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Osteological and histopathological details of unilateral microphthalmia and anophthalmia in juvenile common carp (*Cyprinus carpio*)

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ABSTRACT

We describe and compare normal eye, unilateral microphthalmia (small eye), and unilateral anophthalmia (eye incompletely developed) in young (5–7 month-old) common carp, *Cyprinus carpio*, (Cypriniformes: Cyprinidae). Five of 1058 (0.47%) common carp had grossly-observed eye deformities that we characterized using osteology and histopathology: 2 (81 and 98 mm fork length) had unilateral microphthalmia and 3 (103, 123, and 137 mm in fork length) had unilateral anophthalmia. Grossly, the eye of the microphthalmic common carp was opaque and slightly protruding from a reduced orbit; whereas, only a slight integumental concavity was observed on the deformed side of the anophthalmic common carp. Osteologically, microphthalmic and anophthalmic common carp had an irregular orbit with orbital bones that were laterally-expanded and that formed a discontinuous, uneven orbital perimeter compared to the narrow orbital bones forming a nearly circular orbit in normal common carp. Histologically, microphthalmia manifested as an eye having a thickened cornea with a retina and attached optic nerve but no lens (aphakia); whereas, anophthalmia manifested as lacking a globe, cornea, lens, or retina but having a nerve fascicle and capillaries representing probable optic nerve and its associated choroid. No histological sign of infection was observed in the ocular region of any common carp studied. The etiological agent associated with microphthalmia and anophthalmia is indeterminate, and the hypothesis that electroporation caused the ocular deformity was not tested herein. We suspect that the microphthalmic eye could detect light but not form a focused image. A list of putatively similar abnormalities in other fishes as well as anisophthalmia (incomplete eye development) and symmetrical cyclopia (medially fused eye) in larval and juvenile fishes is provided. This is the first published report providing osteological or histopathological details of microphthalmia or anophthalmia in a non-embryonic or non-larval fish.

1. Introduction

Ophthalmology of fish is little studied relative to that of mammals, and few studies have detailed deformations or pathological changes to fish ocular tissues (Dukes, 1975). In general, and relative to other non-skeletal animal tissues, the eye itself is difficult to study with histology because it includes dense tissues that challenge proper preservation and sectioning. The lens is dense and sectioning it with routine histological methods typically results in poor sections riddled with artifactitious separation of tissues; a dense sclera prevents rapid fixative penetration; and a metabolically active retina is prone to artifact without rapid fixation (Roberts et al., 2012).

Most reports of infectious and non-infectious eye diseases in fishes derive from aquaculture production and experimental culture facilities wherein they can impact fish health, indicate husbandry problems, or comprise endpoints of toxicological studies (Hargis, 1991). They can result from (i) exposure to environmental stressors (e.g., ultraviolet light exposure) that manifest as cataracts (Steucke et al., 1968), (ii) nutritional deficiencies that manifest as cataracts and concomitant ocular degeneration (Allison, 1950; Waagbø et al., 2010; Lee et al., 1976; Satoh et al., 1983; Yokote, 1974), (iii) exposure to toxins, low dissolved oxygen, or heat that manifests as ontogenic ocular deformation (Table 1 and references therein), and (iv) infections of adult ectoparasites or larval endoparasites that are associated with a range of lesions attributable to parasite attachment or encystment (Bullard and Overstreet, 2008). To our knowledge, however, no study has provided osteological and histopathological details of ontogenetic microphthalmia or anophthalmia in a non-larval fish (Table 1).

Herein, we describe the gross, osteological, and histological details of unilateral microphthalmia and unilateral anophthalmia observed among several of the common carp, *Cyprinus carpio*. Sperm, one-cell embryos, or

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Table 1
Eye deformities in fish from the wild and experiments.

Fish	Deformity	Stage	Source	Etiology	Reference(s)
Acipenser baerii, Siberian sturgeon	Ano, cycl	Larva	Yangtze River Fisheries Research Institute, Beijing, China	Triphenyltin	Hu et al. (2009)
Acipenser sinensis, Chinese sturgeon	Ano, cycl	Larva	Yangtze River, Yichang City, China	Triphenyltin	Hu et al. (2009)
Danio rerio, zebrafish	Ano, cycl, micro	Larva	NS	NS; heat, hypoxia	Ingalls and Murakami (1962)
	Micro	Larva	NS	Heat	Dukes (1975)
	Cycl	Embryo	NS	Ethylnitrosourea	Rebagliati et al. (1998)
	Cycl	Embryo	University of Oregon, Eugene, OR, USA	Compared wild type with *cyclops cyc-1* (*b16*) strains	Strähle et al. (2012)
Cyprinus carpio, common carp	Ano, 3 eyes, 4 eyes, SCy	Larva	Inland Fisheries Institute, Żabieniec, Poland	Cu, Pb toxicity	Jezierska et al. (2000)
	NS	Embryo; larva	Research Institute of Fish Culture and Hydrobiology, Vodnany, University of South Bohemia, Czech Republic	NO_2^-	Kroupova et al. (2010)
Fundulus heteroclitus, *mummichog*	Ani, ano, cycl, exo, micro, SCy	Embryo	Horton's Creek, New Brunswick, Canada	17α-Methyltestosterone, cyproterone acetate	Boudreau et al. (2005)
Oryzias latipes, medaka	Ani	Embryo	NS	*N*-nitroso-*N*-methylurea	Marty et al. (1990)
	Ani	Larva	Carolina Biological Supply, Burlington, North Carolina	4-Tert-octylphenol	Gray et al. (1999)
	Ano, cycl	Embryo; Larva	NS	N-ETHYL-N-NITROSOUREA	Loosli et al. (2000)
	Ani	Embryo	Duke Forest Research Facility, Durham, North Carolina	Naphthoic acid	Carney et al. (2008)
	Ani, ano, cycl	Embryo	Carolina Biological Supply, Burlington, North Carolina	Pentachloronitrobenzene	Metcalfe et al. (2008)
	Ani, ano, micro	Larva	Argent Laboratories, Redmond, VA, USA; Marienfeld Superior Laboratory, Germany	Cd^{2+} toxicity	González-Doncel et al. (2003)
Oncorhynchus mykiss × *Salmo trutta* hybrid	Ano, SCy	Larva	Quinnebaug Valley Hatchery, Central Village, CT, USA	NS; heat	Bolker and Thomson (1992)
Oncorhynchus mykiss (as *Salmo irideus*)	Micro	Larva	NS	NS	Corsin (1961)

