Peters (1975, 1977), Möller (1987) |
| *Salmo salar* | P* | Su | J | – | Carlisle (1975, 1977), Bylund et al. (1980), Shchelkunov et al. (1992) |
| *Salvelinus namaycush* | H* | NR | J | + | Bradley et al. (1989), McAllister & Herman (1989) |
| *Osmerus eperlanus* | H, P* | Sp | A | – | Breslauer (1916), Anders & Möller (1985), Anders (1989) |
| *Esox lucius* | H* | Sp | A | – | Yamamoto et al. (1984) |
| | L | – | A | ? | Thompson & Hildén (1988) |
| *Barbus fluviatilis* | P | Sp | NR | ? | Keysselitz (1908) |
| *Cyprinus carpio* | P* | W, Sp? | A | ? | Haempel (1929), Hines et al. (1974) |
| *Leuciscus idus* | H, P* | Sp | NR | ? | McAllister et al. (1985) |
| *Rutilus rutilus* | H, P | Sp | A | ? | Andrews & Bucke (1982), E. T. Valtonen (pers. comm.) |
| *Catostomus commersoni* | P* | NR | A | ? | Sonstegard (1977) |
| *Ictalurus nebulosus* | P* | NR | A | ? | Lucké & Schlumberger (1941) |
| *Lepomis macrochirus* | H, P | Sp | NR | ? | Nigrelli (1948b) |
| *Perca flavescens* | H | NR | A | ? | Walker (1969b) |
| *Stizostedion vitreum* | H* | W, Sp | A | ? | Yamamoto et al. (1985a, b) |
| | S* | Sp | A | – | Bowser et al. (1988), Bowser & Wooster (1991) |
| *Paralichthys olivaceus* | H* | NR | La, J | + | Iida et al. (1989), Masumura et al. (1989) |
| *Limanda limanda* | H, P* | Sp | A | ? | Wolthaus (1984) |

