

Review

Olfactory toxicity in fishes

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ABSTRACT

Olfaction conveys critical environmental information to fishes, enabling activities such as mating, locating food, discriminating kin, avoiding predators and homing. All of these behaviors can be impaired or lost as a result of exposure to toxic contaminants in surface waters. Historically, teleost olfaction studies have focused on behavioral responses to anthropogenic contaminants (e.g., avoidance). More recently, there has been a shift towards understanding the underlying mechanisms and functional significance of contaminant-mediated changes in fish olfaction. This includes a consideration of how contaminants affect the olfactory nervous system and, by extension, the downstream physiological and behavioral processes that together comprise a normal response to naturally occurring stimuli (e.g., reproductive priming or releasing pheromones). Numerous studies spanning several species have shown that ecologically relevant exposures to common pollutants such as metals and pesticides can interfere with fish olfaction and disrupt life history processes that determine individual survival and reproductive success. This represents one of the pathways by which toxic chemicals in aquatic habitats may increasingly contribute to the decline and at-risk status of many commercially and ecologically important fish stocks. Despite our emerging understanding of the threats that pollution poses for chemical communication in aquatic communities, many research challenges remain. These include: (1) the determination of specific mechanisms of toxicity in the fish olfactory sensory epithelium; (2) an understanding of the impacts of complex chemical mixtures; (3) the capacity to assess olfactory toxicity in fish *in situ*; (4) the impacts of toxins on olfactory-mediated behaviors that are still poorly understood for many fish species; and (5) the connections between sublethal effects on individual fish and the long-term viability of wild populations. This review summarizes and integrates studies on fish olfaction-contaminant interactions, including metrics ranging from the molecular to the behavioral, and highlights directions for future research.

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Abbreviations: 11-KT, 11 ketotestosterone; ABS, alkyl benzene sulfonates; ACh, acetylcholine; AChE, acetylcholinesterase; BKME, bleached kraft pulpmill effluent; CYP, cytochrome P450; DOC, dissolved organic carbon; EEG, electro-encephalogram; EOG, electro-olfactogram; GPCR, G-protein coupled receptor; GSH, glutathione; GST, glutathione S-transferase; GtH II, gonadotropin II; KME, unbleached kraft pulpmill effluent; LC₅₀, median lethal concentration; OB, olfactory bulb; OE, olfactory epithelium; OP, organophosphate insecticides; OSN, olfactory sensory neuron; PGF, F-type prostaglandin; ppb, parts per billion; ppm, parts per million; SLS, sodium laurel sulfonate; TChA, taurocholic acid; WHO, whole crude oil; WSF, water-soluble fraction of crude oil.

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1. Introduction

Fish rely upon olfaction to provide invaluable information over long distances and through environmental conditions that can render other sensory modalities unavailable. To receive olfactory information, sensory neurons interface almost directly with the aquatic environment, typically protected only in a covered cavity by mucus. In such an exposed situation, dissolved contaminants can interact with the olfactory neurons as readily as odorants, which is problematic given many of the contaminants presently found in the world's waters are neurotoxic, i.e. impair neuron functionality.

Olfaction consists of three main factors: the source, signal and receiver (Fig. 1). Fish, i.e. the source or the receiver, can receive signals imparting directional, conditional, tactical and genetic information. Directional information may come from stationary or moving sources. A well-known stationary example is the homing salmon exhibit to the odorant bouquet of their natal stream (Scholz et al., 1976), while a moving example is the searching behavior a shark exhibits up a concentration gradient of blood (Gilbert, 1977). Conditional information can indicate the status of either biotic or abiotic sources. A biotic example is the ability of male sticklebacks to discriminate between males and ovulated females (McLennan, 2004), while an abiotic example is the ability of goldfish (*Carassius auratus*) to sense changes in environmental calcium (a correlate of salinity) (Hubbard et al., 2000). Tactical information may concern the presence of prey (Hara, 2006a), or predators, either through the release of an alarm pheromone from a nearby injured fish (Brown, 2003) or through the scent of a predator (Rehnberg and Schreck, 1987; Vilhunen, 2006). Genetic information can enable sibling (Quinn and Hara, 1986) or conspecific identification (Rajakaruna et al., 2006).

Waterborne contaminants can disrupt all of the above olfactory-based responses, although the ways in which this can occur are often complex and involve multiple mechanisms. Contaminants can act as signals, modify odorant perception, and/or act on the nervous system and/or other physiologic responses (i.e., not directly through olfaction), all of which potentially alter normal olfactory-mediated responses (Fig. 2). For example, contaminants might mimic naturally-occurring odorants, or change stream chemistry so that these become biologically unavailable. They may also disrupt the endocrinology of fish, thereby causing them to send

situationally inappropriate cues. Some contaminants may appear to affect olfaction, but are actually impairing responses that can be linked to olfaction, such as directed swimming. Because of this complexity, isolating the manner(s) in which any given toxicant affects olfaction can require assessment of numerous biological endpoints.

It is important to note that even though concentrations of contaminants in the environment are typically quite low (e.g. in the ppb), they are not necessarily below concentrations of other compounds known to elicit biological responses (Fig. 3). For example, for three classes of odorants, a concentration of 10^{-9} M is sufficient to produce detectable responses in the olfactory system of fish (Fig. 3) (Hara, 1992). Similar molar concentrations of pesticides have been detected in surface waters of the United States (Gilliom et al., 2006) and Canada (Harris et al., 2008; Tierney et al., 2008). While exposure to these pesticide concentrations may not necessarily produce toxicity, the comparison to olfaction shows that these concentrations might be capable of producing a biological response.

This review summarizes a diversity of studies on fish olfaction and olfactory toxicity. This review also compares and relates olfactory toxicity endpoints measured at different levels of biological organization to reveal differences in the sensitivity of the levels and determine if lower level responses can be used to predict responses from higher, more ecologically relevant-responses such as behavior. Finally, this comprehensive review will serve well as a foundation for several unexplored research avenues (discussed below) that may ultimately help ensure the longevity of the world's fishes.

2. Fish olfaction

The neurobiology underlying olfaction in fish has been extensively reviewed (e.g. Zippel, 1993; Hara, 1994; Laberge and Hara, 2001; Zielinski and Hara, 2001, 2007; Hamdani and Døving, 2007). The sensitivity of fish olfaction is odorant-dependent. In general, fish can detect natural chemical cues in aquatic environments at concentrations ranging down to the parts per billion (10^{-9} M) or trillion (10^{-12} M) (Belanger et al., 2006). This level coincides with concentrations of natural odorants, such as amino acids (Shoji et al., 2003) and bile salts (Zhang et al., 2001), in surface waters. To provide a basic biological context for considering the impacts of

