

# Chemical Ototoxicity of the Fish Inner Ear and Lateral Line

Allison B. Coffin and John Ramcharitar

**Abstract** Hair cell-driven mechanosensory systems are crucial for successful execution of a number of behaviors in fishes, and have emerged as good models for exploring questions relevant to human hearing. This review focuses on ototoxic effects in the inner ear and lateral line system of fishes. We specifically examine studies where chemical ototoxins such as aminoglycoside antibiotics have been employed as tools to disable the lateral line. Lateral line ablation results in alterations to feeding behavior and orientation to water current in a variety of species. However, neither behavior is abolished in the presence of additional sensory cues, supporting the hypothesis that many fish behaviors are driven by multisensory integration. Within biomedical research, the larval zebrafish lateral line has become an important model system for understanding signaling mechanisms that contribute to hair cell death and for developing novel pharmacological therapies that protect hair cells from ototoxic damage. Furthermore, given that fishes robustly regenerate damaged hair cells, ototoxin studies in fishes have broadened our understanding of the molecular and genetic events in an innately regenerative system, offering potential targets for mammalian hair cell regeneration. Collectively, studies of fish mechanosensory systems have yielded insight into fish behavior and in mechanisms of hair cell death, protection, and regeneration.

**Keywords** Hair cell • Lateral line • Ear • Auditory • Fish

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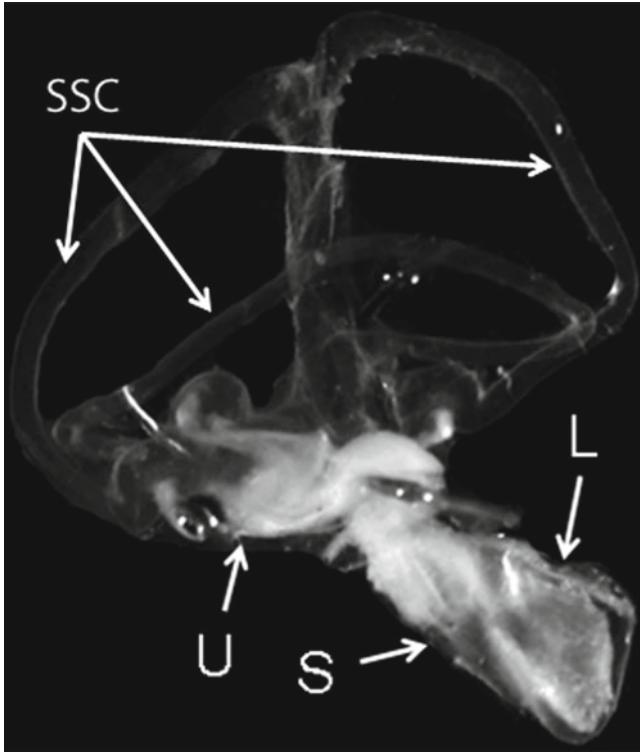
## 1 Introduction

The sensory arsenal of fishes has likely contributed to their tremendous evolutionary success, allowing them to thrive in diverse habitats. Fishes are armed with chemical, visual, tactile, auditory, and vestibular sensory apparatus that are morphologically and functionally comparable to those found in other vertebrate taxa. Sensory hair cells serve as receptors in both the auditory and vestibular systems, and in an additional hair cell-based system, the mechanosensory lateral line found in fishes and aquatic amphibians. Together, these sensory systems operate in concert to provide fishes with a comprehensive picture of the ambient environment. Multisensory integration is a critical feature for successful execution of a host of fish behaviors such as prey detection, predator avoidance, and escape responses (Collins et al. 2003; Mirjany et al. 2011; Van Trump and McHenry 2013).

Here, we review studies of fish hair cell systems, with a focus on how chemical ototoxins, substances that damage hair cells, have been employed as tools to enhance fish hair cell research. We first examine how ototoxins, combined with detailed morphological assessment, have informed our understanding of vertebrate hair cell heterogeneity and evolution. We then look at studies of mechanosensory-mediated behaviors in fishes, again relying on selective ablation with ototoxins to determine the relative contribution of these systems to fish behavior. In addition to understanding fish mechanosensation, the inner ear and lateral line have been deployed as valuable models for human auditory studies. Section 4 describes some of this biomedical research, both the cellular understanding of ototoxicity itself and the use of ototoxic tools for investigating hair cell regeneration. The bulk of both behavioral and biomedical studies use known ototoxins, such as aminoglycoside antibiotics, but several studies suggest that many other compounds are potentially ototoxic (Hirose et al. 2011). We conclude with a brief description of newly identified putative ototoxins, specifically those found in the aquatic environment, and with some of the unresolved questions that ototoxic research may help answer in the future.

The inner ears of fishes comprise three semicircular canals, each with associated sensory cristae and three otolithic end organs, the saccule, utricle, and lagena (Fig. 1; Popper 1977, 1978; Popper and Lu 2000). All end organs contain populations of sensory hair cells interdigitated with non-sensory supporting cells. The semicircular canals and the utricle primarily subserve vestibular functions, although the utricle plays an auditory role in some taxa. On the other hand, the saccule is the primary auditory end organ in most species, while the function of the lagena is poorly understood (Popper and Lu 2000). The vestibular organs provide positional information, while the auditory organ(s) allow the fish to detect both abiotic (e.g., rainfall or wave noise) and biotic (e.g., conspecific calls) acoustic stimuli of biological relevance. Psychophysical studies in the late 1990s demonstrated that fishes are capable of auditory scene analysis, and this finding supports the hypothesis that they are broadly sensitive to sound stimuli from diverse sources (Fay 1998; Fay and Popper 2012).

The second major hair cell-based system in fishes, the lateral line system, is responsive to mechanosensory stimulation in a relatively low and narrow frequency range of ~50–200 Hz (Kalmijn 1988; reviewed in Coombs et al. 2014). In the lateral

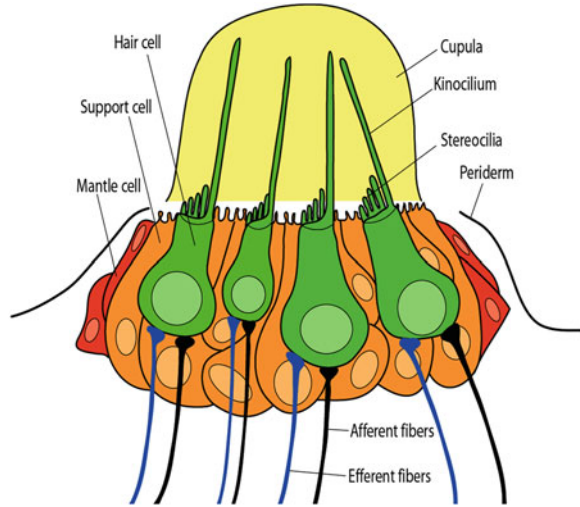


**Fig. 1** Lateral view of the goldfish inner ear (from Ramcharitar and Selckmann 2010, used with permission). The saccule (*S*), lagena (*L*), and utricle (*U*) are indicated. Each of these otolithic structures contains a sensory epithelium with an overlying dense calcareous otolith. Vestibular semicircular canals (*SSC*) are also shown

line system, clusters of sensory hair cells and associated supporting cells are housed in sensory organs called neuromasts that populate the head and body of the fish (Fig. 2). Neuromasts are either contained within canals (canal neuromasts, CN) or are free-standing (superficial neuromasts, SN). The lateral line system mediates schooling, prey capture, and predator avoidance, as well as navigation around inanimate obstacles (Gompel et al. 2001; Bleckmann and Zelick 2009; Coombs et al. 2014). Throughout this chapter we used the terms “lateral line” and “lateral line system” interchangeably to refer to the entire sensory system, with specific reference to subsystems (i.e., CN, canal neuromast; SN, superficial neuromast) where appropriate.

