

Histopathological survey of lesions and infections affecting sick ornamental fish in pet shops in New South Wales, Australia

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ABSTRACT: The objective of this study was to describe the frequency of histopathological lesions and categorize histopathologically evident infections in sick ornamental fish from pet shops in New South Wales, Australia. We examined 108 fish that had evidence of morbidity or mortality, including 67 cyprinids, 25 osphronemids, 11 poeciliids, 4 characids and 1 cichlid, sourced from 24 retail outlets. Conditions frequently observed in the study population included branchitis (62/86, 72.1%), visceral granulomas (41/108, 38.0%), dermatitis (17/55, 30.9%), wasting (31/108, 28.7%), and intestinal coccidiosis (18/104, 17.4%). Branchitis and dermatitis were usually due to monogenean flukes, or flagellate or ciliate protozoa. Intralesional Microsporidia (16/41, 39.0%), mycobacteria (7/41, 17.0%), or Myxosporidia (5/41, 12.2%) were identified in the majority of fish with visceral granulomas; however, special stains were critical in their identification. The proportion of histologically evident infections was remarkably high (77/108, 71.3%), and parasitic infections predominated. Many pathogens identified in the study have low host specificity and/or direct life cycles which would facilitate transmission to exposed naïve fish populations, potentially posing a threat to native and commercial fish populations. Those caring for sick ornamental fish should take appropriate steps to investigate infectious disease and should take precautions that prevent the spread of pathogens.

KEY WORDS: Ornamental fish · Histopathology · Lesion · Parasite · Bacteria

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INTRODUCTION

The ornamental fish industry in Australia has an estimated total value, including accessories, of A\$135 to 150 million yr⁻¹ (Kahn et al. 1999). Despite this, little is known about the patterns of infections affecting ornamental fish in Australia (Whittington & Chong 2007); to the authors' knowledge, there have been no peer-reviewed surveys which catalogue diseases affecting Australian ornamental fish. Moreover, there is very little peer-reviewed literature documenting histopathologically confirmed disease patterns of ornamental fish throughout the world. This information is necessary for those who are diagnosing and

treating disease in sick ornamental fish, and is needed to facilitate disease management and biosecurity.

The objective of this study was to describe the frequency of histopathological lesions and categorize associated histopathologically evident infections in sick ornamental fish from pet shops in New South Wales (NSW), Australia.

MATERIALS AND METHODS

Fish. Study fish were opportunistically sampled from compliant retail outlets in NSW. Sampling tar-

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geted goldfish *Carassius auratus*, livebearers (family Poeciliidae), and gouramis (family Osphronemidae) with clinical signs of disease. To be eligible for inclusion in the study, fish had to fulfil one or more of the following criteria: (1) they had been reported by the retail outlet owner as 'sick' or inappetent; (2) cohorts had experienced unusually high mortalities; (3) the fish had been housed in a tank with visual evidence of treatment including dyes and oxytetracycline; or (4) abnormalities had been detected on visual examination of fish in tanks by a veterinarian. These abnormalities were poor body condition, skin lesions (including change of colour, ulceration or fin erosion), abnormal behaviour (including loss of motility, loss of schooling behaviour or lying on the bottom of the tank) and abnormal locomotion (such as spiral swimming).

Fish were anaesthetised with benzocaine until recumbent and non-responsive. The spinal cord was then severed immediately caudal to the head. This study was approved by the Animal Ethics Committee, University of Sydney, Australia.

Histopathology. Whole fish, with body walls incised to expose the coelomic cavity, were fixed in 10% neutral buffered formalin for at least 48 h. For some fish, portions of viscera were sampled for another study prior to formalin fixation. Fish with abdominal girth <2 cm were transversely sectioned (cranially to caudally) at 2 to 3 mm increments for histology. In the remaining fish, samples of liver, spleen, anterior kidney, posterior kidney, heart, intestine, stomach, skin, and brain were trimmed for histology. Tissues containing bony elements, such as skin or body wall, were decalcified with 12.5% EDTA solution pH 7.0 for 24 to 48 h prior to trimming. Tissues were processed for histology using standard methods. Sections (5 µm thick) were made and stained with haematoxylin and eosin (H&E).

Additional histochemical staining was performed in some cases. Tissues affected with visceral granulomas were stained with Ziehl Nielsen (ZN). The presence of acid-fast bacilli within foci of granulomatous inflammation was considered pathognomonic for mycobacterial infection. If a causative agent was not identified on the ZN section, additional sections were stained with Gram Twort and then Gomori Methenamine Silver (GMS). Skin sections from fish with granulomatous or ulcerative dermatitis were stained with GMS. An assortment of histochemical stains were used to characterize histomorphological features of protistan, myxosporidian and microsporidian parasites, including ZN, Giemsa, Gram Twort, Periodic Acid Schiff (PAS) and either GMS or Warthin-Starry. Fish were categorized as 'infected' if a pathogen was evident histologically.

RESULTS

A total of 108 freshwater ornamental fish were sampled from 24 retail outlets. The study population comprised 62 goldfish *Carassius auratus* Linnaeus, 1758, 15 dwarf gourami *Colisa lalia* Hamilton, 1822, 6 guppy *Poecilia reticulata* Peters, 1860, 4 neon tetra *Paracheirodon innesi* Myers, 1936, 4 blue gourami *Trichogaster trichopterus* Pallas, 1770, 3 platy *Xiphophorus maculatus* Günther, 1860, 2 molly *Poecilia sphenops* Valemcieumes, 1846, 2 rosy barb *Puntius conchonius* Hamilton, 1822, 2 rainbow sharkminnow *Epalzeorhynchus frenatum* Fowler, 1934, 1 pearl gourami *Trichogaster leeri* Bleeker, 1852 and 1 rosy red minnow *Pimephales promelas* Rafinesque, 1820. An additional 5 fish of the family Osphronemidae and 1 fish of the family Cichlidae were not identified to genus level. The mean (SE, range) number of fish collected per retail pet shop (the source) was 5 (1.20, 1 to 27) fish.