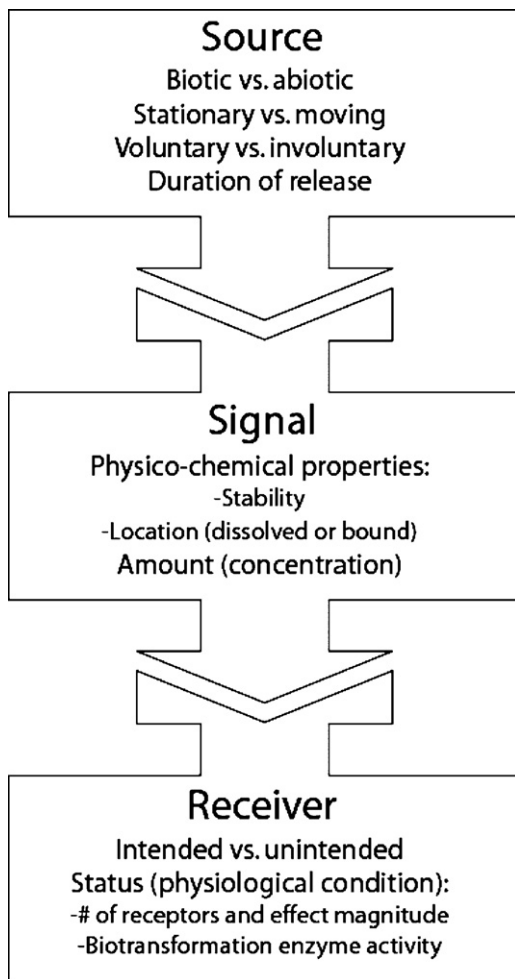


Fig. 1. A general schematic of the transmission of sensory information and the properties of three distinct compartments (with a focus on olfaction). Although sources can emit numerous substances, signals must emerge above background concentration, which will provide them with a location or locations, which receivers must be in or pass into. One example is an alarm pheromone, which is typically released involuntarily (through skin damage), acts locally and is short lived (i.e. is unstable), and is typically very specific to a particular receiver. A second example is a food odor, which likewise may be released involuntarily, come continuously from a moving organism, be very stable (such as an amino acid), and be detected by a range of other organisms.

contaminants, the architecture and key components of this sensory system are briefly described below.

Most teleost fish possess well-developed peripheral olfactory organs. These organs, or rosettes, are paired structures that reside in bilaterally positioned olfactory chambers. Once an odorant is taken into the olfactory chamber, either actively or passively depending on the fish and odorant, olfaction begins with an interaction between an odorant molecule, or ligand, and an olfactory sensory neuron (OSN) located in the olfactory epithelium (OE). Odorants bind to receptor proteins that are differentially expressed among individual OSNs. These G-protein coupled receptors (GPCRs) comprise a superfamily that includes a diverse array of as many as 100 different receptor types (Mombaerts, 1999). In fish and other vertebrates, each neuron generally expresses one receptor type (Sato et al., 2007). Not all fish have the same complement of receptor proteins. For example, rainbow trout (*Oncorhynchus mykiss*) appear insensitive to F-prostaglandins (PGFs), which serve as mating pheromones in other fish species (Laberge and Hara, 2003).

GPCRs have been classified into subfamilies, which include OR, V2R and GFB (reviewed in Hamdani and Døving, 2007). These subfamilies belong to morphologically distinct OSNs, in these

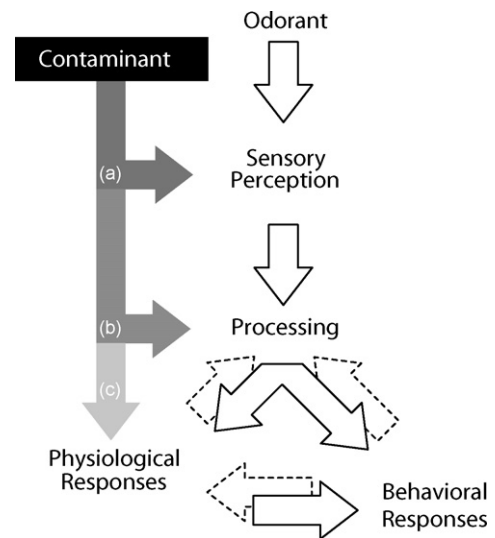


Fig. 2. Odorants are perceived by sensory neurons, the input is then processed and integrated with other sources, which can lead to physiological and/or behavioral responses. Physiological and behavioral responses can feed back on each other, and be integrated into further processing (adapted from: Scott and Sloman, 2004). Contaminants can (a), act as odorants or modify odorant perception, and/or (b), act on the nervous system through other pathways, and/or (c), alter other physiologic responses, all of which potentially translate into altered behavior.

cases, to ciliated, microvillus and crypt OSNs, respectively. Each OSN type can be distinguished microscopically, and is generally named after appearance: ciliated cells have cilia protruding from a knob, microvillus cells have larger, unciliated protruberances, while crypt cells have an apically focused ciliary grouping (Zielinski and Hara, 2001; Schmachtenberg, 2006). These classes have differential responses to five odorant classes: amino acid, bile salt, steroid, prostaglandin and nucleotide (Laberge and Hara, 2001). The different types of OSNs are dispersed across the OE, and OSNs that express a common odorant-binding receptor extend their axons via the olfactory nerve to converge on the olfactory bulb (OB) at discrete subregions containing one or more glomeruli (Friedrich and Korsching, 1998).

The various OSN classes express one of two types of heterotrimeric GPCRs; those that stimulate phospholipase C (PLC), which produces inositol triphosphate (IP₃) and those that stimulate adenylyl cyclase, which produces cAMP (Sorensen and Sato,

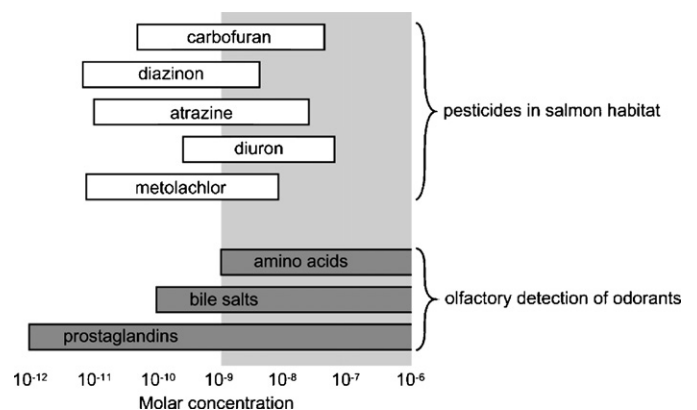


Fig. 3. Comparison of molar concentrations of pesticides measured in the environment and of molar concentrations of odorants required to elicit a detectable olfactory response. The five pesticides shown represent examples of data from surface water monitoring by the United States Geological Survey (reviewed in Gilliom et al., 2006) that have been converted to molar concentrations. The olfactory thresholds for three classes of odorants are representative of data summarized in Hara (1992).

2005). Both second messenger cascades lead to the opening of cation (sodium or calcium) channels, and the subsequent influx of calcium activates calcium-gated chloride channels (Zhainazarov and Ache, 1995). In fishes, these physiological changes in the electrical properties of OSNs have predominantly been characterized using an extracellular recording technique known as an electro-olfactogram (EOG). The EOG is a measure of the generator potential produced by populations of OSNs as they respond to odorant binding in the olfactory epithelium. The generator potential needs to be of sufficient magnitude in order to evoke an action potential. For this reason, factors that reduce the generator potentials conceivably cause fewer action potentials, and so can disrupt olfactory information. The EOG technique is a standard procedure (Baldwin and Scholz, 2005), one which has been in use for more than fifty years (Otto, 1956) (reviewed in Scott and Scott-Johnson, 2002). The terminal, glomerular responses to odorants at the level of the olfactory forebrain have also been monitored using extracellular field potential recordings; in this case as electro-encephalograms (EEGs) (Hara, 1975).

Following the integration of peripheral olfactory responses into the olfactory bulb, aggregate sensory information is relayed from the glomeruli by mitral cells to networks in other brain centers, which can be processed and lead to physiological and/or behavioral responses. In some cases, olfaction can be directly coupled to motion (i.e., lampreys will reflexively respond to a migratory pheromone, Dubuc et al., 2008). Olfaction can serve as the foundation for many complex behaviors, including alarm and avoidance response, feeding, migration, kin and conspecific recognition and mating synchronization, to name a few. Some of these responses involve physiologic components, for example the reception of priming pheromone by male salmon can lead to an increase in plasma testosterone that upregulates milt production (Sorensen, 1992; Waring et al., 1996). In this example, the olfactory-mediated response corresponded to a distinct stage of maturity, which is often the case. In general, olfaction ties fish to their biotic and abiotic environment, permits survival, and helps to facilitate reproduction.