Fish mechanosensory systems are of particular interest in biomedical sciences because they contain hair cells that are structurally and functionally similar to the hair cells of the human inner ear (Chang et al. 1992; Popper 2000; Coffin et al. 2010). In addition, the sensory hair cells of fish have a propensity for regeneration—a feature not observed in mammals (Matsuura et al. 1971; Lombarte et al. 1993; Lanford and Popper 1996; Corwin and Oberholtzer 1997). This is of clinical rele-

**Fig. 2** Schematic of neuromast structure illustrating sensory hair cells (green), support cells (orange), and cupula (yellow). From Chiu et al. (2008), reprinted with permission

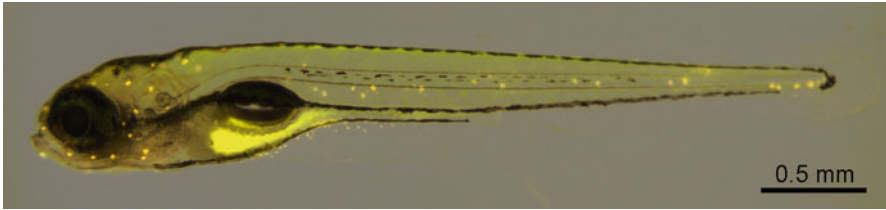


vance as loss of inner ear hair cells accounts for a large majority of acquired and congenital hearing disorders in humans (Behra et al. 2009; Brignull et al. 2009).

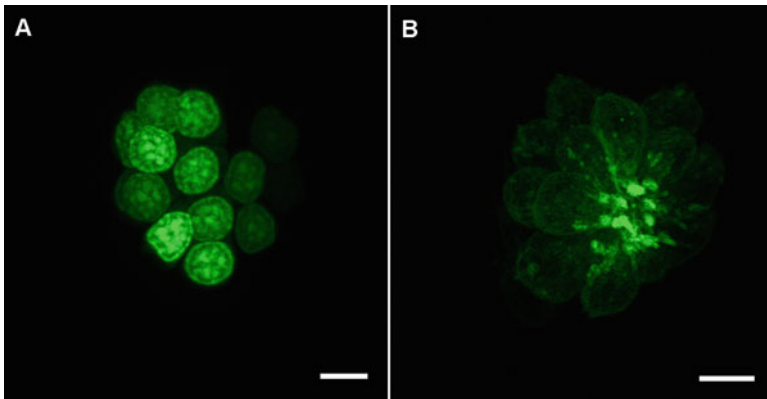
Much of our understanding of fish mechanosensory systems results from the use of chemical ototoxins. Ototoxin studies have yielded valuable insight into the relative contribution of different hair cell sub-populations to fish behaviors, as well as informing mechanistic understanding of hair cell death and regeneration. Aminoglycoside antibiotics were the first class of drugs reported to present the problem of ototoxicity, with the first incidence of human hearing loss noted in the 1940s (Schacht 1993). Gentamicin is currently the most widely used aminoglycoside in clinical settings, but may lead to a 30 % incidence of hearing loss, necessitating the development of otoprotective drugs (Nakashima et al. 2000; Santucci and Krieger 2000). Many classes of drugs are now known to cause ototoxic effects, including cisplatin, a platinum-based compound that is widely used to treat various malignancies (Lynch et al. 2005; Guthrie 2008).

Gentamicin was the agent of choice for initial ototoxic investigations in fishes. These studies focused primarily on the auditory system of the goldfish (*Carassius auratus*) and oscar (*Astronotus ocellatus*) (Yan et al. 1991; Chang et al. 1992; Lombarte et al. 1993). While the majority of these early investigations focused on collection of histological data, several studies have demonstrated changes in auditory thresholds or lateral line evoked potentials elicited by administration of gentamicin (e.g., Ramcharitar and Brack 2010; Ramcharitar and Selckmann 2010; Brack and Ramcharitar 2012).

More recently, larval zebrafish (*Danio rerio*) have taken center stage in ototoxicity investigations. Zebrafish are small, highly fecund tropical fish that breed readily in captivity, allowing for quantitative studies using large numbers of larvae. Lateral line visualization is easily achieved in live larvae with vital dye labeling or by using one of the growing number of transgenic strains that express fluorescent proteins in



**Fig. 3** Five-day-old zebrafish larvae labeled with the vital dye DASPEI. When viewed with fluorescence microscopy, neuromasts are clearly visible as bright *dots* on the head and body of the animal



**Fig. 4** Images of single neuromasts labeled with (a) the vital dye Yo-Pro-1, which labels hair cell nuclei, and (b) green fluorescent protein (*GFP*). This image is from a *Brn3c:mGFP* transgenic fish, which expresses *GFP* in hair cell membranes. Scale bars = 5  $\mu$ m

hair cells (Figs. 3 and 4, and see Coffin et al. 2014b). To date, thousands of compounds with established and potential ototoxic activity have been successfully explored in zebrafish (e.g., Ou et al. 2009; Hirose et al. 2011).

Here we review ototoxicity studies in fishes, examining both neuroethology research aimed at understanding fish sensory function and behavior, as well as biomedical studies that may influence clinical use of agents with demonstrated ototoxicity.

## 2 Evolutionary Perspectives

Do fishes have a homogenous population of hair cells? A combination of ultrastructural and ototoxicity studies suggest that fish hair cells are indeed heterogeneous, falling into characteristic sub-types similar to those found in mammals (Chang et al. 1992; Lanford et al. 2000; Popper 2000). Mammalian utricles have distinct striolar

and extrastriolar regions, with flask-shaped type I hair cells present in the striolar region and cylindrical type II hair cells in extrastriolar areas (Wersäll 1956, 1960). Data from early transmission electron microscopy (TEM) studies suggested that the sensory hair cells of anamniotes were exclusively type II (Wersäll 1961). However, more recent TEM investigations have demonstrated that the sensory epithelia of the lagena and utricle have two distinct populations of hair cells, one within a striolar region, which contains larger hair cells and a distinct line of hair bundle polarity reversal, and the other in an extrastriolar zone (Saidel et al. 1990; Chang et al. 1992).

In the mammalian ear, striolar hair cells are particularly susceptible to aminoglycoside toxicity (Forge and Li 2000; see Wu et al. 2002; Salvi et al. 2008). Similarly, striolar hair cells in fishes are preferentially ablated by gentamicin, while extrastriolar hair cells remain relatively unaffected (Yan et al. 1991; Chang et al. 1992; Lanford et al. 2000). Regional differences in gentamicin-induced hair cell loss have also been demonstrated in the goldfish saccule along with shifts in auditory thresholds (Ramcharitar and Brack 2010; Ramcharitar and Selckmann 2010), suggesting that hair cell heterogeneity is present in all three otolith end organs. Collectively, these data demonstrate similarities in hair cell morphology and associated ototoxin sensitivity across vertebrates, suggesting that inner ear hair cell heterogeneity arose early in vertebrate evolution.