Cycl Cyclopia, single orbit
Micro Microphthalmia, small eye
Ano Anophthalmia (unilateral or bilateral anophthalmia), absence of eye or presence of vestigial eye
Ani Anisophthalmia, unequal eye development
SCy Symmetrical cyclopia, range of eye fusion medially
Exo Exophthalmia, protruding eye
NS Not specified

one-cell embryos fertilized with electroporated sperm of these carp were electroporated with a variety of DNA constructs. Although we lack any replicate or sufficient sample size to confidently assert that these processes caused any ocular deformity, we mention those details herein simply so that future workers can build from these results. Few studies specifically diagnose the cellular nature of seemingly non-infectious, putatively ontogenetic ocular abnormalities in non-embryonic or non-larval fish. Hence, our specific objective herein was to fill that gap in the literature by providing foundational osteological and histological descriptions of these abnormalities in fish.

2. Material and methods

Common carp, *C. carpio* Linnaeus, 1758, (Cypriniformes: Cyprinidae) were artificially spawned at the E. W. Shell Fisheries Center (Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama, USA). Deformed common carp were opportunistically observed during other research activities related to electroporation of gametes and embryos. In specific, sperm, one-cell embryos, or one-cell embryos fertilized with electroporated sperm were electroporated with a variety of DNA constructs including a shRNAi of aromatase, ricin, and red fluorescent protein genes. A non-DNA control was included. For sperm only treatments, sperm was electroporated, and then used to fertilize the eggs. For egg only treatments, eggs were fertilized with non-electroporated sperm, and the resulting embryos electroporated 20 min post fertilization. For the sperm and egg treatments, eggs were fertilized with electroporated sperm and the resulting embryos electroporated again 20 min post-fertilization. Non-electroporated sperm was used to fertilize the non-DNA control, and these embryos were electroporated in a buffer without DNA 20 min after fertilization. Electroporation was performed with the Baekon 2000 macromolecule transfer system (Baekon, Inc. Saratoga, California). Parameters were set at 6 kV, 2^7 pulses, 0.8 s burst, 4 cycles, 160 μs (Powers et al., 1992). Non-contact mode of electroporation with the electrode 1 to 2 mm above the buffer was applied. Embryos were initially incubated in Holtfreter's solution (NaCl 3.5 g, $NaHCO_3$ 0.2 g, KCl 0.05 g, $MgSO_4$ 333 μl (300 g in 500 ml), $CaCl_2$ 333 μl (150 g in 500 ml), pH: 7–7.5 in 1.0 l dechlorinated water) in separate tubs (Armstrong et al., 1989). Water temperature was maintained at 25–26 °C using submersible heaters. After hatching, fry were cultured in the same tubs. Later, they were transferred to and cultured in raceways supplied with dechlorinated city water.

A total of 9 individual common carp (4 grossly normal, 2 having a small orbit [unilateral microphthalmia], and 3 lacking an orbit [unilateral anophthalmia]) were studied for osteological and histopathological details. Normal carp were 80, 88, 95, and 100 mm in fork length, and estimated to be aged 5–6 months. Microphthalmic carp were 81 and 98 mm in fork length. Anophthalmic carp were 103, 123, and 137 mm in fork length and estimated to be 5–7 months old (Vilizzi and Walker, 1999). The deformed common carp were removed from raceways, euthanized with tricainemethansulfonate (MS-222), and immediately fixed whole by immersion in 10% neutral buffered formalin ($n = 6$) or Bouin's fixative ($n = 3$). Studied fishes were allocated for gross photography (Figs. 1–4), whole–body clearing and staining (Figs. 5–10), and histology (Figs. 11–22). Gross photographs were facilitated by a geared rail and track system (StackShot module; Cognisys, Inc., Kingsley, Michigan) at 2 frames/mm with a Canon 5D digital single lens reflex camera mounted to an adjustable boom stand, with resulting images assembled using Zerene Stacker v1.5 (Zerene Systems LLC, Richland, Washington).