3. Olfactory toxicity

The reception of chemical signals in the aquatic environment, the subsequent processing and integration of this information in the fish central nervous system, and the physiological and behavioral changes that subsequently occur together constitute a complex system that is vulnerable to the disruptive effects of toxicants at several levels of biological organization (Fig. 2). The peripheral olfactory system is distinct from most other components of the fish nervous system in that OSNs are in direct contact with an animal's surrounding environment. Because of this, they are particularly vulnerable to environmental changes, including exposure to neurotoxic xenobiotics. These changes in olfactory function can be categorized as (1) anosmia, or an inability to smell; (2) hyposmia, or a reduced capacity to smell; and (3) dysosmia, where olfactory information is processed incorrectly. Most chemical contaminants cause some degree of hyposmia or, at higher exposure concentrations, functional anosmia. Dysosmia is less common, but fish becoming attracted to relatively high concentrations of metal-contaminated waters is an example (e.g., Giattina et al., 1982). In this review we consider olfactory toxicity across several biological scales, from molecular biology to fish behavior.

3.1. Molecular and biochemical indicators of olfactory toxicity

Molecular analyses of contaminant-induced olfactory toxicity in fish have been relatively rare. However, this is likely to change with the advent and increasing refinement of microarray tech-

nologies, bioinformatics, and quantitative methods for measuring changes in the levels of targeted gene products and proteins in the transcriptome and proteome of OSNs, as well as other components of olfactory neural networks. Some of the available endpoints include measurements of cellular enzymatic reactions, DNA or RNA adducts, DNA mutations, or effects on cellular receptors or signal amplification proteins. Furthermore, current research methods, such as those used to profile the transcriptional dynamics of OSNs (for determining changes occurring with olfactory memory, Dukes et al., 2004), and similar approaches, should lend themselves to determine mechanisms of olfactory toxicity in fish.

Several mechanistic studies have focused on toxicants that are known to target acetylcholinesterase (AChE), an enzyme that regulates chemical signaling between cells (via the transmitter acetylcholine; ACh) in fish and other animals. These include, for example, organophosphorus and carbamate classes of pesticides. A variety of 'anticholinesterase' insecticides are known to reduce the responsiveness of OSNs to natural olfactory stimuli (i.e., cause hyposmia; Table 1). It has been suggested that the inhibition of AChE may be involved (e.g. Jarrard et al., 2004; Tierney et al., 2007b). This is because mucous production in the olfactory epithelium is upregulated by the secretion of ACh (Inglis et al., 1997). With reduced transmitter hydrolysis by AChE, mucous production will likely increase, thereby increasing the distance over which dissolved-phase odorants will have to diffuse to come in contact with receptor proteins on cilia and other apical extensions of OSNs. Notably, certain other stressors, such as the irritation of the olfactory epithelium by low pH (in rainbow trout; Miller and Mackay, 1982; Klapat et al., 1988), can also promote increased mucous secretion. Anticholinesterase pesticides can also influence other enzymes in the olfactory system. For example, diazinon exposure reduced the expression of the gene encoding the enzyme tyrosine hydroxylase (TH), a key regulator of catecholamine production, in the olfactory bulb of Japanese medaka (*Oryzias latipes*) (Shin et al., 2001).

Genotoxic compounds have the capacity to form DNA adducts. Although we are unaware of work on fish olfactory DNA adducts, examples exist for mammals exposed to toxicants (Mathison et al., 1995; Segerback et al., 1998). Since the toxicity of carcinogenic compounds typically evolves over long-term or repeated exposures, olfactory dysfunction through genotoxic mechanisms may take time to develop.

Receptor level effects hold promise for determining toxicity mechanisms, since many neurotoxic agents mediate their toxicity through receptor modification (Tierney and Kennedy, 2008). At least one study has already noted that OSNs expressing different GPCR proteins can be differentially affected by certain pesticides (Tierney et al., 2007b). Further studies could use biochemical techniques (e.g. inhibit or stimulate known portions of the GPCR signaling pathway) or molecular techniques (e.g. alter receptor protein expression) to further determine pesticide targets.

Another potential avenue for research is into possible contaminant-caused modification of proteins associated with olfaction. For example, basic research studies have determined changes in receptor protein transcripts and transcripts associated with nerve growth. For example, the transcription factor *otx2* was up-regulated in zebrafish (*Danio rerio*) following phenylethyl alcohol exposure (Harden et al., 2006). A recent study on copper toxicity (also using zebrafish) (Tilton et al., 2008), examined gene expression within olfactory tissues using gene set analysis (GSA) targeting genes in the olfactory signal transduction pathway. Down-regulations were noted in calcium channels, ion transports, g-proteins and olfactory receptors. This methodology could be used in other toxicity studies, such as with pesticides, to potentially isolate the mechanisms by which neurons and other cells of the OE are adversely affected.

Table 1

The effects of various contaminants on the olfactory responses of several fishes.

Contaminant	Species ^a	OSN test ^b	Odorant ^c	[odorant]	[contam.]	Exposure duration	Response (of pre-exp.)	Recovery (time)	(%)	Reference				
pH	<i>S. salar</i>	EOG	Testosterone	10 ⁻⁷ M	9.5	5 min	5%			Moore (1994)				
pH											8.5	62%		
											7.5	100%		
											6.5	76%		
											5.5	38%		
											4.5	5%		
											3.5	0%		
											Ov. female	1 in 10 ⁻⁴ dilution	9.5	48%
											Urine		8.5	64%
													7.5	176%
													6.5	100%
													5.5	80%
													4.5	20%
													3.5	0%
	<i>O. mykiss</i>	EEG	L-serine	10 ⁻⁵ M	4.7	2 wk	50%			Klaprat et al. (1988)				
Metals (pH 4.7+)	<i>O. mykiss</i>	EEG	L-serine	10 ⁻⁵ M	20 µm/L	2 wk	15%			Klaprat et al. (1988)				
Aluminum	<i>S. alpinus</i>	EEG	L-serine	10 ⁻⁵ M	0.1 mg/L	10 min	50%	10 min	80%	Thompson and Hara (1977)				
CdCl ₂	<i>O. kisutch</i>	EOG	L-serine	10 ⁻⁵ M	1 µg/L	30 min	75%			Baldwin et al. (2003)				
					2 µg/L		70%							
					5 µg/L		50%							
					10 µg/L		30%							
					10 µg/L		33%							
	EOG	TChA	10 ⁻⁶ M	5 µg/L	30 min	75%	90 min	65%		Sandahl et al. (2004)				
	EOG	L-serine	10 ⁻⁴ M	10 µg/L		50%								
				20 µg/L		5%								
			TChA	10 ⁻⁵ M	5 µg/L		50%							
					10 µg/L		50%							
					20 µg/L		0%							
		EEG	L-serine	10 ⁻⁴ M	5 µg/L		75%							
					10 µg/L		50%							
					20 µg/L		10%							
			TChA	10 ⁻⁵ M	5 µg/L		100%							
					10 µg/L		50%							
					20 µg/L	30%								
		EOG	L-serine	10 ⁻⁵ M	2 µg/L	3 h	85%			Sandahl et al. (2007)				
					5 µg/L		60%							
					10 µg/L		45%							
					20 µg/L		20%							
			TChA	10 ⁻⁶ M	2 µg/L		60%							
					5 µg/L	25%								
					10 µg/L	20%								
					20 µg/L	10%								
			Skin extract	10 µg of protein/L	2 µg/L		45%							
					5 µg/L		35%							
					10 µg/L		20%							
					20 µg/L		15%							
CuCl ₂	<i>O. keta</i>	EOG	L-serine	10 ⁻³ M	3 µg/L	4 h	86%	1 d	100%	Sandahl et al. (2006)				
					8 µg/L		71%				100%			