In contrast, there are conflicting data on the differential susceptibility of superficial versus canal neuromast hair cells to ototoxic damage. Initial studies using SEM to assess hair cell damage suggested that CN hair cells were preferentially damaged by ototoxic treatment, while SN remained intact, suggesting a possible parallel between CN hair cells and the type I hair cells of the inner ear (Song et al. 1995; Coombs et al. 2001). However, a recent study by Van Trump et al. (2010) used fluorescence-based assays to determine that gentamicin significantly reduced hair cell survival in both superficial and canal neuromasts in zebrafish and Mexican blind cavefish (*Astyanax mexicanus*). Future studies are needed to resolve these conflicting data and clarify the pharmacologic heterogeneity of lateral line hair cells. Nonetheless, as a whole, the data strongly suggest that hair cell heterogeneity arose very early in the evolution of vertebrates (Popper 2000).

### 3 Perception and Behavior

Ototoxic compounds, most often aminoglycoside antibiotics and cobalt chloride, have long been used to disable the lateral line system so as to study the effects of mechanosensory deprivation on fish behavior. These studies have investigated behaviors such as rheotaxis (orientation to water current) and feeding, and the relative contribution of the lateral line to sound reception. This body of work underscores the dynamic use of the lateral line system for a variety of behaviors, depending on species, feeding modality, and availability of additional sensory information. Wersäll and Flock (1964) were the first to report aminoglycoside sensitivity in the

fish lateral line system. Using the gadiform fish *Lota lota*, they demonstrated that local application of streptomycin to CN over several minutes transiently and reversibly suppressed lateral line microphonic potentials, opening up the possibility of using aminoglycoside treatment as a tool in functional studies (Wersäll and Flock 1964). Most recent studies use bath immersion for one or more hours, rather than short-term focal application, to deliver aminoglycosides to the entire lateral line system, resulting in ablation (death) of sensory hair cells rather than short-term functional suppression (e.g., Song et al. 1995; Buck et al. 2012; Suli et al. 2012; Sampson et al. 2013).

Chemical ototoxins are important tools for studying the role of the lateral line in feeding behavior. Both largemouth bass (*Micropterus salmoides*) and muskellunge (*Esox masquinongy*) altered their approach to a prey item when the lateral line was inactivated with cobalt chloride (New et al. 2001; Gardiner and Motta 2012). Under these conditions, bass increased pre-strike velocity and muskellunge changed their approach angle, suggesting that near-field vibratory stimuli contribute to a more nuanced approach to prey capture (New et al. 2001; Gardiner and Motta 2012). Similarly, lateral line ablation altered the orienting response of mottled sculpin (*Cottus bairdi*) to a vibrating (prey-like) stimulus (Coombs et al. 2001). In keeping with the importance of the lateral line for feeding, larval striped bass (*Morone saxatilis*) prey-capture rates decreased following neomycin exposure, particularly for fish tested in the dark (i.e., without visual cues) (Sampson et al. 2013). Furthermore, experimentally blinded muskellunge have similar prey capture rates as sighted ones, providing strong evidence that mechanosensory cues detected via the lateral line are sufficient for feeding (New et al. 2001). Selective physical ablation of superficial neuromasts vs. chemical ablation of canal neuromasts with gentamicin (which selectively damages CN in some species, although see Van Trump et al. 2010 and Brown et al. 2011 for evidence of damage to SN) suggests that orientation to a prey-like stimulus depends primarily on canal neuromasts, at least in sculpin (Coombs et al. 2001). As sculpin are bottom dwelling “lie and wait” predators, it remains to be determined if this finding applies to fishes that use different prey capture strategies or if species occupying similar ecological niches use the lateral line in similar ways.

Further evidence supporting the role of canal neuromasts in feeding comes from recent comparative studies in cichlid fishes, where canal morphology is correlated with lateral line-mediated feeding behavior. *Aulonocara stuartgranti*, which has wide canals and enlarged neuromasts, can feed successfully in both light and dark conditions, based on video analysis of prey capture behavior (Schwalbe et al. 2012). Inactivation of the lateral line with cobalt chloride inhibited the ability of these fish to feed in the dark, demonstrating that without visual cues, the lateral line is necessary for prey detection and/or strike behavior (Schwalbe et al. 2012). In contrast, cichlids from the genus *Tramitichromis*, which have narrow canals and smaller neuromasts, did not strike at prey in lightless conditions, and lateral line ablation did not substantially alter prey capture behavior in the light (Schwalbe and Webb 2014). Collectively, these data demonstrate that different fishes rely on different combinations of sensory modalities for feeding, and that the lateral line is important for prey detection and capture in some fishes.

While canal neuromasts play a role in feeding in some species, superficial neuromasts appear to mediate rheotaxis behavior in a variety of fishes. In a classic study by Montgomery et al. (1997), chemical ablation of the entire lateral line system significantly decreased rheotaxis, such that significantly higher flow velocities were necessary to elicit orienting behavior. CN ablation with gentamicin did not alter rheotaxis, suggesting that only SN are necessary for responding to low velocity flow. Consistent with this finding, Buck et al. (2012) and Suli et al. (2012) demonstrated that in larval zebrafish, which only have SN, chemical ablation of the lateral line resulted in decreased rheotaxis (defined as an increase in the angle of the fish's head relative to current direction) and flow-mediated startle responses. These data are consistent with the presumed function of SN as low frequency, direct current detectors and CN as accelerometers that subservise flow sensing at higher velocities (Coombs et al. 1989, 2014).

One long-standing question is the relative contribution of the lateral line to sound reception. Higgs and Radford (2013) measured auditory evoked potentials (AEPs) in goldfish after streptomycin ablation of the lateral line and found increased thresholds (reduced sensitivity) to low frequency sound stimuli (100–200 Hz). Physical SN ablation had no effect on thresholds, suggesting that CN specifically contribute to the AEP response. Coffin et al. (2014a) examined the role for the lateral line in sound source localization in plainfin midshipman fish (*Porichthys notatus*), a soniferous species for which directional hearing and source localization is critical for reproductive success. Female midshipman showed no changes in the proportion of animals that localized the source after lateral line ablation, although changes in bearing angle during the final approach to the target speaker suggest that the lateral line may help fine-tune the approach, similar to what is seen in feeding studies. Collectively, these data support the hypothesis that many fish behaviors are driven by the multisensory integration of visual, mechanosensory, and other sensory information (Braun and Coombs 2000; Webb et al. 2008; Schwalbe and Webb 2014).

## 4 Biomedical Applications

Fish inner ear and lateral line hair cells are homologous to hair cells in the mammalian inner ear and share a number of properties, including susceptibility to ototoxic drugs (reviewed in Coffin et al. 2004, 2010). This latter feature makes fishes choice models for biomedical studies of drug-induced hair cell death and protection. Unlike mammals, however, fish can fully regenerate lost hair cells, opening up a range of studies exploring the cellular mechanisms underlying hair cell regeneration (Brignull et al. 2009). Chemical ototoxins are effective tools for regeneration studies, as they are usually employed to kill hair cells and trigger the regenerative process. In this section we briefly survey some of the cellular and molecular research on hair cell death, protection, and regeneration using the fish inner ear and lateral line as a model system. More comprehensive reviews are available in Brignull et al. (2009), Coffin et al. (2010, 2014b), and Esterberg et al. (2012).



