Health survey of aquarium fish in Swedish pet-shops

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Abstract

The aim of this study was to conduct a health survey of aquarium fish in pet-shops in order to identify the main causes for disease. In total 720 freshwater aquarium fish from 24 Swedish pet-shops (i.e. 30 fish from each shop) were collected for bacteriological and virological investigations from August 2006 to May 2007. Fish were primarily selected according to signs of illness, otherwise fish were randomly chosen. Of the collected fish, 120 fish belonging to various species and 23 neon tetra (*Paracheirodon innesi*) were in addition thoroughly investigated microscopically regarding occurrence of ecto- and endoparasites and pathological organ alterations. Moreover, 51 fish included in the total 720 and belonging to the family *Cyprinidae*, were investigated for the presence of koi herpes virus by real-time PCR. The most commonly occurring reason for illness in the fish was infections caused by parasites and the most frequent parasitic group was *Monogenea*. Monogenic flukes were not found on the neon tetra, but were found as frequently as on 77 (64%) of the other 120 fish studied. On the other hand, microsporidia infection with *Pleistophora hyphessobryconis* was found especially in the neon tetra. Bacterial infections in the fish were mainly caused by acid-fast bacteria. Probable or established infection with acid-fast bacteria was found in 28 (23%) of the 120 fish and in 5 (22%) of the 23 neon tetra studied more in detail. In three fish typical nodules indicative of lymphocystis were observed microscopically, but no other acute or latent virus infections could be demonstrated by virus cultivation of organ material from all 720 fish or by real-time PCR investigation of organ material from the 51 fish of the family *Cyprinidae*. Oomycete infection was only observed in one swordtail (*Xiphophorus hellerii*). In conclusion, this health survey carried out on aquarium fish in Swedish pet-shops showed that the most common causes of diseases in the fish were infections with parasites and acid-fast bacteria.

Introduction

Since the beginning of the twentieth century, people in Sweden have kept fish in aquariums. Initially, mostly European coldwater fish species and North American sunfish (*Lepomis spp.*) were kept (Brorsson, 1987), but today aquarium fish in Sweden mainly originate from Africa, Asia and South America. In the last decade, the keeping of koi (*Cyprinus carpio koi*) and goldfish (*Carassius auratus auratus*) in garden ponds also has been very popular. In 2002 the number of aquarium fish

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in Sweden was estimated to 4.5 million out of total 8.1 million pet animals (Danielsson, 2003). Most of these fish are bred in Asia and Eastern Europe, but there are also wild-caught freshwater fish originating from Africa, Asia and South America. Out of total 30 385 fish species described worldwide in 2008, as many as 3068 (11 %) are commercialized as aquarium fish and of these approximately 720 species are bred in captivity (http://www.fishbase.se). Diseases of aquarium fish are a common problem for aquarists in Sweden and in many other countries. Some studies concerning causes for diseases among aquarium fish in wholesale business have been made (Engelhardt, 1992; Kim et al., 2002; Levsen et al., 2003; Thilakaratne et al., 2003), but no previous survey has been published that describes the disease-causing agents of aquarium fish exclusively in pet-shops. The aim of this study was therefore to conduct a health survey of aquarium fish in pet-shops in order to identify the main causes for disease such as bacterial-, virus- and parasitic infections as well as water quality problems. This information will give indications for prophylactic measures and treatments that are possible to reduce the practical and financial consequences of the diseases and is an important step to improve aquarium fish welfare.

Materials and methods

Collection of aquarium fish for investigation

Freshwater aquarium fish were collected from 24 pet-shops randomly chosen from the phone book, located in the middle part of Sweden, from August 2006 to May 2007. Twelve of these shops were specialized in selling only aquarium fish, whereas the other 12 shops also held other kinds of animals for sale. The numbers of aquariums in the shops varied from 16 to 200. Thirty fish (i.e. 1 fish from 30 different aquariums) were collected from each pet-shop for bacteriological- and virological investigations. In cases where a shop (n=5) had less than 30 aquariums, fish of different species were selected from the same aquarium to fulfil the number of 30 fish from each shop. Fish were primarily selected according to signs of illness such as emaciation, exophthalmia, fin- and skin erosions, swollen belly, wounds and/ or a deviant behaviour. Otherwise, fish were randomly collected. Of the collected fish from each shop, five fish with signs of illness were in addition thoroughly investigated microscopically regarding occurrence of ecto- and endoparasites and pathological organ alterations, in order to establish diagnosis. These in total 120 (5 x 24) fish were subgrouped according to fish species and are described in Table 1. Additional investigations were also made on the neon tetra (*Paracheirodon innesi*) from total 23 shops (1 fish/ shop) (Table 3).