					24 µg/L		36%		71%	
					58 µg/L		11%		100%	
	<i>O. tshawytscha</i>		L-serine	10 ⁻³ M	25 µg/L	1 h	50%			Hansen et al. (1999b)
					50 µg/L		50%			
					100 µg/L		10%			
					200 µg/L		10%			
	<i>O. mykiss</i>		L-serine	10 ⁻³ M	25 µg/L	1 h	50%			Hansen et al. (1999b)
					50 µg/L		20%			
					100 µg/L		10%			
					200 µg/L		10%			
	<i>S. salar</i>	EOG	L-alanine	10 ⁻³ M	4 mM	5 min	50%	30 min	90%	Winberg et al. (1992)
	CuCl ₂	10 mM	in concentrations of HCO ₃ ⁻		0.4 mM		10%	30 min	70%	
					0.04 mM		0%	30 min	50%	
					0.00 mM		0%	30 min	30%	
CuSO ₄	<i>O. mykiss</i>	EEG	L-serine	10 ⁻⁵ M	0.01 mg/L	4 h	90%			Hara et al. (1976)
					0.05 mg/L	4 h	50%			
					0.1 mg/L	4 h	15%			
					0.1 mg/L	10 min	20%	10 min	80%	Thompson and Hara (1977)
HgCl ₂	<i>S. salar</i>	EEG	D L-serine	10 ⁻³ M	≥10 ⁻⁴ M	10 s	0%	>1-h		Sutterlin and Sutterlin (1971)
	<i>O. mykiss</i>	EEG	L-serine	10 ⁻⁵ M	0.25 mg/L	1 h	78%	20 min	100%	Hara et al. (1976)
						2 h	67%	50 min	100%	
						3 h	47%	60 min	60%	
						4 h	29%	60 min	55%	
Pesticides	<i>S. salar</i>	EOG	L-alanine	340 µM	10 ⁻⁵ M	2 min	35%	NA	50%	Baatrup et al. (1990)
2,4-D	<i>O. kisutch</i>	EOG	L-serine	10 ⁻³ M	1 mg/L	30 min	100%			Tierney et al. (2006a)
					10 mg/L		100%			
					100 mg/L		0%	>60 min		
	<i>O. mykiss</i>	EOG	L-histidine	10 ⁻⁵ M	1 µg/L	30 min	100%			Tierney et al. (2007c)
					10 µg/L		45%	2 min		
					100 µg/L		20%	2 min		
Atrazine	<i>S. salar</i>	EOG	PGF _{2α}	10 ⁻⁹ M	1 µg/L	30 min	100%			Moore and Waring (1998)
					2 µg/L		91%			
					5 µg/L		79%			
					10 µg/L		66%			
					20 µg/L		58%			
	<i>S. salar</i>	EOG	L-serine	10 ⁻⁵ M	2 µg/L	30 min	53%			Moore and Lower (2001)
			PGF _{2α}	10 ⁻⁹ M	1 µg/L		86%			
Carbaryl	<i>O. kisutch</i>	EOG	L-serine	10 ⁻⁵ M	100 µg/L	30 min	70%	>20 min		Tierney et al. (2007b)
			TChA		100 µg/L		75%	5 min		
	<i>O. mykiss</i>		L-serine		100 µg/L		80%	>20 min		
			TChA		100 µg/L		90%	10 min		
	<i>O. nerka</i>		L-serine		100 µg/L		50%	>20 min		
			TChA		100 µg/L		80%			
Carbofuran	<i>O. kisutch</i>	EOG	L-serine	10 ⁻⁵ M	2 µg/L	30 min	67%			Jarrard et al. (2004)
					10 µg/L		52%			
					20 µg/L		48%			

Table 1 (Continued)

Contaminant	Species ^a	OSN test ^b	Odorant ^c	[odorant]	[contam.]	Exposure duration	Response (of pre-exp.)	Recovery (time)	(%)	Reference									
Carbofuran	<i>S. salar</i>	EOG	PGF _{2α}	10 ⁻⁹ M	200 µg/L	30 min	20%			Waring and Moore (1997)									
					0.1 µg/L		61%												
					1 µg/L		88%												
					2 µg/L		73%												
					5 µg/L		82%												
10 µg/L	67%																		
Chlorothalonil	<i>O. kisutch</i>	EOG	L-serine	10 ⁻³ M	1 mg/L	30 min	100%			Tierney et al. (2006a)									
Chlorpyrifos	<i>O. kisutch</i>	EOG	TChA	10 ⁻⁵ M	0.625 µg/L	7 d	75%			Sandahl et al. (2004)									
					0.625 µg/L		75%												
					0.625 µg/L		75%												
					0.625 µg/L		75%												
					1.25 µg/L		30%												
					1.25 µg/L		30%												
					1.25 µg/L		50%												
Cypermethrin	<i>S. salar</i>	EOG	PGF _{2α}	10 ⁻⁸ M	<4 ng/L	5 d	12%			Moore and Waring (2001)									
					L-serine		10 ⁻⁵ M				<4 ng/L	17%							
					Diazinon		<i>S. salar</i>				EOG	PGF _{2α}	10 ⁻⁹ M	0.1 µg/L	30 min	100%	4.5 h	80%	Moore and Waring (1996b)
														1 µg/L		78%			
														2 µg/L		65%			
Endosulfan	<i>O. kisutch</i>	EOG	L-serine	10 ⁻³ M	10 µg/L	30 min	100%	2 min		Tierney et al. (2006a)									
					100 µg/L		60%												
					Glyphosate		<i>O. kisutch</i>				EOG	L-serine	10 ⁻³ M	0.1 mg/L	30 min	100%	>60 min		Tierney et al. (2006a)
														1 mg/L		66%			
														10 mg/L		44%			
IPBC	<i>O. kisutch</i>	EOG	L-serine	10 ⁻⁵ M	0.047 µg/L	30 min	70%			Jarrard et al. (2004)									
					0.47 µg/L		51%												
					4.7 µg/L		35%												
					47 µg/L		24%												
					1 µg/L		72%												
Linuron	<i>O. kisutch</i>	EOG	L-serine	10 ⁻⁵ M	100 µg/L	30 min	40%	30 min		Tierney et al. (2006a)									
					100 µg/L		100%												
					100 µg/L		54%												
					100 µg/L		58%												
					100 µg/L		58%												
Linuron	<i>O. mykiss</i>	EOG	L-histidine	10 ⁻⁵ M	1 µg/L	30 min	45%	2 min		Tierney et al. (2007c)									
					10 µg/L		40%												
					100 µg/L		20%												
Linuron	<i>O. mykiss</i>	EOG	L-serine	10 ⁻⁵ M	100 µg/L	30 min	40%	>60 min		Tierney et al. (2007b)									
					100 µg/L		100%												
					100 µg/L		100%												
Linuron	<i>O. nerka</i>	EOG	L-serine	10 ⁻⁵ M	100 µg/L	30 min	50%			Tierney et al. (2007b)									
					100 µg/L		100%												
					100 µg/L		100%												