#### 4.1 Hair Cell Death and Protection

Inner ear ototoxicity studies generally rely on daily systemic aminoglycoside injections spanning multiple days (e.g., Lombarte et al. 1993; Ramcharitar and Selckmann 2010). Intramuscular injection is simple to administer but often causes morbidity due to nephrotoxic side effects. One group has recently employed direct intrasaccular gentamicin injection using X-ray-assisted needle placement in Atlantic cod (*Gadus morhua*) (Faucher et al. 2008a, 2009), an elegant approach that bypasses systemic toxicity, although the systemic model more closely approximates human clinical use. Using either treatment paradigm, most fish inner ear aminoglycoside research is descriptive rather than mechanistic. Several studies in the oscar employed SEM imaging of dissected sensory epithelia to demonstrate selective loss of striolar hair cells in the utricle and lagena (Yan et al. 1991; Chang et al. 1992; Lombarte et al. 1993), consistent with findings of increased toxin sensitivity in mammalian striolar hair cells (reviewed in Salvi et al. 2008). While these earlier studies did not report evidence of saccular hair cell loss, Ramcharitar and Selckmann (2010) and Uribe et al. (2013) demonstrated hair cell loss in the caudal region of the saccule in goldfish and zebrafish, respectively, correlated with a significant hearing threshold shift in the low frequency range. These ototoxicity studies inform future biomedical work using the fish inner ear as a model system.

In contrast to inner ear research, many lateral line biomedical studies are mechanistic or translational. This body of work primarily relies on a tractable genetic model, the zebrafish. Over a decade ago, Williams and Holder (2000) and Harris et al. (2003) demonstrated that hair cells of 5-day-old zebrafish larvae respond to aminoglycoside damage similarly to mature mammalian hair cells, setting the stage for a myriad of studies aimed at understanding the intracellular death cascade initiated by chemical ototoxins and at identifying novel therapeutics that protect hair cells from drug damage.

Studies on zebrafish larvae demonstrate that mitochondrial swelling and a loss of mitochondrial membrane potential occur shortly after treatment with the ototoxin neomycin. These data are consistent with genetic and pharmacologic evidence for the importance of Bcl-2 family proteins that regulate mitochondrial-dependent cell death pathways (Owens et al. 2007; Coffin et al. 2013a, b). Mitochondria are a major calcium store in many cell types, and an elegant series of experiments with transgenic zebrafish expressing a genetically encoded calcium sensor clearly demonstrate that calcium dysregulation plays an important role in aminoglycoside-induced hair cell loss (Esterberg et al. 2013, 2014). Neomycin treatment first leads to a decrease in calcium in the endoplasmic reticulum, following by an increase in mitochondrial calcium and ending with a cytoplasmic calcium spike immediately preceding hair cell death (Esterberg et al. 2013, 2014). Nuclear condensation, a hallmark of classical apoptosis, occurs in aminoglycoside-damaged hair cells, although the role of caspases (cysteine-dependent proteases associated with apoptosis) has not been conclusively demonstrated (Williams and Holder 2000; Santos et al. 2006; Ou et al. 2009; Coffin et al. 2013a). Collectively, these studies are con-

sistent with hair cell death research in the inner ears of amniotic vertebrates, particularly chickens and rodents, demonstrating conservation of hair cell death mechanisms across mechanosensory systems and taxa (Matsui et al. 2002, 2004; Cunningham et al. 2004). Even the lack of consensus on caspase activation parallels mammalian-based research, as some studies in the rodent inner ear suggest a requirement for caspases in aminoglycoside ototoxicity, while others demonstrate activation of caspase-independent mechanisms (Cunningham et al. 2002; Cheng et al. 2005; Jiang et al. 2006).

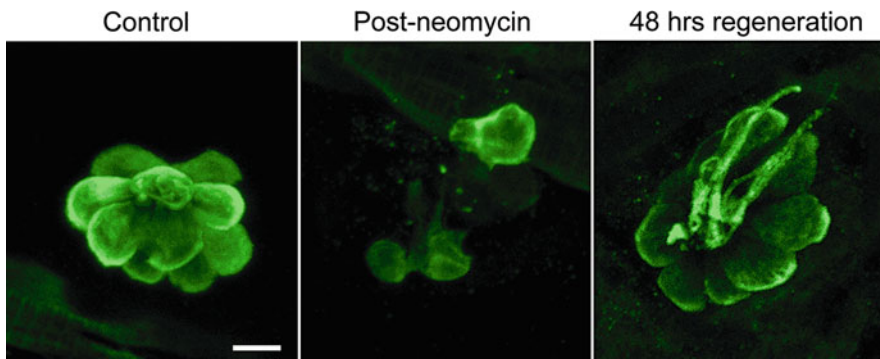
Larval zebrafish are particularly amenable for large-scale drug screening, an unbiased drug discovery process that probes a collection of chemical compounds for a phenotypic effect or behavioral response of interest (Peterson et al. 2000; reviewed in Kaufman et al. 2009). The lateral line also provides a tractable model system for identifying novel compounds that may protect hair cells from ototoxin-induced damage. Ton and Parng (2005) first took advantage of this system in a small-scale drug screen, demonstrating that several antioxidants, including glutathione and D-methionine, attenuated hair cell death from the chemotherapy agent cisplatin. Screens of libraries of FDA-approved drugs and similar bioactive compounds have yielded several otoprotective molecules for potential translational development, including antidepressants such as paroxetine (Paxil) and anticholinergics (Tacrine) (Ou et al. 2009; Vlasits et al. 2012). Other otoprotection screens have cast a wider net, including a screen of 10,000+ small molecules with diverse chemical structures that discovered a novel benzothiophene carboxamide, now called PROTO-1, which robustly protects zebrafish hair cells from aminoglycoside toxicity (Owens et al. 2008). Together, these studies have identified new potential uses for several approved drugs and uncovered new drug candidates for future development. The majority of drug discovery studies have relied on morphological criteria for hair cell protection, but recent advances in physiology and behavior set the stage for functional studies of newly identified protective compounds (Zeddies and Fay 2005; Trapani and Nicolson 2010; Brack and Ramcharitar 2012; Buck et al. 2012; Suli et al. 2012; Bhandiwad et al. 2013).

## 4.2 *Regeneration*

All fishes examined to date robustly regenerate hair cells, although the time course of recovery depends on the sensory system in question (inner ear vs. lateral line), developmental stage of the test organism, and ototoxin administered (Lombarte et al. 1993; Ma et al. 2008; reviewed in Brignull et al. 2009; Lush and Piotrowski 2014). In the ear of adult oscars, structural regeneration post-gentamicin treatment is complete 10 days after observation of maximum damage (20 days from first gentamicin injection; Lombarte et al. 1993). A similar recovery time course is seen in Atlantic cod that received an intrasaccular gentamicin injection (Faucher et al. 2009), suggesting that ~20 days post-trauma is a typical regeneration period for adult fishes. In contrast, larval zebrafish manifest complete lateral line

regeneration 3–4 days after aminoglycoside insult (Harris et al. 2003; Ma et al. 2008; MacKenzie and Raible 2012; Fig. 5). Experiments with cell division markers and cell cycle inhibitors demonstrate that lateral line regeneration primarily results from a wave of supporting cell proliferation and differentiation of newly born progeny into hair cells (Ma et al. 2008; MacKenzie and Raible 2012), although non-proliferative regeneration has been observed following copper ototoxicity (Hernández et al. 2007). Interestingly, the time course of regeneration is delayed when some ototoxins (e.g., copper) are employed to ablate hair cells, suggesting that at high concentrations, copper (and perhaps cisplatin) damages supporting cells as well as hair cells (Hernández et al. 2006; Linbo et al. 2006; MacKenzie and Raible 2012).