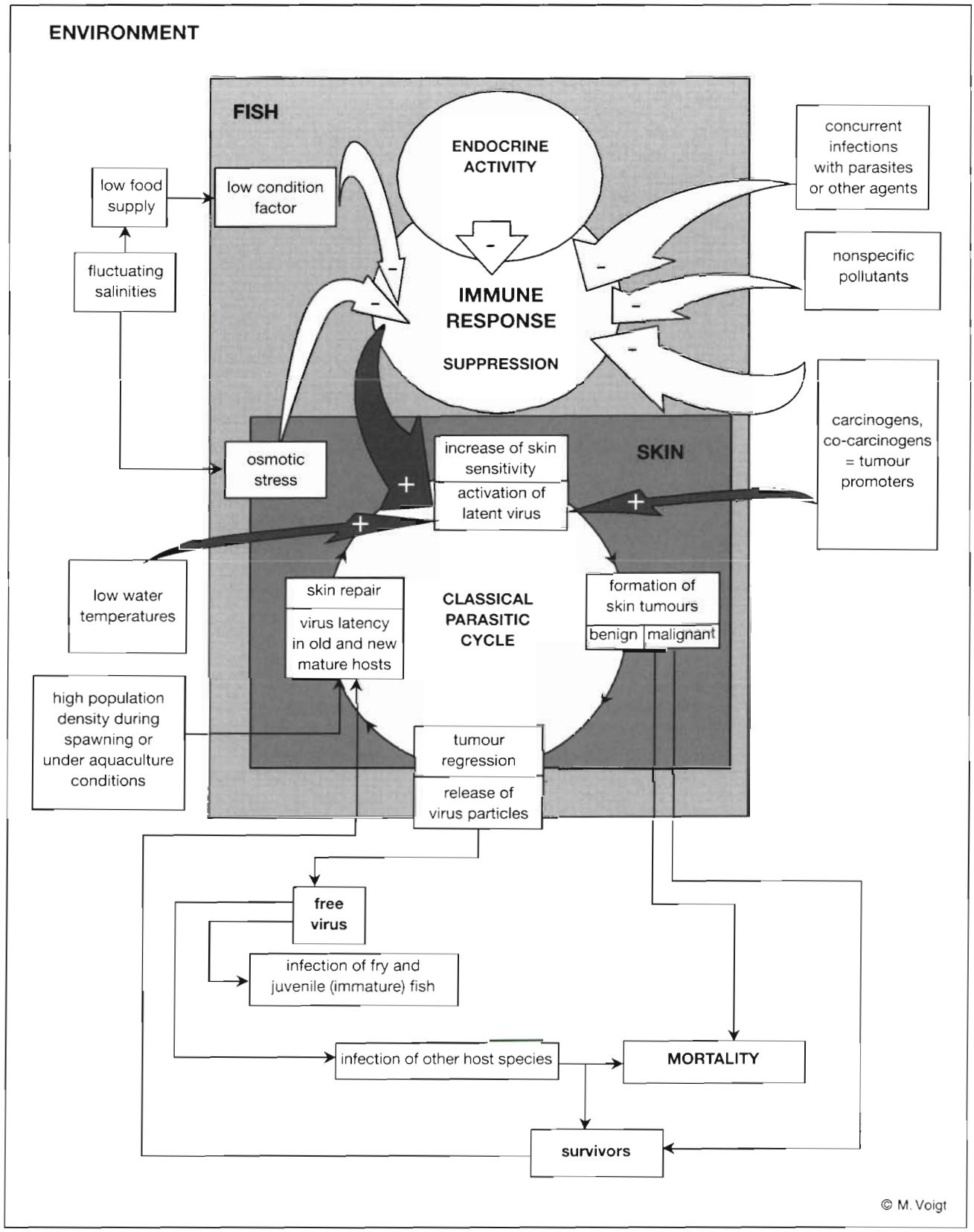



Fig. 7. Proposed hypothesis outlining the interactions between skin, skin tumour viruses, biological and environmental factors. (+) Stimulating factors; (–) diminishing factors

Secondly, it is considered that the marked seasonal variation in skin tumour prevalence is a normal, expected feature of sexual maturation, spawning and metamorphosis in certain species of fish, mediated by suppression of the immune system which is itself induced by an increase in endocrinological activity. Different environmental factors may then contribute to an intensification of the process, even to the point of malignancy and subsequent death of host fish.

### Role of endocrine activity

It seems likely from many epidemiological findings that the seasonal cycle of endocrine activity mediates the cyclical changes of the immune system and is the predominant factor influencing the defense mechanisms of fish. Direct evidence for this is provided by studies on the haematology of brown trout *Salmo trutta* which revealed significant depression of lymphocyte numbers during each of 3 separate spawning seasons (Pickering 1986) indicating a general suppression of the immune response.

It also seems likely that high levels of sex hormones in the blood of fish stimulate proliferation of the epidermis and thus act as tumour promoters in forming a sensitive substrate for the multiplication of acute and persistent infectious agents, as observed for pathogenic fungi (Richards & Pickering 1978) and a variety of skin ectoparasites of brown trout (Pickering & Christie 1980). An excellent review of this subject has been given by Pickering & Richards (1980).

### Role of immune suppression

Antibodies, specific agglutinins and molecules with possible antibiotic activity are almost exclusively found in skin mucus (Pickering & Richards 1980) whereas macrophages and lymphocytes are frequent inhabitants of the epidermis (Bullock & Roberts 1974, Roberts & Bullock 1980).

It is widely accepted that depression of the immune response in fish favours the outbreak of infectious diseases. As the formation of skin tumours of fish is understood to be an infectious process, it may therefore be inferred that persistent tumour viruses are activated at times when the immune system is destabilized. Under normal circumstances this occurs naturally at times of spawning, smoltification and metamorphosis. Adverse environmental factors such as salinities close to the maximum tolerance level (Thompson 1982), fluctuating salinities resulting in a reduction of food supply for host fish and subsequent lowering of the condition factor (Möller 1984, 1990), high population densities on spawning grounds and high concentrations of non-specific pollutants and carcinogens in water, sediments and food organisms may contribute to a further depression of defense mechanisms, thus enhancing prevalence rates. According to the present hypothesis, immune suppression by both natural and man-made environmental factors alone cannot stimulate expression of the tumour viruses.

### Viral induction of tumour formation

The key factor of immune suppression allows activation of latent endogenous viruses. A latent infection is one in which at least the genome of the virus is present but infectious virions cannot be recovered except during episodes of overt disease (Rapp & Jerkofsky 1973). Subsequently, virus-stimulated proliferation of epidermal and fibroblastic cells takes place leading to the formation of tumours. Additionally, carcinogenic substances and/or low water temperatures (Brown et al. 1976, Papas et al. 1976) may further enhance this process. The lesions normally regress spontaneously without leaving scars (= response of permissive cells to infection) but, in the case of malignant growths (= response of nonpermissive cells to infection, leading to a permanently transformed cell progeny), may lead to mortalities among the affected population. Lysis of benign tumour cells, especially those induced by herpesviruses, results in passive release of infective exogenous virus particles into the environment. High population densities, as may occur during spawning seasons, favour the transmission of virus particles to new host specimens. At the next stage, intracellular virus particles become dormant until they are activated once more.