Mancozeb	<i>O. kisutch</i>	EOG	L-serine	10 ⁻⁵ M	0.22 mg/L 2.2 mg/L	30 min	57% 50%			Jarrard et al. (2004)
Roundup®	<i>O. mykiss</i>	EOG	L-histidine	10 ⁻⁵ M	10 µg/L 100 µg/L 1000 µg/L	30 min	70% 40% 25%	2 min 2 min 2 min	50%	Tierney et al. (2007c)
Simazine	<i>S. salar</i>	EOG	L-serine PGF _{2α}	10 ⁻⁵ M 10 ⁻⁹ M	2 µg/L 0.1 µg/L 2 µg/L	30 min	50% 101% 72%			Moore and Lower (2001)
Simazine + Atrazine	<i>S. salar</i>	EOG	L-serine PGF _{2α}	10 ⁻⁵ M 10 ⁻⁹ M 10 ⁻⁹ M	1 + 1 µg/L 0.5 + 0.5 1 + 1		51% 82% 70%			
Trifluralin	<i>O. kisutch</i>	EOG	L-serine	10 ⁻³ M	30 µg/L 300 µg/L	30 min	100% 70%	2 min		Tierney et al. (2006a)
Pesticide complex mixture Mixture of: Dimethoate, Simazine, Methamidophos, Diazinon, Chlorpyrifos, Endosulphan, Malathion, Atrazine, Linuron, Parathion	<i>O. mykiss</i>	EOG	L-serine	10 ⁻³ in 10 ⁻⁵	0.186 µg/L 1.01 µg/L 13.9 µg/L	96 h	-14% -42% -53%			Tierney et al. (2008)
Surfactants SLS	<i>C. clupeaformis</i>	EEG	Food extract L-serine	NA 10 ⁻⁵ M	0.1 mg/L 0.5 mg/L 1 mg/L 5 mg/L 10 mg/L 0.1 mg/L 0.5 mg/L 1 mg/L 5 mg/L 10 mg/L	15 min	20% 50% 70% 60% 80% 10% 50% 55% 60% 90%			Hara and Thompson (1978)
<i>S. salar</i> /EEG/10 ⁻⁵ M DL-alanine Alkyldimethyl-3,4-dichloro-benzyl ammonium chloride Alkyldimethyl-3,4-dichloro-benzyl ammonium chloride B-hydroxyethylbenzyl coco imidazolinium chloride B-hydroxyethylbenzyl coco imidazolinium chloride B-hydroxyethylbenzyl stearyl imidazolinium chloride B-hydroxyethylbenzyl stearyl imidazolinium chloride Branched sodium dodecylbenzene sulfonate Calcium dodecylbenzene sulfonate Di hydrogenated tallow dimethyl ammonium chloride DL-coco dimethyl ammonium chloride DL-coco dimethyl ammonium chloride Lauryldimethylbenzyl ammonium chloride					1 mg/L 10 mg/L 1 mg/L 10 mg/L 1 mg/L 10 mg/L 10 mg/L 10 mg/L 10 mg/L 10 mg/L 1 mg/L 10 mg/L 10 mg/L	15 s	90% 0% 50% 0% 90% 27% 60% 60% 90% 39% 0% 85%			Sutterlin et al. (1971)

Table 1 (Continued)

Contaminant	Species ^a	OSN test ^b	Odorant ^c	[odorant]	[contam.]	Exposure duration	Response (of pre-exp.)	Recovery (time)	(%)	Reference
Linear sodium dodecylbenzene sulfonate					10 mg/L		50%			
Methyldodecylbenzyl-trimethyl ammonium chloride					1 mg/L		70%			
Methyldodecylbenzyl-trimethyl ammonium chloride					10 mg/L		0%			
N-coco-propylenediamine					10 mg/L		30%			
N-soya-propylenediamine					10 mg/L		18%			
N-tallow-propylenediamine					10 mg/L		26%			
Octylcresoxyethoxyethyl-dimethylbenzyl ammonium chloride					1 mg/L		33%			
Octylcresoxyethoxyethyl-dimethylbenzyl ammonium chloride					10 mg/L		0%			
Octylphenoxyethoxyethyl-dimethylbenzyl ammonium chloride					1 mg/L		85%			
Octylphenoxyethoxyethyl-dimethylbenzyl ammonium chloride					10 mg/L		0%			
Sodium kerylbenzene sulfonate					10 mg/L		80%			
Sodium toluene sulfonate					10 mg/L		80%			
Sodium tridecylbenzene sulfonate					10 mg/L		60%			
Stearyldimethylbenzyl ammonium chloride					10 mg/L		90%			
Triethanolammonium dodecylbenzene sulfonate					10 mg/L		48%			
Other contaminants										
Hydrocarbons (monocyclic aromatic)	<i>O. kisutch</i>	EEG	L-serine	10 ⁻³ M	4 mg/L	20 min	NS			Maynard and Weber (1981)
Morpholine	<i>O. mykiss</i>	EEG	L-serine	10 ⁻⁵ M	10 g/L	2 min	70%			Hara (1974)

^a Fish key: *C. clupeaformis* = Lake whitefish, *O. keta* = Chum salmon, *O. kisutch* = Coho salmon, *O. mykiss* = Rainbow trout, *O. nerka* = Sockeye salmon, *O. tshawytscha* = Chinook salmon, *S. alpinus* = Arctic char, *S. salar* = Atlantic salmon.

^b Two types of olfactory neuron tests are included: EOG (electro-olfactogram), which are field potentials taken from the nasal tissue, and EEG (electro-encephalogram), which are field potentials taken from the olfactory bulb (i.e. brain).

^c Various odorants were used to evoke EOG and EEG responses, these include amino acids (L-serine, L-histidine, DL-alanine), bile salt (taurocholic acid; TChA), food extract, hormones (testosterone), ovulated (Ov.) female urine and pheromones (prostaglandin F_{2α}; PGF_{2α}).

3.2. Neurophysiological indicators of olfactory toxicity

Direct, *in vivo* measurements of OSN function within the olfactory rosette, or the integration of peripheral OSN activity into the olfactory bulb, have been widely used for many years to characterize olfactory toxicants in fish. The ability of a toxicant to impair the physiology of the cells can be measured as reductions in the amplitudes of the responses to odorants following exposure. The sections that follow will consider specific examples for pH, metals, pesticides and surfactants on cells of the olfactory rosette and bulb. Data from both rosette (EOG) and bulbar (EEG) recordings typically vary proportionately with each other (Sandahl et al., 2004; Hara, 2006b), and are considered together below.

3.2.1. pH

Acid rain, mining waste and industrial discharges are among some of the factors that can alter the pH of an aquatic environment. Both acidity and alkalinity appear to alter fish OSN responses, and the effects do not appear specific to OSN class. The EOG responses of male Atlantic salmon (*S. salar*) to testosterone and dilute female urine were reduced in a concentration-dependent manner within 5 min of exposure to pH changes $>$ or $<$ 7.5 (Moore, 1994) (Table 1). Chronic exposure to acidified water also results in EOG reduction. For example, rainbow trout exposed to pH 4.7 water for two weeks showed a 50% reduction in their olfactory responsiveness to the amino acid L-serine (Klaprat et al., 1988). Together, these data suggest pH can cause acute and persisting impairment of olfactory function.