Lesions were present in 105/108 (97.2%) fish and histologically evident infection was present in 77/108 (71.3%) fish. Of infected fish, mixed infections were common, with 21/77 (27.3%) fish infected with 2 pathogens and 14/77 (18.2%) fish infected with 3 or more. The maximum number of pathogens identified in a single fish was 5. The proportions of infectious agents identified in the study are listed in Table 1.

Gill tissue

Sufficient gill tissue was present in sections to allow evaluation of 86 fish (Table 2). Gill lesions included branchitis and telangiectasis (Table 2). Gill infection was present in 41/86 (47.7%) fish. Of these, 15/41 (36.6%) fish had mixed infection. Forty out of 41 (97.6%) fish with gill infection had associated branchitis, as indicated by lamellar epithelial hyperplasia, leukocyte infiltration, and/or lamellar fusion or synechia. Parasitic infection was diagnosed most commonly including metazoans (n = 20) (Fig. 1), flagellates (n = 18) (Figs. 2 & 3), ciliates (n = 7) (Figs. 4 & 5) and Microsporidia (n = 1). Bacterial infections included epitheliocystis (n = 4) (Fig. 6) and Gram-negative filamentous rods (consistent with *Flavobacterium* spp.) (n = 2). There were 4 fish with lesions consistent with cyprinid herpes virus-2 (CyHV-2) infection, 2 with characteristic amphophilic intranuclear inclusion bodies within degenerate or necrotic lamellar epithelial cells and 2 with branchial epithelial necrosis without inclusions; however, inclusions were detected within other organs in these fish (Table 2).

Granulomas

Granulomas were present within the internal organs of 41/108 (38.0%) fish, and in nearly every case

Table 1. Histologically evident infectious agents detected in 108 freshwater ornamental fish sourced from 24 pet shops (source) in New South Wales, Australia

Infectious agent	Cyprinidae n = 67 No. (%)	Osphronemidae n = 25 No. (%)	Poeciliidae n = 11 No. (%)	Characidae n = 4 No. (%)	Cichlidae n = 1 No. (%)	Total infected No. (%)	No. of sources with infected fish, n = 24
PARASITIC	46 (68.7)	13 (52.0)	3 (27.3)	4 (100)		66 (61.1)	19
Apicomplexans							
<i>Coccidia</i>	17 (25.4)	1 (4.0)				18 (16.7)	7
<i>Cryptosporidium</i> sp.		2 (8.0)				2 (1.9)	1
Ciliates							
<i>Chilodonella</i> sp.	3 (4.5)					3 (2.8)	2
<i>Ichthyophthirius multifiliis</i>	3 (4.5)					3 (2.8)	3
<i>Trichodina</i> sp.		1 (4.0)				1 (0.9)	1
Flagellates							
<i>Ichthyobodo</i> sp.	13 (19.4)		1 (9.1)			14 (13.0)	9
<i>Piscinoodinium</i> sp.	2 (3.0)	2 (8.0)	1 (9.1)			5 (4.6)	3
Metazoans							
Monogeneans	19 (28.4)		1 (9.1)			20 (18.5)	10
Myxozoa	16 (23.9)					16 (14.8)	8
Other metazoans	1 (1.5)	2 (8.0)	1 (9.1)			4 (3.7)	2
Other							
Microsporidia	11 (16.4)	5 (20.0)		4 (100)		20 (18.5)	8
Unidentified parasite		1 (2.0)				1 (0.9)	1
BACTERIAL	7 (10.4)	8 (32.0)	1 (9.1)			16 (14.8)	11
Epitheliocystis	4 (6.0)					4 (3.7)	3
Mycobacteria	1 (1.5)	7 (28.0)				8 (7.4)	7
Gram-negative rods	3 (4.5)		1 (9.1)			4 (3.7)	3
Gram-positive cocci	1 (1.5)					1 (0.9)	1
Epitheliocystis-like		1 (4.0)				1 (0.9)	1
VIRAL	4 (6.0)	2 (8.0)				6 (5.6)	4
CyHV-2	4 (6.0)					4 (3.7)	3
Lymphocystis		2 (8.0)				2 (1.9)	2

involved more than one organ including kidney (n = 34), liver (n = 26), spleen (n = 16), gastrointestinal tract (n = 11), and heart (n = 9). Intralesional aetiologic agents were identified in the majority, 30/41 (73.2%), of cases. Mixed infections were detected in 2 of these fish.

Parasitic infection was the most commonly identified cause of granulomas (Table 2). Microsporidia were identified in 16/41 (39.0%) fish based on observation of intralesional spores. Microsporidian spores are ellipsoidal, 2 to 10 µm in length and contain a refractile posterior vacuole and a polar tubule which appears as a horizontal band. Spores were difficult to discern with H&E, but stained light blue with Giemsa (Fig. 7) and were Gram positive (purple) (Fig. 8). There was some variation in intensity of Gram stain, depending on the stage of maturation: immature spores stained red. The polar cap stained pink with PAS (Fig. 9), the spore coat stained black with GMS. The polar tubule stained acid-fast with ZN; however, this was variable and did not occur in all cases.

Intralesional myxosporidian spores were identified in 5/41 (12.2%) fish. Spores were generally 10 to 20 µm in length, had one or more polar capsules, and were difficult to discern with H&E. Mature spores stained

acid-fast with ZN (Fig. 10), and the polar capsules stained Gram positive (Fig. 11), while both the polar capsules and spore wall stained black with GMS. With Giemsa, spores stained blue with dark blue polar capsules (Fig. 12).