Before examination, the majority of fish were euthanized with Tricaine methane sulphonate (MS222®, Novartis AG, Täby, Sweden, 140-230 mg L^{-1}), followed by a mechanical destruction of the brain. A few larger fish were instead killed by a blow to the head, followed by mechanical destruction of the brain before examination.

Microscopic investigation

Investigation regarding parasites, oomycete and pathological organ alterations was made with microscopy directly after the fish were put to death through examination of the whole surface of the fish and the whole mesentery,

Table 1. Fish species examined more thoroughly (except neon tetra).

Group 1. Live bearers	Group 2. Cyprinids	Group 3. Labyrinth fish	Group 4. Cichlids	Group 5. Other fish
Family*: Poeciliidae*	**Family: *Cyprinidae***	**Family: *Helostomatidae***	**Family: *Cichlidae***	**Family: *Callichthyidae***
Blackmolly, Sailfin molly	**Bala shark**	**Kissing gourami**	**Altolamprologus compressiceps**	**Bronze corydoras**
(Poecilia sphenops,	*(Balantiocheilos melanoptereus)*	*(Helostoma temminkii)*	*(Altolamprologus compressiceps)*	*(Corydoras aeneus)*
Poecilia latipina)	**Cherry barb**		**Angelfish**	**Peppered corydoras**
Guppy	*(Puntius titteya*)	**Family: *Osphronemidae***	*(Pterophyllum scalare)*	*(Corydoras paleatus)*
(Poecilia reticulata)	**Comet goldfish/ goldfish**	**Banded gourami**	**Banded cichlid**	**Other corydoras**
Montezuma swordtail	*(Carassius auratus auratus)*	*(Colisa fasciata)*	*(Heros efasciatus)*	*(Corydoras spp.)*
(Xiphophorus montezumae)	**Fantail goldfish**	**Blue-, Cosby-, Gold gourami**	**Demansoni cichlid**	
Platy	*(Carassius auratus auratus)*	*(Trichogaster trichopterus)*	*(Pseudotropheus demansoni)*	**Family: *Characidae***
(Xiphophorus maculatus)	**Glowlight-/ Lampchop rasbora**	**Dwarf gourami**	**Discus fish**	**Black tetra**
Sailfin molly	*(Trigonostigma hengeli,*	*(Colisa lalia)*	*(Symphysodon aequifasciatus)*	*(Gymnocorymbus ternetzi)*
(Poecilia velifera)	*Trigonostigma espei)*	**Honey gourami**	**Golden mbuna**	**Cardinal tetra**
Swordtail	**Goldbarb**	*(Colisa chuna)*	*(Melanochromis auratus)*	*(Paracheirodon axelrodi)*
(Xiphophorus hellerii)	*(Punctius semifasciolatus)*	**Pearl gourami**	**Labidochromis yellow**	**Colombian tetra**
	Harlequin rasbora	*(Trichogaster leeri)*	*(Labidochromis caeruleus)*	*(Hyphessobrycon columbianus)*
	(Trigonostigma heteromorpha)	**Siamese fighting fish**	**Pseudotropheus liliancinus**	**Cuchu´s blue tetra**
	Koi	*(Betta splendens)*	*(Pseudotropheus lombardoi)*	*(Boehlkea fredcochui)*
	(Cyprinus carpio koi)	**Snakeskin gourami**	**Rainbow krib**	**Glowlight tetra**
	Rosy barb	*(Trichogaster pectoralis)*	*(Pelvicachromis pulcher)*	*(Hemigrammus erythrozonus)*
	(Puntius conchonius)	**Thick-lipped gourami**	**Ramirez´s dwarf cichlid**	**Lemon tetra**
	Tiger barb	*(Colisa labiosus)*	*(Microgeophagus ramirezi)*	*(Hyphessobrycon pulchripinnis)*
	(Puntius tetrazona)			**Swegles tetra**
	Zebra fish			*(Hyphessobrycon sweglesi)*
	(Danio rerio)			
				Family: *Eleotridae*
				Peacook gudgeon
				(Tateurdina ocellicauda)
				Family: *Melanotaeniidae*
				Rainbow fish (*Melanotaenia sp.)*
				Red rainbow fish
				(Glossolepis incisus)
				Family: *Pangasiidae*
				Sutchi catfish
				(Pangasius hypophthalmus)
				Family: *Pimelodidae*
				Pictus cat *(Pimelodus pictus)*