3.2.2. Metals

Metals are well-known for their effectiveness as blockers of ion channels, such as sodium or calcium channels (reviewed in Florea and Busseberg, 2006). In studies of fish olfactory toxicity, copper has received the most attention (Table 1). This metal can cause a concentration-dependent decrease in EOG/EEG, with negative effects occurring at concentrations below 10 μ g/L. For example, the perfusion of copper caused a steady decline in coho salmon (*O. kisutch*) OSN responses over 30-min exposures (Baldwin et al., 2003). In particular, 5 min into an exposure of 10 μ g/L copper, EOG responses were \sim 80% of the pre-exposure value. At 30 min, the value was \sim 30% of pre-exposure. Recovery was not rapid, as even 90 min following the return of clean water flow to the OSNs, the responses were only \sim 60%. Recovery from copper toxicity over longer intervals (several days) was explored in chum salmon (*O. keta*). EOG responses appeared to return to control within 1 d following exposure to concentrations less than 24 μ g/L (Sandahl et al., 2006). The toxicity of copper does not appear specific to OSN receptor class, as exposure caused similar decreases in responses to L-serine and taurocholic acid (TChA; a bile salt) alone, as well as to an amino acid mixture (Baldwin et al., 2003), and a skin extract (Sandahl et al., 2007). These findings imply that copper exposure can have a general effect on olfactory tissue, as suggested by Thompson and Hara (1977).

The inhibitory effects of copper on the odor-evoked responsiveness of OSNs are influenced to a certain degree by changes in water chemistry such as hardness, alkalinity, and dissolved organic carbon (DOC) (Baldwin et al., 2003; Bjerselius et al., 1993; Winberg et al., 1992). Recently, McIntyre et al. (2008) evaluated a range of water chemistries encompassing habitat conditions for salmon in the western United States. They found that environmentally relevant changes in water hardness (as calcium carbonate) and alkalinity had only modest influences on copper neurotoxicity, as indicated by changes in the amplitudes of EOGs evoked by L-serine. The ameliorative effects of DOC were more pronounced. Specifically, DOC levels above \sim 6.0 mg/L were protective against copper toxicity.

There have been mixed results to the question of whether certain fish species are more sensitive than others to the olfactory neurotoxicity of copper. In experiments using EEG recordings, Hansen et al. (1999b) found that rainbow trout (*O. mykiss*) were more vulnerable than juvenile chinook salmon (*O. tshawytscha*). Thus, while there may be modest differences in sensitivity for some species, the available evidence suggests that copper is a general-purpose olfactory toxicant for all freshwater fish.

Although copper has received considerable attention in recent years, the basic phenomenon of copper-induced olfactory toxicity has been known for more than three decades (Hara et al., 1976). Studies even earlier had used the EOG technique to show that mercuric chloride (HgCl_2) blocks the olfactory response of Atlantic salmon to various amino acids (Sutterlin and Sutterlin, 1971). Here 10^{-4} M (\sim 27 mg/L) mercuric chloride effectively eliminated all amino acid responses up to 1-h post-exposure. Subsequent studies also focused on the toxicity of HgCl_2 for this purpose, including Hara et al. (1976), Thompson and Hara (1977) and Baatrup et al. (1990). The latter study found that within 2 min of exposure to 10^{-5} M HgCl_2 (\sim 2.7 mg/L), the EOG responses of Atlantic salmon to L-alanine were reduced to 35% of the pre-exposure amplitude. These responses recovered to 50% when the mercury-containing perfusate was switched to clean water. Methylation appears to enhance mercury's toxicity, as EOG responses did not recover within 10 min of exposure to an equivalent concentration of methyl mercury (CH_3HgCl) (Baatrup et al., 1990).

Aluminum has also been shown to influence the electrical properties of OSNs. In a study on rainbow trout, Klaprat et al. (1988) found aluminum exposures in low pH water caused reductions in trout EOGs that were greater than those produced by an equivalent change in pH alone (a 35% differential). Although other studies have shown that the olfactory-mediated behaviors of fish are impaired in low pH waters (discussed below), more work is needed on the peripheral sensory toxicity of aluminum, independent of pH. Finally, other metals have been shown to interfere with chemoreception in fish, albeit at exposure concentrations that are higher than those for copper (e.g., mg/L vs. μ g/L). This includes cadmium (CdCl_2) (Thompson and Hara, 1977). Interestingly, gold reduces the responsiveness of chemoreceptors in the mouths of carp (*Cyprinus carpio*) to taste stimuli (Hidaka, 1970).

Compared to the examples above for individual metals, only a few studies have assessed the toxicity of mixtures of metals, such as those associated with surface waters contaminated with mining effluent, industrial discharges, or stormwater runoff from roadways. For example, an investigation into the toxicity of lake water degraded by mining effluent, consisting of cadmium, copper, nickel and zinc, to Arctic char (*Salvelinus alpinus*) determined that cadmium but not nickel or zinc also can impair OSNs (Thompson and Hara, 1977).

3.2.3. Pesticides

Several classes of pesticides affect fish olfactory responses (Table 1). Those that do, typically have a rapid effect that manifests within minutes. Pesticide-mediated OSN impairments often remain constant throughout the exposure period, and the recovery of sensory function occurs more quickly for pesticides than for metals such as copper (minutes vs. hours). Although direct evidence is still lacking, this suggests that pesticides and metals may have different targets in the olfactory epithelium.

In one of the few analyses of pesticide toxicity to fish in seawater, Labenia et al. (2007) found that the olfactory system of seawater-phase cutthroat trout (*O. clarki*) is unresponsive to relatively high concentrations ($>$ 0.5 mg/L) of the insecticide carbaryl. Several studies in freshwater fish have also found that OSNs do not respond to several other pesticides, including chlorpyrifos (Sandahl et al., 2004), esfenvalerate (Sandahl et al., 2004), and atrazine (Tierney et

al., 2007c). If fish are unable to detect pesticides using their sense of smell, they may not be able to behaviorally avoid aquatic habitats contaminated with these chemicals (Labenia et al., 2007). This would appear to set pesticides apart from metals such as copper, which fish actively avoid (e.g., Hansen et al., 1999a).

Carbamate insecticides—EOG responses can be rapidly decreased by exposure to parts per billion concentrations of carbamates (Table 1). The fungicide IPBC (3-iodo-2-propynyl butyl carbamate) is the most toxic chemical in this class that has been examined thus far, with a 30-min exposure to 0.1 µg/L IPBC reducing coho EOGs by as much as 40% (Jarrard et al., 2004). Other carbamates appear less toxic. Carbofuran, for example, required almost 10 µg/L to reach the same level of impairment (Jarrard et al., 2004). These findings are not unsurprising given the mechanism of toxicity for IPBC is believed to differ from other carbamates (i.e. may not be mediated through anti-AChE effects) (Juergensen et al., 2000). Carbaryl impaired both L-serine and TChA-evoked EOGs of coho, rainbow trout and sockeye salmon (*O. nerka*) within 30 min, although the subset of OSNs responding to TChA was less sensitive (Tierney et al., 2007b). Here the differences across OSN class were not large enough to suggest that the mechanism of toxic action of carbamates may be specific to certain OSNs. Yet, with OSN classes often differing in morphology and receptor and transduction machinery, it would be surprising if some pesticides did not have OSN class-specific effects.

Organophosphate (OP) insecticides—As with carbamates, these anti-AChE agents can rapidly reduce EOG responses (Table 1). Chlorpyrifos appears to be the most toxic. 30-min exposure to 625 ng/L chlorpyrifos exposure reduced coho L-serine and TChA-evoked EOGs and EEGs to 75% of control (Sandahl et al., 2004). A third receptor class, those that respond to pheromones, was also tested with diazinon. A 30-min exposure to this OP caused a concentration-dependent decrease in EOG responses to prostaglandin F_{2α} (PGF_{2α}) in precociously maturing male Atlantic salmon (Moore and Waring, 1996b) (Table 1). Fish that had a reduced olfactory sensitivity to this priming pheromone also had reduced milt production.