An unidentified parasite was observed within granulomas in the posterior kidney of a dwarf gourami *Colisa lalia* (Table 1). It was also identified in the brain of the same fish (Table 2).

Bacterial granulomas were identified in 9/41 (22.0%) fish (Table 2). Intralesional mycobacteria were identified in 7/41 (17.1%) fish. Gram-positive cocci in chains (consistent with *Streptococcus* spp.) and Gram-negative rods were both within the granulomas of 1/41 (2.4%) fish, and intralesional Gram-negative rods were present in 1/41 (2.4%) fish.

Gastrointestinal tract

The stomach and/or intestine was present in sections evaluated from 104 fish (Table 2). Gastrointestinal disease was present in 35/104 (33.7%) fish, not including fish with granulomas of the gastrointestinal tract.

Table 2. Proportion of histopathological lesions identified in 108 ornamental fish from pet shops in New South Wales, Australia. CNS: central nervous system

Tissue Histopathological lesion	Cyprinidae No. evaluated No. affected (%)	Osphronemidae No. evaluated No. affected (%)	Poeciliidae No. evaluated No. affected (%)	Characidae No. evaluated No. affected (%)	Cichlidae No. evaluated No. affected (%)	Total no. No. evaluated No. affected (%)
Gills	56	14	11	4	1	86
Branchitis	48 (85.7)	5 (35.7)	8 (72.7)		1 (100)	62 (72.1)
Branchial epithelial necrosis	4 (7.1)					4 (4.7)
Telangiectasis	2 (3.6)		2 (18.2)			4 (4.7)
Granulomas^a	67	25	11	4	1	108
Parasitic granulomas	15 (22.4)	6 (24.0)		1 (25.0)		22 (20.4)
No aetiology identified	6 (9.0)	2 (8.0)	2 (18.2)		1 (100)	11 (10.2)
Bacterial granulomas	3 (4.5)	6 (24.0)				9 (8.3)
Gastrointestinal tract	65	24	10	4	1	104
Gastritis		1 (4.2)				1 (1.0)
Enteritis	12 (18.5)	3 (12.5)				15 (14.4)
Mucosal atrophy	14 (21.5)	2 (8.3)				16 (15.4)
Skin	18	22	10	4	1	55
Dermatitis	9 (50.0)	5 (22.7)	1 (10.0)			15 (27.3)
Ulcer	1 (5.6)	3 (13.6)				4 (7.3)
CNS	52	17	11	1	1	82
Encephalitis	5 (9.6)	2 (11.8)				7 (8.5)
Other encephalopathy ^b		1 (5.9)				1 (1.2)
Liver	64	22	11	4	1	102
Hepatic lipidosis	5 (7.8)	3 (13.6)	7 (63.6)	2 (50.0)		17 (16.7)
Telangiectatic hepatopathy	1 (1.6)	1 (4.5)				2 (2.0)
Kidney	64	25	10	4	1	104
Renal tubular proteinosis		4 (16.0)		1 (25.0)		5 (4.8)
Renal tubular myxosporidiasis	4 (6.3)					4 (3.8)
Herpesviral haematopoietic necrosis	3 (4.7)					3 (2.9)
Renal haematopoietic necrosis (unknown origin)			1 (10.0)			1 (1.0)
Glomerulonephritis		1 (4.0)	1 (10.0)			2 (1.9)
Wasting^c	65	24	11	4	1	105
Wasting	25 (38.5)	4 (16.7)	1 (9.1)	1 (25.0)		31 (29.5)

^aGranulomas were localized to more than 1 organ in nearly every case; organs involved included kidney (n = 34), liver (n = 26), spleen (n = 16), gastrointestinal tract (n = 11), and heart (n = 9). ^bAn unidentified parasite within CNS tissue that was not associated with tissue damage or response. ^cFish with hepatocellular and/or pancreatic acinar atrophy, no ingesta present in the gastrointestinal tract and no fat deposits in the coelomic cavity were classified as wasting

Gastric abnormalities were present in 3/104 (2.9%) fish. Two out of 104 (1.9%) fish, both dwarf gourami *Colisa lalia*, suffered from gastric cryptosporidiosis. Sporogonial stages were observed in the mid to basal portion of the epithelium. All stages stained dark blue with Giemsa. Amylopectin granules in the macrogamonts and oocyst residuum stained pink with PAS (Fig. 13). No apparent host response was observed in the stomach of either fish. One out of 104 (1.0%) fish had gastric helminthiasis associated with eosinophilic granular cell gastritis (Table 2).

Thirty-two out of 104 (30.8%) fish were affected with intestinal abnormalities (Table 2). Intestinal coccidial infection occurred in 18/104 (17.3%) fish: 17 *Carassius auratus* and 1 *Trichogaster trichopterus*. In *C. auratus*, oocysts were located in the basal portion of the lamina epithelialis and in the lamina propria.