Both mutagenesis and chemical screens have attempted to identify the underlying molecular factors responsible for hair cell regeneration in the lateral line. The novel gene *Phoenix* is one such factor, as *Phoenix* mutants demonstrate normal lateral line development but reduced supporting cell proliferation after ototoxic insult (Behra et al. 2009). The underlying cause of this proliferative defect is unknown, as the mutated gene in *Phoenix* encodes a novel protein. In contrast, chemical screens have provided tantalizing hints into innate regenerative mechanisms. The glucocorticoids dexamethasone and prednisolone enhance regeneration by increasing supporting cell proliferation, suggesting that the inflammatory response may modulate regenerative potential (Namdaran et al. 2012). Low molecular weight fucoidan, an extract from marine algae that also has anti-inflammatory properties, similarly enhances proliferative regeneration (Moon et al. 2011; Kim et al. 2012). Interestingly, caudal fin regeneration is not affected in *Phoenix* mutants and fin regeneration is reduced by glucocorticoid exposure, suggesting that hair cell regeneration proceeds by a mechanism distinct from other regenerative processes (Behra et al. 2009; Namdaran et al. 2012).



**Fig. 5** Hair cells in the larval zebrafish lateral line quickly regenerate after ototoxic damage. (a) Intact neuromast, (b) neuromast following one hour of exposure to 300  $\mu$ M neomycin, and (c) neuromast 48 h after neomycin treatment. Hair cells were labeled with an antibody to parvalbumin. The scale bar in A = 5  $\mu$ m and applies to all images

In an experimental *tour de force*, two recent studies examined transcriptome-level changes in isolated lateral line supporting cells from ototoxin-treated larval zebrafish as a major step towards identifying the complete set of molecular factors responsible for innate regenerative capacity (Jiang et al. 2014; Steiner et al. 2014). These studies saw changes in several signaling pathways important for hair cell development and regeneration, including Notch and Wnt signaling, consistent with previous pharmacologic and genetic manipulation studies demonstrating the importance of these pathways for hair cell regeneration (Ma et al. 2008; Head et al. 2013; Wada et al. 2013; Jacques et al. 2014). Genomics tools have also been applied to insightful regeneration studies in the adult zebrafish inner ear. These studies, which used intense noise exposure to ablate hair cells, found that activation of *stat3/socs3* and growth hormone signaling pathways were involved in proliferative regeneration in the adult zebrafish saccule (Schuck et al. 2011; Liang et al. 2012). It is unclear if these pathways are also important for lateral line regeneration, and conversely if pathways identified in the lateral line are required for regeneration of inner ear hair cells. Moreover, it is possible that different ototoxins activate different regenerative mechanisms, although this hypothesis has not been fully tested (but see Mackenzie and Raible 2012).

## 5 Environmental Toxins

While many in the auditory field think of “chemical ototoxicity” as it relates to aminoglycoside antibiotics and platinum-based chemotherapy agents, some environmental contaminants, particularly metals, have damaging effects on hair cells. Exposure to these contaminants may have long-term consequences for the fish by disrupting lateral line function and reducing lateral line-mediated behaviors. Environmentally relevant concentrations of cadmium (0.5–2 µg/ml) damage hair bundles and reduce rheotaxis and startle responses in banded kokopu (*Galaxias fasciatus*) and sea bass (*Dicentrarchus labrax*), respectively (Baker and Montgomery 2001; Faucher 2006, 2008b). Copper concentrations as low as 10 µM rapidly kill hair cells in larval zebrafish, likely by inducing oxidative stress (Linbo et al. 2006; Olivari et al. 2008). Zinc may similarly damage hair cells, while other metals such as silver or manganese have no visible effect. Both cadmium and copper are also toxic to fish olfactory receptors (Hansen et al. 1999; Baker and Montgomery 2001; Blechinger et al. 2007), suggesting that direct contact with the aquatic environment plays an important role in sensory receptor susceptibility to toxins. Recent evidence suggests that the ubiquitous contaminant bisphenol-A, a component of many plastics, is also a hair cell toxin and may impede hair cell regeneration (Hayashi et al. 2015). Collectively, these findings underscore the vulnerability of the lateral line to aquatic pollutants. Furthermore, these studies inform human health, as environmental contaminants in food and water sources may exert similar effects on mammalian hair cells.

## 6 Looking Forward: Where Do These Studies Lead Us?

The use of fish models in ototoxicity research has come a long way since the initial testing of gentamicin-mediated effects on auditory and vestibular sensory epithelia in the research laboratory of Dr. Arthur Popper (Yan et al. 1991; Chang et al. 1992). Notwithstanding the burgeoning larval zebrafish model in this arena of scientific investigation, several important questions remain unresolved, including:

1. What are the relative contributions of the different inner ear end organs and lateral line neuromasts to the detection and processing of auditory, vestibular, and current-mediated stimuli? Ablation studies demonstrate that the lateral line is not required for sound source localization in one fish species (the plainfin midshipman; Coffin et al. 2014a), but it is not known if this finding applies to other fishes, or if the lateral line and inner ear act in concert to perform non-localization tasks. Further, the role of the lagena remains a mystery.
2. What are the relative contributions of particle motion and pressure stimuli to the auditory, vestibular, and lateral line-mediated senses? Comprehensive assessment of mechanosensory stimuli remains elusive in the complex soundscape of aquatic media. Differential ototoxin-induced ablation of superficial versus canal neuromasts may yield insight into the role of particle versus pressure signals in mediating sensory hair cell transduction.
3. What accounts for variation in data concerning differential susceptibility of fish sensory hair cell sub-types to ototoxic damage? Differences in aminoglycoside sensitivity in the lateral line are still a puzzle—are these differences based on species, ototoxic treatment paradigm, or detection method? Future comparative studies are needed to differentiate between these possibilities.
4. How is neuromast size regulated during development and regeneration? Following chemical ablation of the lateral line in larval zebrafish, neuromasts regenerate to their original size, with larger neuromasts pre-neomycin damage also possessing more hair cells after regeneration (Ma et al. 2008). However, the molecular mechanisms that regulate neuromast size are unknown. Pharmacological or genetic manipulation of cell patterning pathways during the regeneration process can help answer these questions.
5. Could auditory- and lateral line-evoked potentials yield greater insight into neural correlates of ototoxic damage? Auditory- and lateral line-evoked potentials from fishes may be used to investigate response thresholds, although little is known about the specific neural activities that underlie the various waveforms that characterize these responses. Standardization of techniques (per species) may go a long way in establishing waveform criteria for investigating auditory- and lateral line-evoked potentials. This may lead to more powerful analysis of ototoxic effects.
6. How do we advance the use of initial screening of potential pharmaceutical agents for toxicity in larval zebrafish assays for later assessment of identified compounds in mammalian models, and then potentially to clinical trials? Chemical screens in zebrafish have yielded a wealth of novel protective com-

pounds, but few have advanced to mammalian testing. Close collaboration between fish and mammalian researchers is necessary to facilitate this “bench to bedside” pipeline.

Thanks to decades of study in fish mechanosensory systems using chemical ototoxins, we are closer to understanding (1) hair cell evolution and the different subtypes found across vertebrate taxa, (2) the role(s) of the fish inner ear and lateral line to behavior, (3) how chemical ototoxins kill hair cells, and (4) how fish regenerate hair cells following ototoxic damage. We believe the next several decades will see continued progress in these areas, leading to answers to the questions above and to many others we do not yet foresee.