Phenylurea herbicides—One phenylurea herbicide has been evaluated, and it is the first example of marked OSN class-specific toxicity (Table 1). Exposure to the phenylurea herbicide linuron caused toxicity to one class of OSNs but not another (Tierney et al., 2007b). Specifically, the L-serine-evoked EOG responses of coho, rainbow trout and sockeye salmon were reduced from 50 to 80% of their pre-exposure values following a 30-min exposure to 10 µg/L. However, the TChA-evoked responses were not affected for any of the species, even by a 100 µg/L exposure. Since ciliated OSNs respond to at least three odorant classes (pheromone, amino acid and bile salt) and microvillar respond to just amino acids (Sato and Suzuki, 2001), it is possible that microvillar OSNs are more susceptible to this herbicide. This differential toxicity highlights the potential dissimilarity between the general effects of metal exposure and the potentially specific effects of a subset of pesticides.

Pyrethroid insecticides—There is reason to expect that these pesticides will affect salmon olfaction, since they act by delaying closure of sodium channels (Narahashi, 1996). In fact, effects on salmon EOG responses have been noted in separate studies of cypermethrin and esfenvalerate, although very different results were found for each. A strict comparison cannot be made though, as the concentration and exposure periods were considerably different. Peripheral changes in coho OSN responses were not observed following exposure to 0.2 µg/L of esfenvalerate (Sandahl et al., 2004). However, simultaneous recordings from the olfactory bulb revealed bursts of abnormal activity in response to the stimulation of the sensory epithelium. This central hyperexcitation is consistent with actions of pyrethroids on voltage-gated sodium channels. Following exposure of Atlantic salmon to cypermethrin, EOG effects

were noted (Moore and Waring, 2001). For exposure to <4 ng/L, PGF_{2α}-evoked EOGs were only 12% of control, and L-serine evoked EOGs were 17%. The reasons for these differences in the olfactory toxicity of esfenvalerate and cypermethrin are not presently known. The pyrethroids are a large and increasingly important class of current use insecticides, and these chemicals merit additional study.

Triazine herbicides—As with carbamate, OP and phenylurea pesticides, triazine herbicides are effective at impairing EOG responses in the µg/L range (Table 1). Moore and Waring (1998) noted decreases in Atlantic salmon EOGs evoked by PGF_{2α} following atrazine exposures greater than 2 µg/L. Amino acid responses can be similarly affected, as decreased EOGs evoked by both L-serine and L-histidine have been noted (Moore and Lower, 2001 and Tierney et al., 2007c, respectively). For example, in Atlantic salmon, a 30-min exposure to 2 µg/L simazine reduced L-serine-evoked EOGs to ~70% of pre-exposure (Moore and Lower, 2001), while for rainbow trout, a 30-min exposure to 10 µg/L of atrazine reduced L-histidine responses to 45% of pre-exposure amplitudes (Tierney et al., 2007c). Simazine and atrazine likely share a common olfactory mechanism of EOG toxicity, since in combination their effects were additive (Moore and Lower, 2001) (Table 1).

Other pesticides—There are numerous other current-use pesticides that are delivered to fish habitats via surface runoff and other transport pathways. However, the remaining chemicals that have been examined to date do not appear to cause olfactory toxicity over a concentration range in the low parts per billion, which is typically of the greatest environmental relevance for fish (excepting accidental chemical spills). The 2,6-dinitroaniline herbicide trifluralin and organochlorine insecticide endosulfan affected coho EOGs. However, hyposmia was marginal and only occurred at concentrations approaching the solubility of the two pesticides (Tierney et al., 2006a) (Table 1). Exposure to a high (100 mg/L) concentration of the phenoxyacetic herbicide 2,4-D appeared to eliminate coho EOG responses altogether (Tierney et al., 2006a). A similar concentration of glyphosate also reduced EOGs, but recovery followed (Tierney et al., 2006a). Given that glyphosate resembles the amino acid glycine, the reductions and recovery in L-serine-evoked EOGs may have been caused by some receptor specificity overlap. One of the pesticide formulations in which glyphosate serves as the active ingredient, Roundup®, is considerably (>10-fold) more toxic than the active ingredient alone (Tierney et al., 2007c). An exposure of 100 µg/L (of active ingredient in formulation) resulted in a persisting (>20 min) 50% EOG impairment in rainbow trout (Tierney et al., 2007c). The greater effects observed with Roundup® may have been due to 'inert ingredients' such as surfactants, as many of these chemicals are known to be olfactory toxicants (Sutterlin et al., 1971).

Pesticide mixtures—Capturing environmentally realistic exposure scenarios involves testing pesticide mixtures, since these are typically encountered (Gilliom et al., 2006; Harris et al., 2008). However, testing pesticides in mixtures, especially complex ones, greatly limits (or abolishes altogether) the mechanistic determination of pesticide effects. At least two studies have attempted to use EOG effects to measure mixture effects, one of which focused on two pesticides of the same class (mentioned already above under triazine) (Moore and Lower, 2001), and the other of pesticides across several classes (Tierney et al., 2008). In the latter, rainbow trout exposed to a combination of ten of the most frequently occurring pesticides in a salmon-bearing waterway (i.e. a complex mixture) (Table 1), did experience diminished EOG responses, although EOG recordings were used in a slightly different manner (to detect the ability of the olfactory system to respond to a change in L-serine background concentration). The overall message from the complex mixture study was that a low, realistic concentration of pesticides has the ability to affect OSNs. Future studies may wish to increas-

ingly focus on mixtures, and through the simultaneous testing of individual pesticide effects, develop predictive models of OSN impairment.

3.2.4. Other contaminants

The effects of many contaminants, including hydrocarbons, on EOG/EEG remain unknown. However, there have been at least two studies that examined the effects of surfactants. An early electrophysiology study into the effects of contaminants on fish olfaction tested the effect of 150 surfactants on the EEG responses of Atlantic salmon (Sutterlin et al., 1971). Because of the volume of tests it is not possible to present the results for each surfactant. Rather, the effects of several classes are presented (Table 1). Examples of chemicals affecting EEGs include anionic surfactants such as alkyl benzene sulfonates (ABS), diamines and quaternary ammonia compounds. A subsequent EOG study found that exposure to sodium laurel sulfonate (SLS) at 0.5 mg/L depressed L-serine evoked responses in lake whitefish (*Coregonus clupeaformis*) by 50% (Hara and Thompson, 1978). Surfactants, adjuvants and emulsifiers are widely used in pesticide formulations. Given that Roundup® was found to be 10-fold more toxic than its active ingredient alone (Tierney et al., 2007c), these chemicals should be a focus of future study. Our understanding of the impacts of surfactants on the sensory biology of fish also benefit from improved environmental monitoring of surfactants from various sources, including pesticide use, municipal wastewater discharges and urban stormwater runoff.

3.3. Anatomical indicators of olfactory toxicity

The effects of toxic agents may be evident as changes to internal or external cellular appearance, or in cellular death or growth. As with other techniques, quantification of the extent of alteration through histochemical and ultrastructural/SEM means can represent a challenge (Bernet et al., 1999). Nevertheless, just as *in situ* cellular responses such as EOG/EEG temporally captures changes in condition, histology or immunocytochemistry may be used to identify disruption of a natural state by toxic agents. It should be noted, however, that disruption in physiological function may occur at exposure concentrations that are lower than those that cause overt physical damage.

3.3.1. pH

Moderate acid (pH 4.7) exposure alone does not appear to cause ciliary loss (Klaprat et al., 1988). Data are unavailable for the effects of alkalinity on the olfactory epithelium. Given that pH alterations have an effect on EOG responses, not all pH-mediated effects may be apparent through structural observation.