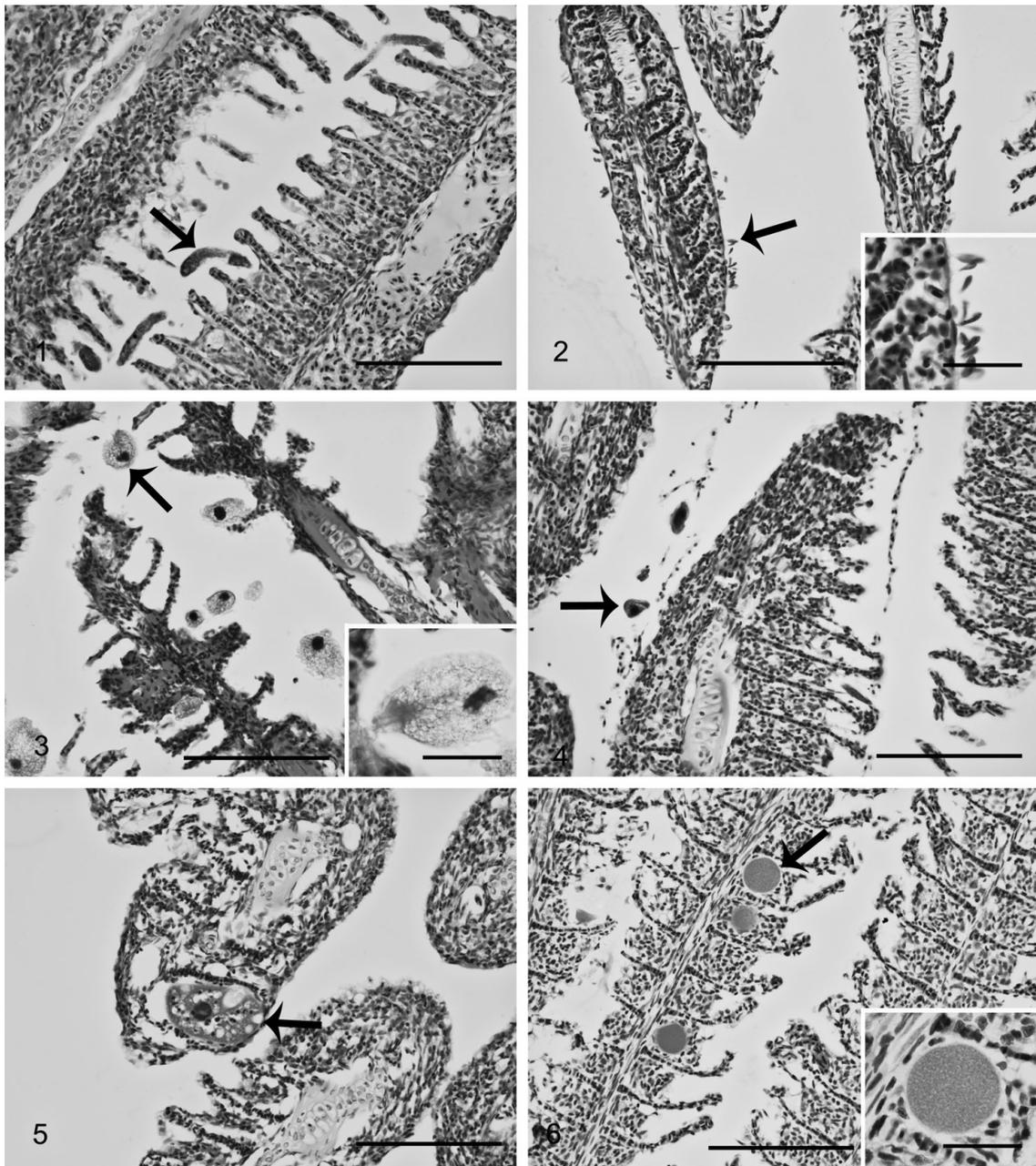
Oocysts contained 4 sporocysts, each containing 2 sporozoites and a residual body (Fig. 14). Sporocysts stained black with Warthin-Starry, acid-fast with ZN, pink with PAS and blue with Giemsa. In *T. trichopterus* the morphology of the coccidia were consistent with *Goussia trichogasteri* (Kim & Paperna 1993). Six out of 18 (33.3%) fish with intestinal coccidiosis had concurrent enteritis affecting areas where coccidia were present, characterised by increased numbers of mononuclear leukocytes within the lamina propria. Ten out of 18 (55.6%) fish with coccidiosis had concurrent intestinal mucosal atrophy, characterised by blunting and fusion of the mucosal folds and decreased mucosal thickness. One out of 104 (1.0%) fish had intestinal helminthiasis associated with severe mucosal atrophy and eosinophilic granular cell enteritis. Eight out of 104 (7.7%) fish had

enteritis and 5/104 (4.8%) fish had intestinal mucosal atrophy of unknown aetiology.