or in the case of larger fish, selected parts of the skin, fins, gills, muscle and viscera (i.e. gallbladder, heart, intestine, kidney, liver, spleen and stomach).

Bacteriological investigation

Bacteriological investigation was made on fish with signs of infection like wounds, skin erosions and morphological changes in internal organs. Cultivation of tissue samples were carried out on horse blood (5%) agar and/or Cytophaga agar (Bernardet & Kerouault, 1989), TYES agar (Holt et al., 1993) and KDM-C agar (Daly & Stevenson, 1985). Agar plates were incubated at 20°C and examined for up to 7 days. Suspected colonies of *Aeromonas sobria, A. hydrophila, Listonella anguillarum and Vibrio sp.* were verified through their reactions in standard biochemical tests. *L. anguillarum* were further identified through sensitivity for the vibriostatic agent 0129 and by slide agglutination with verified polyclonal rabbit serum directed to *L. anguillarum* serotype 01 and 02. Either native tissue smears or formalin fixed tissue sections from the organs with indications of granuloma formations or lesions were stained with Gram- and Ziehl-Nielsen and microscopically investigated. In the cases, the granuloma had an appearance typical for acid-fast bacteria infection, but no acid-fast bacteria could be detected by means of Gram- and Ziehl-Nielsen staining and microscopy, the granuloma were classified as a granuloma probably due to acid-fast bacteria.

Virological investigation

Materials for virological investigation were taken from parts of the fishes' brain, gills, heart, kidney, liver and spleen, or in the case of very small fish, the whole mesentery was chosen. The organ materials from the 30 fish from each pet-shop were then pooled into three samples consisting of organ materials from 10 fish in each. The pooled samples were stored in Earle´s Minimal Essential Medium (EMEM) supplemented with 10% calf serum, without antibiotics, at +1°C to +4°C, until virological investigation. When it was impossible due to practical reasons to start the virological investigation within 24 hours after collecting the fish, the pooled samples were kept in -20°C, or if stored longer than a week, in -70°C until investigation. The pooled tissue samples were homogenized in a polytron homogenizer (Kinematica AG, Littau, Switzerland) and the supernatants, after centrifugation at 2500g for 20 min, were inoculated on the bluegill fry-2 (BF-2)-, epithelioma papulosum cyprini (EPC)- and rainbow trout gonad (RTG-2) fish cell lines (Roberts, 1989b), for incubation at 13-14.5°C. Samples were passed on to the homologous cell lines after seven days and the primary inoculated cells and the passage cells were regularly red for further seven days.

In addition, 51 fish of the family *Cyprinidae* [i.e. golden orfe (*Leuciscus idus*), goldfish and koi] and one sutchi catfish (*Pangasius hypophtalmus*) of the total 720 fish were investigated for the presence of koi herpes virus (KHV) by detection of the DNA polymerase gene of the virus by an unpublished real-time PCR (M. Y. Engelsma, Central Veterinary Institute, Lelystad, The Netherlands, personal communication). Briefly, fish tissue samples were grinded with beads in a Qiagen TissueLyser and thereafter genomic DNA was extracted with the Qiagen Mag Attrach Virus Mini kit (QIAGEN, Hilden, Germany). Detection of KHV DNA was then carried out by real-time Taqman PCR in an

AmpliTaq Gold system (Applied Biosystems, Foster City, CA, USA), using forward primer 5'-TGC-CCC-GAG-ACC-AAA-CAC-3', reverse primer 5'-GCG-TGC-GGT-CAA-AGA-TGT-C-3' and probe 6-FAM 5'-TGT-GGA-CGG-GCA-GCG-GGT-GTA-3' BHQ. Tissue samples from the fish were stored at -20°C for 1 to 12 months until investigated. Each tissue sample analysed consisted of 0.1 to 1.6 mg of gills, and in one case also skin, pooled from 1 to 4 fish.