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## References

- Baker CF, Montgomery JC (2001) Sensory deficits induced by cadmium in banded kokopu, *Galaxias fasciatus*, juveniles. *Environ Biol Fish* 62:455–464

- Behra M, Bradsher J, Sougrat R, Gallardo V, Allende ML, Burgess SM (2009) Phoenix is required for mechanosensory hair cell regeneration in the zebrafish lateral line. *PLoS Genet* 5(4), e1000455
- Bhandiwad AA, Zeddies DG, Raible DW, Rubel EW, Sisneros JA (2013) Auditory sensitivity of larval zebrafish (*Danio rerio*) measured using a behavioral prepulse inhibition assay. *J Exp Biol* 216(18):3504–3513
- Blechinger SR, Kusch RC, Haugo K, Matz C, Chivers DP, Krone PH (2007) Brief embryonic cadmium exposure induces a stress response and cell death in the developing olfactory system followed by long-term olfactory deficits in juvenile brafish. *Toxicol Appl Pharmacol* 224(1):72–80
- Bleckmann H, Zelick R (2009) Lateral line system of fish. *Integr Zool* 4(1):13–25
- Brack CL, Ramcharitar J (2012) Assessment of lateral line function: a potential technique for studies on ototoxicity. *J Clin Neurosci* 19:333–335
- Braun CB, Coombs S (2000) The overlapping role of the inner ear and lateral line: the active space of dipole source detection. *Philos Trans R Soc Lond* 355:1115–1119
- Brignull HR, Raible DW, Stone JS (2009) Feathers and fins: non-mammalian models for hair cell regeneration. *Brain Res* 1277:12–23
- Brown AD, Mussen TD, Sisneros JA, Coffin AB (2011) Reevaluating the use of aminoglycoside antibiotics in behavioral studies of the lateral line. *Hear Res* 272(1–2):1–4
- Buck LMJ, Winter MJ, Redfern WS, Whitfield TT (2012) Ototoxin-induced cellular damage in neuromasts disrupts lateral line function in larval zebrafish. *Hear Res* 284:67–81
- Chang JSY, Popper AN, Saidel WM (1992) Heterogeneity of sensory hair cells in a fish ear. *J Comp Neurol* 324:621–640
- Cheng AG, Cunningham LL, Rubel EW (2005) Mechanisms of hair cell death and protection. *Curr Opin Otolaryngol Head Neck Surg* 13(6):343–348
- Chiu LL, Cunningham LL, Raible DW, Rubel EW, Ou HC (2008) Using the zebrafish lateral line to screen for ototoxicity. *J Assoc Res Otolaryngol* 9(2):178–190
- Coffin AB, Kelley MW, Manley GA, Popper AN (2004) Evolution of sensory hair cells. In: Manley GA, Fay RR, Popper AN (eds) *Evolution of the auditory system*. Springer-Verlag, New York
- Coffin AB, Ou H, Owens KN, Santos F, Simon JA, Rubel EW, Raible DW (2010) Chemical screening for hair cell loss and protection in the zebrafish lateral line. *Zebrafish* 7(1):3–11
- Coffin AB, Rubel EW, Raible DW (2013a) Bax, Bcl2, and p53 differentially regulate neomycin- and gentamicin-induced hair cell death in the zebrafish lateral line. *J Assoc Res Otolaryngol*. doi:10.1007/s10162-013-0404-1
- Coffin AB, Williamson KL, Mamiya A, Raible DW, Rubel EW (2013b) Profiling drug-induced cell death pathways in the zebrafish lateral line. *Apoptosis*. doi:10.1007/s10495-013-0816-8
- Coffin AB, Zeddies DG, Fay RR, Brown AD, Alderks PW, Bhandiwad AA, Mohr RA, Gray MD, Rogers PH, Sisneros JA (2014a) Use of the swim bladder and lateral line in near-field sound source localization by fishes. *J Exp Biol*. doi:10.1242/jeb.093831
- Coffin AB, Brignull H, Raible DW, Rubel EW (2014b) Hearing loss, protection, and regeneration in the larval zebrafish lateral line. In: Coombs S, Bleckmann H, Fay RR, Popper AN (eds) *The lateral line system*. Springer, New York
- Collins SP, Marshall JN, Atema J, Fay RR (2003) *Sensory processing aquatic environments*. Springer, New York
- Coombs S, Görner P, Münz H (1989) *The mechanosensory lateral line: neurobiology and evolution*. Springer-Verlag, New York
- Coombs S, Braun CB, Donovan B (2001) The orienting response of Lake Michigan mottled sculpin is mediated by canal neuromasts. *J Exp Biol* 204:337–348
- Coombs S, Bleckmann H, Fay RR, Popper AN (2014) *The lateral line system*. Springer, New York
- Corwin JT, Oberholtzer JC (1997) Fish n' chicks: model recipes for hair-cell regeneration? *Neuron* 19(5):951–954
- Cunningham LL, Cheng AG, Rubel EW (2002) Caspase activation in hair cells of the mouse utricle exposed to neomycin. *J Neurosci* 22(19):8532–8540

- Cunningham LL, Matsui JI, Warchol ME, Rubel EW (2004) Overexpression of Bcl-2 prevents neomycin-induced hair cell death and caspase-9 activation in the adult mouse utricle in vitro. *J Neurobiol* 60(1):89–100
- Esterberg R, Coffin AB, Ou H, Simon JA, Raible DW, Rubel EW (2012) Fish in a dish: drug discovery for hearing habilitation. *Drug Discov Today Dis Models*. doi:10.1016/j.ddmod.2012.02.001
- Esterberg R, Hailey DW, Coffin AB, Raible DW, Rubel EW (2013) Disruption of intracellular calcium regulation is integral to aminoglycoside-induced hair cell death. *J Neurosci* 33(17):7513–7525
- Esterberg R, Hailey DW, Rubel EW, Raible DW (2014) ER-mitochondrial calcium flow underlies vulnerability of mechanosensory hair cells to damage. *J Neurosci* 34(29):9703–9719
- Faucher K, Fichet D, Miramand P, Lagardère J-P (2006) Impact of acute cadmium exposure on the trunk lateral line neuromasts and consequences on the “C-start” response behaviour of the sea bass (*Dicentrarchus labrax* L.; Teleostei, Moronidae). *Aquat Toxicol* 76:278–294
- Faucher K, Aas-Hansen O, Damsgard B, Stenklev NC (2008a) Effects of systemic versus local gentamicin on the inner ear in the Atlantic cod, *Gadus morhua* (L.), relevance for fish hearing investigations. *Hear Res* 240:12–21
- Faucher K, Fichet D, Miramand P, Lagardère J-P (2008b) Impact of chronic cadmium exposure at environmental dose on escape behaviour in sea bass (*Dicentrarchus labrax* L.; Teleostei, Moronidae). *Environ Pollut* 151:148–157
- Faucher K, Aas-Hansen O, Damsgard B, Laukli E, Stenklev NC (2009) Damage and functional recovery of the Atlantic cod (*Gadus morhua*) inner ear hair cells following local injection of gentamicin. *Int J Audiol* 48(7):456–464
- Fay RR (1998) Auditory stream segregation in goldfish (*Carassius auratus*). *Hear Res* 120:69–76
- Fay RR, Popper AN (2012) Fish hearing: new perspectives from two ‘senior’ bioacousticians. *Brain Behav Evol* 79:215–217
- Forge A, Li L (2000) Apoptotic death of hair cells in mammalian vestibular sensory epithelia. *Hear Res* 139:97–115
- Gardiner JM, Motta PJ (2012) Largemouth bass (*Micropterus salmoides*) switch feeding modalities in response to sensory deprivation. *Zoology* 115:78–83
- Gompel N, Dambly-Chaudière C, Ghysen A (2001) Neuronal differences prefigure somatotopy in the zebrafish lateral line. *Development* 128(3):387–393
- Guthrie OW (2008) Aminoglycoside induced ototoxicity. *Toxicology* 249:91–96
- Hansen JA, Rose JD, Jenkins RA, Gerow KG, Bergman HL (1999) Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) exposed to copper: neurophysiological and histological effects on the olfactory system. *Environ Toxicol Chem* 18(9):1979–1991
- Harris JA, Cheng AG, Cunningham LL, MacDonald G, Raible DW, Rubel EW (2003) Neomycin-induced hair cell death and rapid regeneration in the lateral line of zebrafish (*Danio rerio*). *J Assoc Res Otolaryngol* 4(2):219–234
- Hayashi L, Sheth M, Young A, Kruger M, Wayman GA, Coffin AB (2015) The effect of the aquatic contaminants bisphenol-A and PCB-95 on the zebrafish lateral line. *Neurotoxicology* 46:125–136
- Head JR, Gocioch L, Pennisi M, Meyers JR (2013) Activation of canonical Wnt/ $\beta$ -catenin signaling stimulations proliferation in neuromasts in the zebrafish posterior lateral line. *Dev Dyn* 242(7):832–846
- Hernández PP, Moreno V, Olivari FA, Allende ML (2006) Sub-lethal concentrations of waterborne copper are toxic to lateral line neuromasts in zebrafish (*Danio rerio*). *Hear Res* 213(1–2):1–10
- Hernández PP, Olivari FA, Sarrazin AF, Sandoval PC, Allende ML (2007) Regeneration in zebrafish lateral line neuromasts: expression of the neural Progenitor cell marker sox2 and proliferation-dependent and-independent mechanisms of hair cell renewal. *Dev Neurobiol* 67(5):637–654