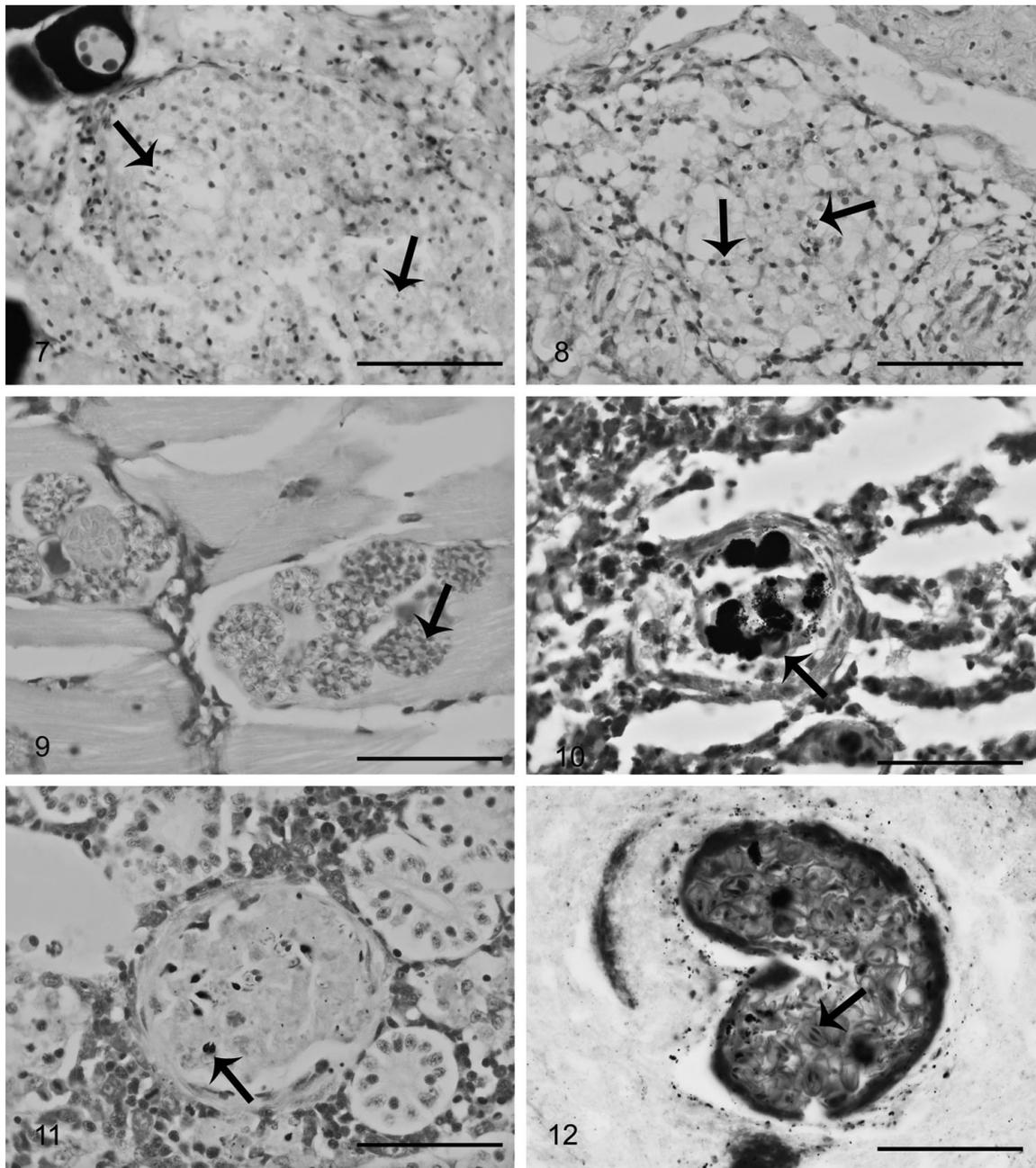
Integument

Sufficient skin was present for evaluation in the sections of 55 fish (Table 2). Skin lesions were present

in 17/55 (30.9%) fish. Six out of 15 (40.0%) fish with dermatitis had concurrent cutaneous ectoparasitism, including *Piscinoodinium* spp. (n = 3), Monogenea (n = 2) and *Ichthyobodo* sp. (n = 1). Aggregates of vegetative myxosporidian stages were present in the dermis and subcutis of 3/15 (20.0%) fish with dermatitis. Hypertrophied dermal fibroblasts with intracytoplasmic basophilic inclusion bodies consistent with



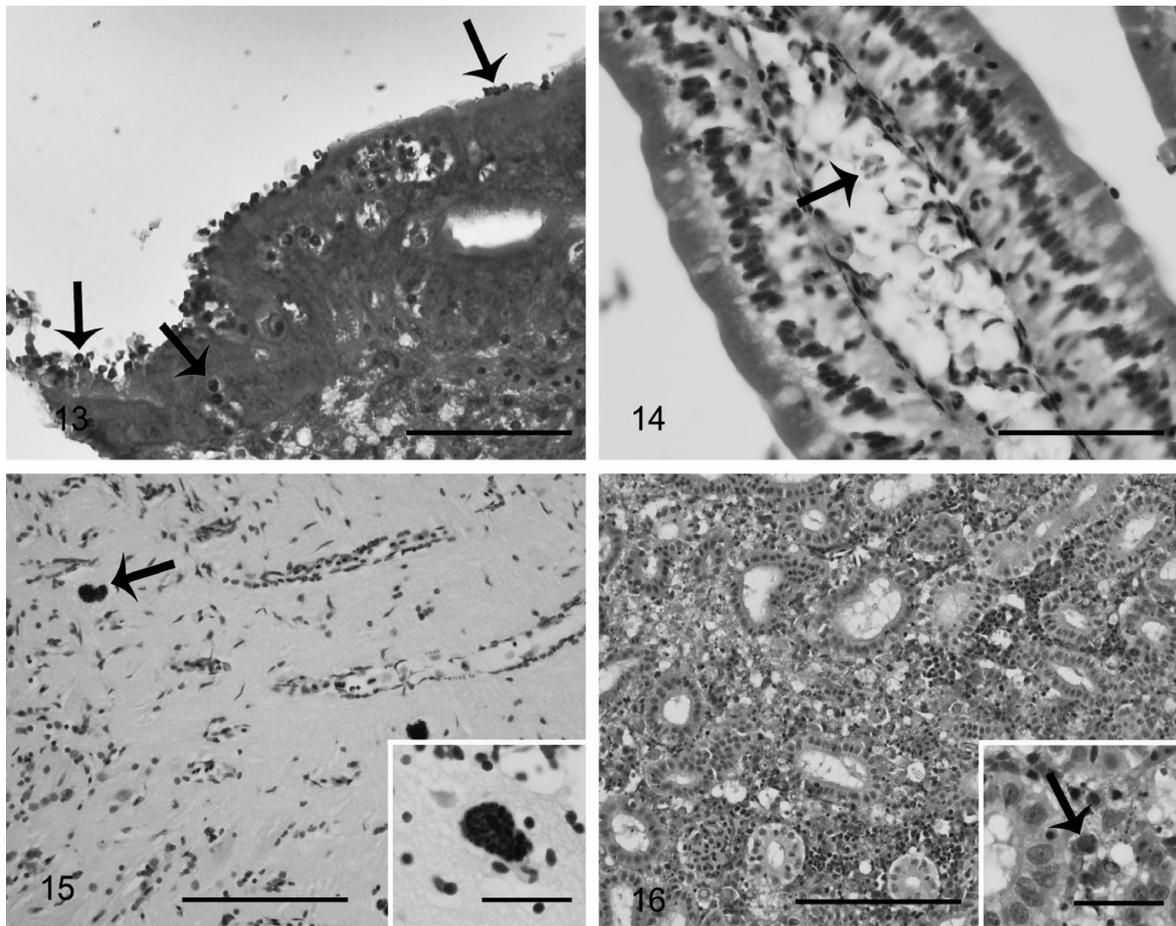
Figs. 1 to 6. Gill tissue with branchitis. Fig. 1. *Carassius auratus*. Parasitic monogenean trematode is shown (arrow). Fig. 2. *Carassius auratus* infected with *Ichthyobodo* sp. (arrow). Inset: *Ichthyobodo* sp. on surface of gill. Fig. 3. *Epalzeorhynchus frenatum* infected with *Piscinoodinium* sp. (arrow). Inset: *Piscinoodinium* sp. Fig. 4. *Carassius auratus* infected with *Chilodonella* sp. (arrow). Fig. 5. *Carassius auratus* infected with *Ichthyophthirius multifiliis* (arrow). Fig. 6. *Carassius auratus*. Lesions consistent with *Epitheliocystis* are shown (arrow). Inset: *Epitheliocystis* lesion in gill. All sections were stained with H&E. All scale bars = 120 μ m, except inset scale bars (all 25 μ m)