Water investigation

Water samples were collected from selected aquarium tanks as well as from the tap water in 22 of the 24 pet-shops. The water samples were taken in 50 or 250 mL polyethene (PE-LD) bottles (Kautex Textron, GMBH & CO., Bonn, Germany) without air layer at the top. The samples were stored at +4°C for 1 to 14 days until examination of ammonium (NH_4), copper (Cu), iron (Fe), nitrate (NO_3), nitrite (NO_2), phosphate (PO_4), carbonate hardness and total hardness by use of conventional colorimetric aquarium test kits (Sera, Heinsberg, Germany). The detection limits were 0.5 mg/L for NH_4, 0.1 mg/L for Cu, 0.1 mg/L for Fe, 5 mg/L for NO_3, 0.9 mg/L for NO_2, 0.1 mg/L for PO_4, 1° dKH for carbonate hardness and 1° dGH for total hardness. Measurement of pH was made with a pH meter [HI 8314], and of conductivity with an EC/TDS & Temperature Meter [HI 98311] (both from Hanna Instruments Inc., Woonsocket, Rhode Island, USA).

Results

Bacterial infections

In the group of 720 fish, potentially pathogenic gram-negative rods such as *A. sobria* and *A. hydrophila* were found in several samples taken from wounds and gills. In a bile sample from a Chinese algae-eater (*Gyrinocheilus aymonieri*) the fish pathogenic gram-negative rod *L. anguillarum* serotype II was found, and in a bile sample from a platy (*Xiphophorus maculatus*) a *Vibrio* sp was encountered. Another finding of a *Vibrio* sp was made in a liver sample from a harlequin rasbora (*Trigonostigma heteromorpha*).

In the group of 120 fish which were more thoroughly investigated in order to establish a diagnosis, acid-fast bacteria were found in 13 (11 %) of the fish (Table 2). Additionally, granuloma formations probably due to acid-fast bacteria were observed in 15 (13 %) of the 120 fish (Table 2). In the separately investigated group of neon tetra from total 23 pet-shops, granuloma formations probably due to acid-fast bacteria were observed in 5 (22 %) of the 23 fish (Table 3).

Parasitic infections

Parasitological investigation of the 120 fish and the 23 neon tetra showed that protozoan parasites like ciliates and flagellates were common (Tables 2 and 3). Microsporidia infection with *Pleistophora hyphessobryconis* was found only in the neon tetra in which a total of seven cases were encountered (Table 3: 1 [9 %] of 11 fish in specialized shops versus 6 [50 %] of 12 fish in non-specialized shops were infected, p=0.07, Fisher's exact test). Monogenic flukes were not found on the neon tetra, but occurred as frequently as on 77 (64 %) of the 120 fish studied (Table 2). Other metazoan parasites such as nematodes were found in the intestine, liver, stomach and other internal organs in 13 (11 %) of the

Table 2. Diagnostic causes for disease in the group of 120 fish studied more thoroughly.

Diagnostic causes for disease[a]	Group 1 Live bearers (total 24 fish)	Group 2 Cyprinids (total 24 fish)	Group 3 Labyrinth fish (total 24 fish)	Group 4 Cichlids (total 24 fish)	Group 5 Other fish (total 24 fish)	∑ Fish with diagnosis
Bacterial infections						
Gram-negative rods (*Vibrio* sp.)		1				1
Gram-negative rods (other species)	3			1		4
Acid-fast bacteria (rod-/ pleomorphic configured)	1	1	3	1	7	13
Parasitic infections						
Protozoan parasites						
Ciliophora: Apiosoma sp.				1		1
Ciliophora: Chilodonella spp.	1		1	2	1	5
Ciliophora: Ichthyophthirius multifiliis	2		2	2		6
Opalinida: Protopalina sp.			1			1
Ciliophora: Tetrahymena spp.				2		2
Ciliophora: Trichodina spp.	3			1	1	5
Dinozoa: Piscinoodinium spp.	1	1			1	3
Euglenozoa: Cryptobia spp.	3		4	5	4	16
Euglenozoa: Ichthyobodo spp.	5			1	1	7
Metamonada: Spironucleus spp.				9		9
Metazoan parasites						
Cestoda: Pleurocercoid				1		1
Monogenea: Dactylogyridae	11	8	11	9	6	45
Monogenea: Gyrodactylidae	8	7	11	2	4	32