The seasonal, cyclical appearance of skin tumours is valid for mature specimens only and is usually a benign process. In some cases, however, mortalities have occurred among affected adults which were mainly attributed to the formation of skin ulcerations which frequently followed spontaneous sloughing of benign skin tumours, as described for European smelt and Atlantic salmon (Carlisle & Roberts 1977, Bylund & al. 1980, Anders 1989). In larvae and juveniles, tumour-associated viruses can give rise to an acute systemic disease with subsequent severe mortalities as has been shown for the 4 herpesviruses OMV, *Herpesvirus cyprini*, herpesvirus of Japanese flounder, and Epizootic Epitheliotropic Disease virus of lake trout (Shchelkunov et al. 1992).

In the case of spawning papillomatosis of smelt, a seasonality in the occurrence of exogenous virus particles has been demonstrated by electron microscopy, with peak prevalences in the most differentiated

tumour stages (Anders 1989). Furthermore, there was a marked increase of papilloma prevalence with increasing size of host fish, which can be explained by an increasing spread of persistent virus within the smelt population with successive cycles of virus activation and release.

### Are fish tumour viruses 'perfect parasites'?

The cyclic process described above most closely resembles a classical parasitic life cycle which is commonly characterized by a free-living stage and a change of host species in some cases. As yet, it is not known whether tumour-associated viruses of fish are infective for more than 1 species or whether other aquatic organisms can serve as transmitters.

A non-tumour-associated calicivirus is the only known example of successful expression in different hosts, occurring in both marine fish and marine mammals (Smith et al. 1986, Barlough et al. 1988). Although this finding may seem surprising at first glance, it is likely to be explained by the phylogenetic position of the host system. Assuming a close co-evolution of tumour viruses and hosts through vertical transmission (Farley 1981), the naive status of their relationship may well reflect the relative simplicity of the fish host's physiology. Examples for a co-evolution of fish viruses and their host species have been summarized by Anders & Darai (1985). It is tempting to speculate that tumour-associated viruses at the piscine level may be considered true parasites and that as the phylogenetic level of the host rises, so the 'life cycle' of associated tumour viruses becomes more specialized, culminating in the perfect endosymbiotic integration of viral genomes in the genetic substance of mammalian host cells.

### Malignant tumour development

It can be further concluded from our hypothesis that carcinogenic substances in water, sediment or biota may affect the development of a benign tumour by mutational modification of cellular functions, resulting in a progress to malignancy.

## CONCLUDING REMARKS

In summary, presently available evidence supports a key role for viruses in the induction of skin tumours of fish. It is concluded that other biological and environmental factors mediate either expression of the tumour virus, tumour development or both.

Overall, there are 2 broad fields for the potential application of these findings.

One area is the investigation and monitoring of environmental effects of contaminants in aquatic ecosystems, although the incidence of benign skin tumours of fish is often argued not to be an ideal indicator parameter in this respect. Unless experimental designs allow distinctions between the effects of the different biological and environmental factors influencing the immune status, the technical problems in 'detecting even a fractional increase (or decrease) in tumour incidence, which may be related to exogenous carcinogens, are enormous, and in many cases impossible to overcome' (Mix 1986). However, according to our hypothesis, and assuming that all but the 'pollution parameters' do not vary more than normal, it should be possible to detect differences in prevalence rates evidently attributable to changes in pollution levels. If so, 'biomarkers' for the presence of carcinogens in the marine environment, such as activation of cellular oncogenes and oxyradicals, could prove to be successful tools for future effect monitoring programmes.

Secondly, tumour diseases of fish may provide a sensitive animal system for exploring man-made and natural environmental factors in oncogenesis. Furthermore, studies of tumours in poikilotherms might play a unique role in developing new model systems for elucidating basic mechanisms underlying neoplastic processes. Such systems could be of value for human cancer research and offer excellent opportunities for collaborative research between fisheries ecologists and specialists in human oncology.

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