Figs. 7 to 12. Visualisation of microsporidian and myxosporidian spores with special stains useful for their identification. Figs. 7 & 8. *Colisa lalia*. Granulomatous oophoritis with intralesional microsporidian spores. Giemsa stains microsporidian spores light blue (arrows, Fig. 7) and Gram Twort stains mature spores purple (arrows, Fig. 8). Fig. 9. *Paracheirodon innesi*. Microsporidian infection of skeletal muscle myocytes, consistent in histological appearance with infection with *Pleistophora hypheobryconis*. PAS stains polar capsule of microsporidian spores pink (arrow). Figs. 10 to 12. *Carassius auratus*. Fig. 10. ZN stains mature myxosporidian spores (arrow) within granuloma in posterior kidney acid-fast. Fig. 11. Polar capsules (arrow) of myxosporidian spores within granuloma in posterior kidney stain purple with Gram Twort. Fig. 12. Granuloma in anterior kidney with intralesional myxosporidian spores (arrow). Giemsa stains spores blue, polar capsules stain darker blue. Scale bars = 50 µm

lymphocystis virus infection were present in the dermis of 2/15 (20.0%) fish with dermatitis. Ulcerative and granulomatous dermatitis contained intralesional mycobacteria in 2/15 (20.0%) fish with dermatitis.

One had concurrent visceral granulomas with intralesional mycobacteria. No intralesional pathogens were detected in 2 fish with dermatitis and 2 fish with ulcers.



Figs. 13 to 16. Fig. 13. *Colisa lalia*. Gastric cryptosporidiosis. PAS stains cryptosporidial stages on the gastric mucosa pink (arrows). Scale bar = 50 μ m. Figs. 14 to 16. *Carassius auratus*. Fig. 14. Coccidial oocysts (arrow) in the basal portion of the lamina epithelialis and lamina propria of intestine. Scale bar = 50 μ m. Fig. 15. Aggregate of vegetative Myxosporidia (arrow) in brain. Scale bar: 120 μ m. Inset: Vegetative myxosporidian stages in brain. Scale bar = 25 μ m. Fig. 16. Renal haematopoietic necrosis with karyomegalic haematopoietic cells with intranuclear amphophilic inclusion bodies surrounding necrotic areas. Scale bar: 120 μ m. Inset: haematopoietic cell with karyomegaly and intranuclear amphophilic inclusion body (arrow) and renal haematopoietic necrosis. Scale bar = 25 μ m

Central nervous system

Adequate brain tissue was present for evaluation in the sections of 82 fish (Table 2) and 8/82 (9.8%) had a central neurological abnormality. Seven fish had non-suppurative encephalitis; mononuclear leukocytes cuffed cerebral vessels infrequently associated with foci of necrosis. Of fish with non-suppurative encephalitis, 5 goldfish *Carassius auratus* from 3 sources had intralesional Myxosporidia, 1 dwarf gourami *Colisa lalia* had an intralesional organism resembling those of the Chlamydiales family, and 1 *C. lalia* had encephalitis of unknown aetiology. In myxosporidian encephalitis, aggregates of multicellular vegetative myxosporidian stages were multifocally distributed within grey and white matter of the brain (Table 2, Fig. 15) and were associated with mild non-suppurative

inflammation in adjacent tissue. An unidentified parasite was present in the brain of 1 *C. lalia*, but was not associated with tissue damage or response (Table 2).