- Higgs DM, Radford CA (2013) The contribution of the lateral line to 'hearing' in fish. *J Exp Biol* 216:1484–1490
- Hirose Y, Simon JA, Ou HC (2011) Hair cell toxicity in anti-cancer drugs: evaluating an anti-cancer drug library for independent and synergistic toxic effects on hair cells using the zebrafish lateral line. *J Assoc Res Otolaryngol*. doi:10.1007/s10162-011-0278-z
- Jacques BE, Montgomery WH 4th, Uribe PM, Yatteau A, Asuncion JD, Resendiz G, Matsui JI, Dabdoub A (2014) The role of Wnt/ $\beta$ -catenin signaling in proliferation and regeneration of the developing basilar papilla and lateral line. *Dev Neurobiol* 74(4):438–456
- Jiang H, Sha SH, Forge A, Schacht J (2006) Caspase-independent pathways of hair cell death induced by kanamycin in vivo. *Cell Death Differ* 13(1):20–30
- Jiang L, Romero-Carvajal A, Haug JS, Seidel CW, Piotrowski T (2014) Gene-expression analysis of hair cell regeneration in the zebrafish lateral line. *Proc Natl Acad Sci*. doi:10.1073/pnas.1402898111
- Kalmijn AJ (1988) Hydrodynamic and acoustic field detection. In: Fay RR, Popper AN, Tavolga WN (eds) *Sensory biology of aquatic animals*. Springer-Verlag, New York
- Kaufman CK, White RM, Zon L (2009) Chemical genetic screening in the zebrafish embryo. *Nat Protoc* 4(10):1422–1432
- Kim K-J, Yoon K-Y, Lee B-Y (2012) Low molecular weight fucoidan from the sporophyll of *Undaria pinnatifida* suppresses inflammation by promoting the inhibition of mitogen-activated protein kinases and oxidative stress in RAW264.7 cells. *Fitoterapia* 83(8):1628–1635
- Lanford PJ, Popper AN (1996) A unique afferent structure in the crista ampullaris of the goldfish. *J Comp Neurol* 366:572–579
- Lanford PJ, Platt C, Popper AN (2000) Structure and function in the saccule of the goldfish (*Carassius auratus*): a model of diversity in the non-amniote ear. *Hear Res* 143:1–13
- Liang J, Wang D, Renaud G, Wolfberg TG, Wilson AF, Burgess SM (2012) The stat3/socs3a pathway is a key regulator of hair cell regeneration in zebrafish. *J Neurosci* 32(31):10662–10673
- Linbo TL, Stehr CM, Incardona JP, Scholz NL (2006) Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. *Environ Toxicol Chem* 25(2):597–603
- Lombarte A, Yan HY, Popper AN, Chang JS, Platt C (1993) Damage and regeneration of hair cell ciliary bundles in a fish ear following treatment with gentamicin. *Hear Res* 64(2):166–174
- Lush ME, Piotrowski T (2014) Sensory hair cell regeneration in the zebrafish lateral line. *Dev Dyn* 243:1187–1202
- Lynch ED, Gu R, Pierce C, Kil J (2005) Reduction of acute cisplatin ototoxicity and nephrotoxicity in rats by oral administration of allopurinol and ebselen. *Hear Res* 201:81–89
- Ma EY, Rubel EW, Raible DW (2008) Notch signaling regulates the extent of hair cell regeneration in the zebrafish lateral line. *J Neurosci* 28(9):2261–2273
- Mackenzie SM, Raible DW (2012) Proliferative regeneration of zebrafish lateral line hair cells after different ototoxic insults. *PLoS One* 7(10), e47257
- Matsui JI, Ogilvie JM, Warchol ME (2002) Inhibition of caspases prevents ototoxic and ongoing hair cell death. *J Neurosci* 22(4):1218–1227
- Matsui JI, Gale JE, Warchol ME (2004) Critical signaling events during the aminoglycoside-induced death of sensory hair cells in vitro. *J Neurobiol* 61(2):250–266
- Matsuura S, Ikeda K, Furukawa T (1971) Effects of streptomycin, kanamycin, quinine, and other drugs on the microphonic potentials of goldfish sacculus. *Jpn J Physiol* 21:579–590
- Mirjany M, Preuss T, Faber DS (2011) Role of the lateral line mechanosensory system in directionality of goldfish auditory escape response. *J Exp Biol* 214(20):3358–3367
- Montgomery JC, Baker CF, Carton AG (1997) The lateral line can mediate rheotaxis in fish. *Nature* 389:960–963
- Moon IS, So JH, Jung YM, Lee WS, Kim EY, Choi JH, Kim CH, Choi JY (2011) Fucoidan promotes mechanosensory hair cell regeneration following amino glycoside-induced cell death. *Hear Res* 282(1–2):236–242
- Nakashima T, Teranishi M, Hibi T, Kobayashi M, Umemura M (2000) Vestibular and cochlea toxicity of aminoglycosides. *Acta Otolaryngol* 120:904–911