Microspora		1				1
Myxozoa: Myxidium spp.	1					1
Myxozoa: Myxobolus spp.	3					3
Nematoda: Camallanus sp.				1		1
Nematoda: adult larvae	1	1	2	6	2	12
Trematoda, metacercaria: *Centrocestus* spp.	1	3	1		1	6
Trematoda, metacercaria: others			1			1
Virus infection						
Lymphocystis (*Iridoviridae*)			1	1		2
Oomycete infection						
Oomycetes	1					1
Pathological alterations in internal organ						
Granuloma (probably due to acid-fast bacteria)		5	4	2	4	15
Granuloma (probably due to parasites)				3		3
Granuloma (unknown pathogen)	2	3	4		3	12
Nephrocalcinosis	4	3	5	2		14
Mechanical injury						
Trauma		1				1
Unknown diagnosis						
Emaciation				1	2	3
Gill hyperplasia	1	2	1	2		6
Others	1	1		1	3	6
Total number of diagnoses in all 120 fish	53	38	52	56	40	239

[a]according to Poppe (1999).

Table 3. Diagnostic causes for disease in neon tetra (*Paracheirodon innesi*).

Diagnostic causes for disease	Group A: Fish with signs of illness collected from shops specialized in selling only aquarium fish (in total 8 fish)	Group B: Fish randomly collected from shops specialized in selling only aquarium fish (in total 3 fish)	Group C: Fish with signs of illness collected from pet-shops (in total 10 fish)	Group D: Fish randomly collected from pet-shops (in total 2 fish)
Parasitic infections				
Ciliophora: *Ichthyophthirius multifiliis*		1	1	
Euglenozoa: *Cryptobia spp.*	6		5	
Metamonada: *Spironucleus spp.*	2		3	
Microspora: *Pleistophora hyphessobryconis*	1		6	
Pathological alterations				
Granuloma (probably due to acid-fast bacteria)	2		3	
Granuloma (unknown pathogen)	1			1
Nephrocalcinosis			1	
Signs with unknown genes				
Emaciation	1		1	
Malformation of the fins	1			
Malformation of the ribs			1	
Fin erosion			1	

120 fish, and infections with trematodes were encountered in the gills and viscera in 7 (6 %) of the 120 fish (Table 2).

Virus infections

In a total of 720 fish (=72 pooled samples) no cases of virus infections were found. Neither in the organ material from the 52 selected fish investigated with real-time PCR was any koi herpes nucleic acid detected. However, typical nodules indicative of lymphocystis were observed microscopically on fins and skin of three out of the 720 fish. The affected species were Ramirez's dwarf cichlid (*Microgeophagus ramirezi*), gold gourami (*Trichogaster trichopterus*) and pearl gourami (*Trichogaster leeri*).

Oomycete infections

Oomycete infections were rarely seen in the 120 fish- and neon tetra groups. Only one infection was found in the skin of a swordtail (*Xiphophorus hellerii*) (Table 2).

Other findings

Nephrocalcinosis occurred relatively frequently. Fourteen fish (12 %) in the 120 fish group and one fish in the neon tetra group had nephrocalcinosis (Tables 2 and 3). Moreover, fin injuries were noted on a comet goldfish, probably caused by trauma as a result from an attack by a turtle living in the same aquarium tank (Table 2).

Water quality parameters

Water samples from tap water showed low Cu content (0.1 mg/L) in seven out of 22 pet-shops. In one of these seven shops, low Cu content (0.1 mg/L) in combination with low carbonate hardness was in addition found in one aquarium tank, in which survival problems in the fish population was reported. Increased levels of NH_4 (0.5-5 mg/L, corresponding to 0.015-0.110 mg NH_3 at present pH) were found in water samples from 20 of 153 aquarium tanks. A high level of NO_2 (16.5 mg/L) was found in one aquarium, harbouring neon tetra which showed signs of oxygen depletion such as dyspnea and listless behaviour. Some of these fish were also infected with microsporidia (*Pleistophora hyphessobryconis*).