Other organs

Liver was present in sections of 102 fish (Table 2), and lesions consisted of hepatic lipidosis (n = 17, 16.7%), and telangiectatic hepatopathy (n = 2, 2.0%) (Table 2). Anterior kidney and/or posterior kidney was present in the sections of 104 fish, and lesions consisted of renal tubular myxosporidiasis (n = 4, 6.3%), renal tubular proteinosis (n = 5, 4.8%), glomerulonephritis (n = 2, 1.9%), herpesviral haematopoietic necrosis (n = 3, 2.9%) and renal haematopoietic necrosis of unknown origin (n = 1, 1.0%) (Table 2). No renal tubule pathology was associated with vegetative myxosporidian

stages. In 1 fish with vegetative myxosporidian stages in the renal tubules, these stages were also present in the dermis and subcutis (reported under 'Integument' above). Sections of kidney were available for 3/4 fish with CyHV-2 infection, and in each the haematopoietic cells surrounding areas of multifocal haematopoietic necrosis had karyomegaly with intranuclear amphophilic inclusion bodies (Fig. 16).

Spleen sections were available for 3/4 fish with CyHV-2; haematopoietic necrosis was present in 2/3 (66.7%) fish. Karyomegaly and intranuclear inclusion bodies were not as prominent in the spleen relative to kidney.

Histopathological lesions consistent with neon tetra disease caused by *Pleistophora hypheobryconis* were present in 4 neon tetra *Paracheirodon innesi*. Merogonial plasmodia, sporogonial plasmodia and sporocysts were present within skeletal muscle myocytes in all fish. Sporocysts contained large numbers of spores (>16), which is consistent with *Pleistophora* spp. (Canning & Nicholas 1980). One fish had concurrent visceral granulomas with intralesional microsporidian spores (reported under 'Granulomas' above).

Wasting

Fish with the following combination of findings were classified as suffering from wasting: hepatocellular atrophy, pancreatic acinar atrophy, no ingesta present in the gastrointestinal tract, and no fat deposits in the coelomic cavity. Exocrine pancreatic atrophy comprised reduced pancreatic acinar cell size, decreased number or size of cytoplasmic zymogen granules and overall reduction in exocrine pancreatic tissue mass. Hepatocellular atrophy comprised reduced hepatocyte cell size, reduced nuclear size with condensed chromatin, thinning of hepatic cords and overall reduction in hepatic tissue mass. In total, 31 fish were affected with wasting (Table 2). Concurrent systemic illness—including visceral granulomas, gastrointestinal parasitism or enteritis—was present in 19/31 (61.3%) fish with wasting. The remaining 12 of these 31 (38.7%) fish with wasting were suspected as suffering from malnutrition or inanition.

DISCUSSION

This study documents diseases and associated infections that are present among sick freshwater ornamental fish in retail outlets of New South Wales. Australia imported 17.7 million ornamental fish in 2006–2007 and produced 8.3 million domestically; 91% of the latter were goldfish *Carassius auratus* (O'Sullivan et al. 2008). Therefore, it is likely that many of the fish other

than *C. auratus* in the present study were imported; however, some of the *C. auratus* may also have been imported. Conditions frequently observed in the study population included branchitis (62/86, 72.1%), visceral granulomas (41/108, 38.0%), dermatitis (17/55, 30.9%), wasting (31/108, 28.7%), and intestinal coccidiosis (18/104, 17.4%).

Branchitis and dermatitis were usually due to parasitic infestation, including monogenean flukes *Ichthyobodo* sp., *Piscinoodinium* sp., *Chilodonella* sp. and *Ichthyophthirius multifiliis*. The occurrence of these infections was probably underestimated in the study population due to reliance on histopathology for detection; examination of wet-mounted gill and skin preparations are more sensitive than histology for detecting these infections. No aetiology was histologically evident for several fish with severe branchitis, emphasizing the importance of evaluating wet-mounted gill preparations and water quality when investigating illness in aquarium fish. Epitheliocystis was also a notable cause of branchitis, and mycobacteria and lymphocystis virus were important aetiologies for dermatitis as well.

Intralesional aetiologic agents, especially Microsporidia, Myxosporidia, and mycobacteria, were present in the majority of fish with visceral granulomas; however, special stains were critical in their identification. ZN was especially helpful for identifying mature myxosporidian spores and mycobacteria, whereas Gram stain was especially useful for identifying Microsporidia. The proportion of mycobacteria-infected fish in the study population was 7.4%, which is slightly lower than that reported from Sweden (11%, Hongslo & Jansson 2009), and Germany (12%, Engelhardt 1992). The most common isolates from ornamental fish are *Mycobacterium marinum*, *M. fortuitum* and *M. chelonae* (Pate et al. 2005, Gómez 2008). Since aquatic mycobacteria can cause localized and disseminated infection in man (Noga 1996b, Pate et al. 2005, Gómez 2008), carers should consider wearing gloves as a precautionary measure when handling sick ornamental fish and aquarium contents (Noga 1996b). Microsporidia and Myxosporidia were much more common in the present study than has previously been reported from freshwater ornamental fish examined by histopathology in Australia (Stephens et al. 2009) and Sweden (Hongslo & Jansson 2009). However, Stephens et al. (2009) did not use special stains to facilitate organism recognition. Relatively little is known regarding the status of microsporidian species in ornamental fish in Australia (Kahn et al. 1999), and further work in this area is warranted given the frequency of infection, low host specificity and direct transmission of certain species (Feist & Longshaw 2006). In contrast to Microsporidia, transmission of Myxosporidia generally requires passage through a definitive host, usually an oligochaete or

bryozoan (Feist & Longshaw 2006), and may be more likely to occur in fish fed live food or held in outdoor ponds (Kahn et al. 1999, Lowers & Bartholomew 2003). In Australia, goldfish *Carassius auratus* and poeciliids are commonly raised in outdoor ponds, and many ornamental fish are imported from Southeast Asia where use of outdoor ponds is widespread (Kahn et al. 1999). Vegetative stages of Myxosporea, not necessarily associated with visceral granulomas, also caused encephalitis and/or dermatitis in several study fish. It is unclear whether the Myxosporea observed in this study were single or multiple species, or were endemic or emerging species. Definitive speciation is warranted and would require examination of spores in wet mount preparations, electron microscopy, or molecular characterization (Feist & Longshaw 2006).