- Namdaran P, Reinhart KE, Owens KN, Raible DW, Rubel EW (2012) Identification of modulators of hair cell regeneration in the zebrafish lateral line. *J Neurosci* 32(10):3516–3528
- New JG, Fewkes LA, Khan AN (2001) Strike feeding behavior in the muskellunge, *Esox masquinongy*: contributions of the lateral line and visual sensory systems. *J Exp Biol* 204:1207–1221
- Olivari FA, Hernández PP, Allende ML (2008) Acute copper exposure induces oxidative stress and cell death in lateral line hair cells of zebrafish larvae. *Brain Res* 1244:1–12
- Ou HC, Cunningham LL, Francis SP, Brandon CS, Simon JA, Raible DW, Rubel EW (2009) Identification of FDA-approved drugs and bioactives that protect hair cells in the zebrafish (*Danio rerio*) lateral line and mouse (*Mus musculus*) utricle. *J Assoc Res Otolaryngol* 10(2):191–203
- Owens KN, Cunningham DE, MacDonald G, Rubel EW, Raible DW, Pujol R (2007) Ultrastructural analysis of aminoglycoside-induced hair cell death in the zebrafish lateral line reveals an early mitochondrial response. *J Comp Neurol* 502(4):522–543
- Owens KN, Santos F, Roberts B, Linbo T, Coffin AB, Knisely AJ, Simon JA, Rubel EW, Raible DW (2008) Identification of genetic and chemical modulators of zebrafish mechanosensory hair cell death. *PLoS Genet* 4(2), e1000020
- Peterson RT, Link BA, Dowling JE, Schreiber SL (2000) Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proc Natl Acad Sci U S A* 97(24):12965–12969
- Popper AN (1977) A scanning electron microscope study of the sacculus and lagena in the ears of fifteen species of teleost fishes. *J Morphol* 153:397–418
- Popper AN (1978) Scanning electron microscope study of the otolith organs in the birchir (*Polypterus bichir*) and shovel-nose sturgeon (*Scaphirhynchus platorhynchus*). *J Comp Neurol* 18:117–128
- Popper AN (2000) Hair cell heterogeneity and ultrasonic hearing: recent advances in understanding fish hearing. *Philos Trans R Soc Lond B* 355:1277–1280
- Popper AN, Lu Z (2000) Structure and function relationships in fish otolith organs. *Fish Res* 46:15–25
- Ramcharitar JU, Brack CL (2010) Physiological dimensions of ototoxic responses in a model fish species. *J Clin Neurosci* 17:103–106
- Ramcharitar J, Selckmann GM (2010) Differential ablation of sensory receptors underlies ototoxin-induced shifts in auditory thresholds of the goldfish (*Carassius auratus*). *J Appl Toxicol* 30(6):536–541
- Saidel WM, Popper AN, Chang JS (1990) Spatial and morphological differentiation of trigger zones in afferent fibers to the teleost utricle. *J Comp Neurol* 302:629–642
- Salvi RJ, Popper AN, Fay RR (2008) Hair cell regeneration, repair, and protection. Springer, New York
- Sampson JA, Duston J, Croll RP (2013) Superficial neuromasts facilitate non-visual feeding by larval striped bass (*Morone saxatilis*). *J Exp Biol* 216:3522–3530
- Santos F, MacDonald G, Rubel EW, Raible DW (2006) Lateral line hair cell maturation is a determinant of aminoglycoside susceptibility in zebrafish (*Danio rerio*). *Hear Res* 213(1-2):25–33
- Santucci RA, Krieger JN (2000) Gentamicin for the practicing urologist: review of efficacy, single daily dosing and “switch” therapy. *J Urol* 163:1076–1084
- Schacht J (1993) Biochemical basis of aminoglycoside ototoxicity. *Otolaryngol Clin North Am* 26:845–856
- Schuck JB, Sun H, Penberthy WT, Cooper NG, Li X, Smith ME (2011) Transcriptomic analysis of the zebrafish inner ear points to growth hormone mediated regeneration following acoustic trauma. *BMC Neurosci* 12:88. doi:10.1186/1471-2202-12-188
- Schwalbe MAB, Webb JF (2014) Sensory basis for detection of benthic prey in two Lake Malawi cichlids. *Zoology* 117:112–121
- Schwalbe MAB, Bassett DK, Webb JF (2012) Feeding in the dark: lateral-line-mediated prey detection in the peacock cichlid *Aulonocara stuartgranti*. *J Exp Biol* 215:2060–2071

- Song J, Yan HY, Popper AN (1995) Damage and recovery of hair cells in fish canal (but not superficial) neuromasts after gentamicin exposure. *Hear Res* 91(1-2):63–71
- Steiner AB, Kim T, Cabot V, Hudspeth AJ (2014) Dynamic gene expression by putative hair-cell progenitors during regeneration in the zebrafish lateral line. *Proc Natl Acad Sci U S A* 111:E1393–E1401. doi:[10.1073/pnas.1318692111](https://doi.org/10.1073/pnas.1318692111)
- Suli A, Watson GM, Rubel EW, Raible DW (2012) Rheotaxis in larval zebrafish is mediated by lateral line mechanosensory hair cells. *PLoS One* 7(2), e29727
- Ton C, Parg C (2005) The use of zebrafish for assessing ototoxic and otoprotective agents. *Hear Res* 208(1-2):79–88
- Trapani JG, Nicolson T (2010) Physiological recordings from zebrafish lateral-line hair cells and afferent neurons. *Methods Cell Biol* 100:219–231
- Uribe PM, Sun H, Wang K, Asuncion JD, Wang Q, Chen CW, Steyger PS, Smoth ME, Matsui JI (2013) Aminoglycoside-induced hair cell death of inner ear organs causes functional deficits in adult zebrafish (*Danio rerio*). *PLoS One* 8(3):e58755
- Van Trump WJ, McHenry MJ (2013) The lateral line system is not necessary for rheotaxis in the Mexican blind cavefish (*Astyanax fasciatus*). *Integr Comp Biol* 53(5):799–809
- Van Trump WJ, Coombs S, Duncan K, McHenry MJ (2010) Gentamicin is ototoxic to all hair cells in the fish lateral line system. *Hear Res* 261(1-2):42–50
- Vlasits AL, Simon JA, Raible DW, Rubel EW, Owens KN (2012) Screen of FDA-approved drug library reveals compounds that protect hair cells from aminoglycosides and cisplatin. *Hear Res* 294(1-2):153–165
- Wada H, Ghysen A, Asakawa K, Abe G, Ishitani T, Kawakami K (2013) Wnt/Dkk negative feedback regulates sensory organ size in zebrafish. *Curr Biol* 23(16):1559–1565
- Webb JF, Fay RR, Popper AN (2008) *Fish bioacoustics*. Springer, New York
- Wersäll J (1956) Studies on the structure and innervation of the sensory epithelium of the cristae ampullaris in the guinea pig. *Acta Otolaryngol Suppl* 126
- Wersäll J (1960) Electron micrographic studies of vestibular hair cell innervation. In: Rasmussen GL, Windle WF (eds) *Handbook of sensory physiology*. Springer, New York
- Wersäll J (1961) Vestibular receptor cells in fish and mammals. *Acta Otolaryngol Suppl* 126:1–85
- Wersäll J, Flock Å (1964) Suppression and restoration of the microphonic output from the lateral line organ after local application of streptomycin. *Life Sci* 3:1151–1155
- Williams JA, Holder N (2000) Cell turnover in neuromasts of zebrafish larvae. *Hear Res* 143(1-2):171–181
- Wu W-J, Sha S-H, Schacht J (2002) Recent advances in understanding aminoglycoside ototoxicity and its prevention. *Audiol Neurootol* 7:171–174
- Yan HY, Saidel WM, Chang JS, Presson JC, Popper AN (1991) Sensory hair cells of a fish ear: evidence of multiple types based on ototoxicity sensitivity. *Proc R Soc Lond B* 245:133–138
- Zeddies DG, Fay RR (2005) Development of the acoustically evoked behavioral response in zebrafish to pure tones. *J Exp Biol* 208(7):1363–1372