Discussion

This health survey on aquarium fish in Swedish pet-shops shows that the most common reason for illness in the fish is infections caused by parasites. The most frequent parasitic group was *Monogenea*, followed by flagellates and in third place ciliates. Bacterial infections were mainly caused by acid-fast bacteria. In the group of 120 fish belonging to 52 different species that were studied more in detail, established or probable infection with acid-fast bacteria was found in as many as 28 (23%) of the fish. Similarly, probable infection with acid-fast bacteria was also found in slightly more than 20% of the neon tetra. In addition, in the group of 720 fish, smaller numbers of bacterial infections caused by gram-negative bacteria such as *A. hydrophila, A. sobria, L. anguillarum* and *Vibrio* spp were found, of which all can cause acute fatal infections in aquarium fish.

Concerning virus infections, we cultured organ material from fish on the cell lines BF-2, EPC and RTG-2, as these cell lines are sensitive for several viruses belonging to the families aquabirnavirus, iridovirus and rhabdovirus,

which all are known to cause virus infections in aquarium fish (Adair & Ferguson, 1981; Hedrick & McDowell, 1995; Betts et al., 2003). No virus was detected in any of the pooled samples investigated including in total 720 fish. However, three fish showed microscopically typical cell changes on fins and skin which were consistent with the iridovirus infection lymphocystis (Poppe, 1999). The lymphocystis virus from freshwater fish can be grown in the BF-2 cell line (Roberts, 1989a; Hedrick & McDowell, 1995), but only materials from internal organs, and not from the fins and skin from these three fish were included in the samples investigated by virus cultivation, which may explain why the iridovirus not could be isolated. Moreover, using real-time PCR, no nucleic acid of KHV was detected in organ material from 52 selected fish.

The selection of fish species in the survey was done in order to reflect the most commonly occurring species of aquarium fish in Swedish pet-shops. These fish species are also mostly cultivated. By now in Sweden, the neon tetra is the most popular and sold aquarium fish, followed by fantail- and comet goldfish and live bearers such as guppy (*Poecilia reticulata*) and platy (*Xiphophorus maculatus*).

An interesting question is whether any differences exist in regard to the occurrence of diseases between fish in pet-shops specialized in selling only aquarium fish, and those selling both aquarium fish and other animals. We diagnosed "Neon Tetra Disease", caused by the microsporidian parasite *Pleistophora hyphessobryconis* (Dykowa & Lom, 1980) in six out of 12 neon tetra, collected from non-specialized shops compared with in only one out of 11 neon tetra, collected from shops specialized in aquarium fish. This finding may suggest that staff working in shops specialized in aquarium fish have a more pronounced experience to identify, handle and treat common diseases in aquarium fish, such as the "Neon Tetra Disease", as this disease usually develops slowly and the affected fish in most cases exhibit noticeable distinct external signs of illness such as fading of the skin coloration and development of white skin patches.

Regarding the finding of survival problems in the fish population in one aquarium tank with a Cu content of 0.1 mg/ L, it has to be noted that Cu can be lethal at concentrations as low as 0.1 mg/L, depending on other water chemistry parameters — for example carbonate hardness (Stoskopf, 1993a). Moreover, the increased levels of NH_4 in the water which were found in 20 of 153 aquarium tanks investigated can be harmful to fish in the long term and cause gill epithelial hyperplasia (Stoskopf, 1993a; Tomasso, 1993). Furthermore, a NO_2 level of 16.5 mg/L that was found in one aquarium, harbouring neon tetra, is a concentration known to be toxic (Tucker, 1993).

In conclusion, this health survey, carried out on aquarium fish in Swedish pet-shops, demonstrated that the most common causes of diseases among the fish are infections caused by parasites and acid-fast bacteria. Improvement of hygiene routines in connection with both the handling of fish, and cleaning of the aquariums and their equipment, are important to avoid infections. Prophylactic treatment against ectoparasitic protozoans and monogeneic flukes on skin and gills of new fish, and regularly on

existing fish populations in the shops, will also reduce these infections. Fish with signs indicating disease have to be identified and rapidly isolated from healthy fish in order to decrease the risk of spreading the infection. Diseased fish should thereafter be treated with appropriate medications in special aquariums suitable for the actual treatment (Scott, 1991; Stoskopf, 1993b). Increasing the knowledge about commonly occurring infections in aquarium fish to people working in these shops will be an important step to improve aquarium fish welfare.