Intestinal coccidiosis, a condition common among study goldfish *Carassius auratus* (17/65, 26.2%), was often associated with enteritis, intestinal mucosal atrophy, and/or wasting, as reported by others (Molnar 2006, Steinhagen & Davies 2008, Roberts et al. 2009). Again, histopathology is not a sensitive technique for diagnosis of this infection, and although special stains may have facilitated its recognition, its occurrence in the study population may have been underestimated. Nonetheless, intestinal coccidiosis was more common than reported by other histopathological surveys (Honglo & Jansson 2009, Stephens et al. 2009). Definitive identification requires examination of fresh oocysts (Lom & Dyková 1995); therefore, it is unclear whether the coccidia identified in the present study population were *Goussia carpelli* (Leger & Stankovich 1921), which has been reported in Australia (Lom & Dyková 1995), or another species such as *Eimeria aurati* (Hoffman 1965) or *E. carassiusaurati* (Romero-Rodriguez 1978). Piscine coccidia are relatively host specific, and it is unlikely that infection would be transmitted to fish in other genera (Molnar 2006, Steinhagen & Davies 2008). However, the frequency of coccidiosis amongst *C. auratus* could be problematic for the domestic goldfish industry, and investigations of illness in New South Wales goldfish should probably include examination of fecal smears.

Wasting was usually associated with concurrent disease; however, no explanatory conditions were found in a substantial proportion of fish with wasting. Those investigating wasting in ornamental fish should consider husbandry issues which may lead to malnutrition or inanition.

The frequency of histologically evident infection in study fish was remarkably high (71.3%) since histopathology is not a sensitive technique for identifying many infectious agents. The frequency of microsporidian and myxosporidian infection in particular was noteworthy and was higher than in histopathologic obser-

vations of sick ornamental fish in Germany (Engelhardt 1992), Sweden (Honglo & Jansson 2009) and in border quarantine premises in Australia (Stephens et al. 2009). A number of factors likely contribute to the frequency of infection in aquarium fish. Stress factors—including poor nutrition, poor water quality, inappropriate water temperature for a particular species and changes in water pH—especially during and immediately post transport, suppress the immune system and predispose fish to infection, or can lead to breakdown of carrier states resulting in sub-clinically infected fish developing clinical disease (Noga 1996a). Other factors which facilitate infection in an aquarium setting include handling and use of nets (Dror et al. 2006), high stocking density, poor biosecurity, and mixing of fish of multiple species and from multiple sources (Goodwin 2002). Aquarium fish carers and health providers should be aware that infectious disease is common among sick fish, and mitigation strategies should aim to address factors which favour infection.

In line with the high frequency of parasitic infection in the present study (66/108, 61.1%), parasites are considered to be the most common infectious agents affecting ornamental fish (Roberts et al. 2009). However, the present study targeted sick fish, and therefore the proportion of parasite infection is probably lower in the general ornamental fish population. Other studies have reported lower prevalences of parasite infection, from 32% (Engelhardt 1992) to 54% (Fuchs 1983). On the other hand, bacterial and viral infections were less common than parasite infections in the present study, but their occurrence would have been underestimated because ancillary microbial isolation techniques are more sensitive than histopathology.

Several pathogens of ornamental fish have low host specificity and can be transmitted directly. These pathogens are more likely to become established in naïve domestic fish, including native and commercial fish populations (Dove 2000). Exposure can occur via release or disposal of ornamental fish or aquarium contents into waterways, use of ornamental fish as bait and cohabitation of ornamental and other aquaculture species in commercial premises (Kahn et al. 1999, Whittington & Chong 2007). Twenty-two species of ornamental fish have established breeding populations in Australian waterways (Lintermans 2004). Many pathogens causing disease outbreaks in Australian native fish are thought to have been introduced through contact with ornamental fish; these include *Ichthyophthirius multifiliis*, *Ichthyobodo necator*, *Trichodina* spp., *Chilodonella cyprini*, *Chilodonella hexasticha* (Kahn et al. 1999), and *Megalocytivirus* (Whittington & Chong 2007). Many pathogens documented in this study are suspected to have been introduced to Australia via imported ornamental fish including some

species of monogenean flukes (Dove & Ernst 1998, Fletcher & Whittington 1998), *Trichodina* sp. (Dove 2000, Dove & O'Donoghue 2005), *I. multifilis* (Kahn et al. 1999, Dove 2000), *I. necator* (Kahn et al. 1999), *Chilodonella* spp. (Kahn et al. 1999), *Goussia carpelli* (Kahn et al. 1999), *Pleistophora hypheobryconis* (Kahn et al. 1999), and CyHV-2 (Stephens et al. 2009). CyHV-2 was only recently introduced to Australia with imported goldfish, and is now clearly present in goldfish in retail outlets in New South Wales. Unusual infections were identified in the present study, including gastrointestinal cryptosporidiosis and chlamydial encephalitis. Cryptosporidiosis was only diagnosed in dwarf gourami *Colisa lalia* in the present study and it is uncertain whether this represents a new species. Novel *Cryptosporidium* spp. have recently been identified in a number of ornamental fish species in Western Australia; however, *C. lalia* were not included in the study (Zanguee et al. 2010). Given the limited size of the study population, it is likely that other unexpected infectious diseases exist in ornamental fish in Australia. It is important that diagnosticians are able to investigate and diagnose disease in ornamental fish so that exotic or emerging infectious diseases are detected promptly, before spread to other populations occurs.

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