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References

Adair BM and Ferguson HW (1981). Isolation of infectious pancreatic necrosis (IPN) virus from non-salmonid fish. *Journal of Fish Diseases* **4**, 69-76.

Bernardet J-F and Kerouault B (1989). Phenotypic and genomic studies of "*Cytophaga psychrophila*" isolated from diseased rainbow trout (*Oncorhynchus mykiss*) in France. *Applied and Environmental Microbiology* **55**, 1796-1800.

Betts AM, Stone DM, Way K, Torhy C, Chilmonczyk S, Benmansour A and de Kinkelin P (2003). Emerging vesiculo-type virus infections of freshwater fishes in Europe. *Diseases of Aquatic Organisms* **57**, 201-212.

Brorsson E (1987). I remember … [in Swedish]. *Akvariet* **1**, 11.

Daly JG and Stevenson RM (1985). Charcoal agar, a new growth medium for the fish disease bacterium *Renibacterium salmoninarum*. *Applied and Environmental Microbiology* **50**, 868-871.

Danielsson M (2003). The number and importance of pet animals in Europe (Sweden) [in Swedish]. *PetScandinavia* **3**, 10-14.

Dykova´ I and Lom J (1980). Tissue reactions to microsporidian infections in fish. *Journal of Fish Diseases* **3**, 265-283.

Engelhardt VA (1992). Causes of diseases and mortality in aquarium fishes – frequency and importance. *Berliner Münchener tierärztliche Wochenschrift* **105**, 187-192.

Hedrick RR and McDowell TS (1995). Properties of iridoviruses from ornamental fish. *Veterinary Research* **26**, 423-427.

Holt RA, Rohovec JS and Fryer JL (1993). Bacterial coldwater disease. In "**Bacterial diseases of fish**" (V. Inglis, R.J. Roberts & N.R. Bromage, Eds.), pp. 3-23. Blackwell Scientific Publications, Oxford, UK.

Kim J-H, Hayward CJ, Joh S-J and Heo G-J (2002). Parasitic infections in live freshwater tropical fishes imported to Korea. *Diseases of Aquatic Organisms* **52**, 169-173.

Levsen A, Mo TA and Sterud E (2003). The most common infections in ornamental fish imported to Norway – a possible threat against Norwegian fauna. *Norsk Veterinaertidsskrift* **115**, 639-649.

Poppe T (1999). "**Fiskehelse og fiskesykdommer**" [in Norwegian]. Universitetsforlaget, Oslo, Norway.

Roberts RJ (1989a). The virology of teleostas. In "**Fish Pathology**", 2nd edn. (R.J. Roberts, Ed.), pp. 192-195. Bailliere Tindall, W.B. Saunders, London, UK.

Roberts RJ (1989b). Laboratory methods. In "**Fish Pathology**", 2nd edn. (R.J. Roberts, Ed.), pp. 374-405. Bailliere Tindall, W.B. Saunders,

London, UK.

Scott PW (1991). Ornamental fish. In "**Manual of Exotic Pets"** (P.H. Beynon, J.E. Cooper, Eds.), pp. 272-285. British Small Animal Veterinary Association, Shurdington, UK.

Stoskopf MK (1993a). Environmental requirements of freshwater tropical fishes. In "**Fish Medicine**" (M.K. Stoskopf, Ed.), pp. 545-553. W.B. Saunders Company, Philadelphia, USA.

Stoskopf MK (1993b). Freshwater tropical fish pharmacology. In "**Fish Medicine**" (M.K. Stoskopf, Ed.), pp. 593-601. W.B. Saunders Company, Philadelphia, USA.

Thilakaratne IDSIP, Rajapaksha G, Hewakopara A, Rajapakse RPVJ and Faizal ACM (2003). Parasitic infections in freshwater ornamental fish in Sri Lanka. *Diseases of Aquatic Organisms* **54**, 157-162.

Tomasso JR (1993). Environmental requirements and diseases of temperate freshwater and estuarine fishes. In "**Fish Medicine**" (M.K. Stoskopf, Ed.), pp. 240-246. W.B. Saunders Company, Philadelphia, USA.

Tucker CS (1993). Water analysis. In "**Fish Medicine**" (M.K. Stoskopf, Ed.), pp. 166-197. W.B. Saunders Company, Philadelphia, USA.