3.3.2. Metals

Metals are the focus of several histological studies. Copper toxicity has been explored histochemically in a variety of ways, and the effects are concentration-dependant. The number of OSNs in chum salmon taking up fluorescent dye increased following 3 and 8 µg/L exposures but decreased with 24 and 58 µg/L 4-h exposures (Sandahl et al., 2006), which suggests that membrane function may be affected. Recovery was not complete even after 10 d. For brown trout (*Salmo trutta*), a concentration of 18 µg/L caused ciliary loss within a day, and recovery took up to 8 d (assessed using TEM and SEM) (Moran et al., 1992). For rainbow trout, a similar concentration (20 µg/L) caused changes in OSNs consistent with apoptotic responses following a 15-d exposure (Julliard et al., 1996). Copper exposure concentrations of ≥50 µg/L reduced the number of ciliated and microvillar cells in chinook and rainbow trout within 1–4-h of exposure (Hansen et al., 1999b). This exposure was associated with loss of cilia and rupture of microvillar

cells. Lengthy copper exposure can alter cell growth and death. For example, increases were noted in the number of goblet cells and degenerating cells in sections of rainbow trout olfactory epithelium chronically (40 wks) exposed to 20 and 40 µg/L copper (Saucier and Astic, 1995). These changes gradually reversed, with 6 and 14 wks required for recovery from the respective concentrations. Combining these various results for copper exposure, it appears that copper causes anatomical changes in the olfactory epithelium that range from slight to severe following low to high µg/L range exposure, respectively.

Aluminum may cause anatomical alteration in the olfactory epithelium at similar concentrations as copper. Klaprat et al. (1988) found that aluminum (9.5 µg/L) in combination with moderate acid (pH 4.7) caused significant ciliary destruction in rainbow trout. Further studies need to test aluminum on its own to determine its effects.

Metal accumulation may occur in the olfactory epithelium, which may give rise to longer term metal toxicity. Mercuric chloride (HgCl₂) accumulated around the cellular borders of OSNs in Atlantic salmon, while methyl mercury (CH₃HgCl) given in food accumulated in OSN lysosomes and inclusion bodies (Baatrup and Døving, 1990). Furthermore, through anterograde (forward moving) transport up the axons, such metals can make their way into the olfactory bulb (Tallkvist et al., 1998), potentially causing impairment in bulbar olfactory responses.

3.3.3. Pesticides

There do not appear to be any studies that have shown anatomical injury to fish OSNs.

3.3.4. Other contaminants

The first documented study into the effects of contaminants on fish olfactory epithelium did not use metals, but rather surfactants. Yellow bullheads (*Ictalurus natalis*) exposed to hard (degradation resistant) and soft (degradable) ABS surfactants experienced a thickening of the OSNs that was not repaired within 6 wks of exposure to 4 and 5 µg/L (Bardach et al., 1965). This resulted in impaired olfaction, as exposed fish were unable to locate distant food pellets as well as control fish. Exposure to 0.03–0.1% of the non-ionic detergent Triton X-100 caused loss of olfactory epithelium cells in channel catfish (*Ictalurus punctatus*), with regeneration apparent within 4 d (Cancalon, 1983). These authors suggested that membrane receptor proteins were solubilized by the detergent.

Hydrocarbon exposure appears to alter cellular turnover in olfactory tissue. For tidewater silverside (*Menidia beryllina*) exposed for 7 d to whole crude oil (WHO) and water-soluble fractions (WSF) of crude oil, aberrant growth (hyperplastic; i.e. increase in cell number) and death of supporting (sustentacular) olfactory cells, as well as death of sensory cells, occurred at concentrations of 5 mg WHO and 5% WSF (21–30-d exposure) (Solangi and Overstreet, 1982). The hogchoker (*Trinectes maculatus*) exhibited similar cell death, however at higher concentrations of 100 mg/L WHO and 50% WSF (Solangi and Overstreet, 1982). Increased cell death may be attributable to the oxidative stress that hydrocarbons can impart (Xue and Warshawsky, 2005). Differences across cell types may be partially due to intrinsic differences in the expression of enzymes that protect against such stress (e.g. glutathione S-transferases; GSTs).

3.4. Behavioral indicators of olfactory toxicity

Behavioral responses are intended to improve an organism's position with respect to survival. Unpleasant or painful stimuli will stereotypically and reflexively evoke avoidance behavior. However, there is no guarantee that nociception (pain) is associated with contaminant exposure. For example, fish sometimes exhibit attraction

Table 2
The preference or avoidance responses of a variety of fishes to various contaminants.

Contaminant		Concentration	Response	Species ^a	Reference
pH					
pH	pH	5.5	Avoidance	<i>S. alpinus</i>	Jones et al. (1985a)
Metals					
Arsenic	Na ₂ AsO ₂ (Ar III)	28 µg/L	Avoidance	<i>N. crysoleucas</i>	Hartwell et al. (1989)
Cadmium	CdCl ₂	68 µg/L	NR	<i>N. crysoleucas</i>	
Chromium	K ₂ Cr ₂ O ₇ (Cr IV)	73 µg/L	Avoidance	<i>N. crysoleucas</i>	
Cobalt	CoCl ₂	180 µg/L	Avoidance	<i>O. mykiss</i>	Hansen et al. (1999a)
		24 µg/L	Avoidance	<i>O. tshawytscha</i>	
Copper	CuCl ₂	0.7 µg/L	Avoidance	<i>O. tshawytscha</i>	Hansen et al. (1999a)
		1.6 µg/L	Avoidance	<i>O. mykiss</i>	
		26 µg/L	Avoidance	<i>N. crysoleucas</i>	Hartwell et al. (1989)
		330 µg/L	Attraction	<i>O. mykiss</i>	Giattina et al. (1982)
		6.4 µg/L	Avoidance	<i>O. mykiss</i>	
	CuSO ₄	0.1 µg/L	Avoidance	<i>O. mykiss</i>	Folmar (1976)
		16 mg/L	Avoidance	<i>P. pungitius</i>	Jones (1947)
Iron	Fe (total dissolved sp.)	4.25–6.45 mg/L	Avoidance	<i>O. kisutch</i>	Updegraff and Sykora (1976)
Mercury	HgCl ₂	272 mg/L	Avoidance	<i>P. pungitius</i>	Jones (1947)
Nickel	NiCl ₂	23.9 µg/L	Avoidance	<i>O. mykiss</i>	Giattina et al. (1982)
		6 µg/L	Attraction	<i>O. mykiss</i>	
Selenium	Na ₂ SeO ₃	3489 µg/L	NR	<i>N. crysoleucas</i>	Hartwell et al. (1989)
Zinc	ZnSO ₄	48 mg/L	Avoidance	<i>P. pungitius</i>	Jones (1947)
		5.6 µg/L	Avoidance	<i>O. mykiss</i>	Sprague (1968)
Mixture	Cu:Co mixture	1.0:0.9 µg/L	Avoidance	<i>O. tshawytscha</i>	Hansen et al. (1999a)
		2.6:2.4 µg/L	Avoidance	<i>O. mykiss</i>	
Mixture	12 Cu:1.1 Cd:3.2 Pb:50 Zn	6.6 µg/L (total)	Avoidance	<i>O. mykiss</i>	Hansen et al. (1999c)
Mixture	1 Cu:0.54 Cr:1.85 Ar:0.38 Se	29 µg/L (in lab)	Avoidance	<i>P. promelas</i>	Hartwell et al. (1987a)
Mixture	1 Cu:0.54 Cr:1.85 Ar:0.38 Se	71.1 µg/L (in field)	Avoidance	<i>P. promelas</i>	
	Spring (simulated stream)	34.3 µg/L (in field)	Avoidance	<i>P. promelas</i>	
	Summer (simulated stream)	73.5 µg/L (in field)	Avoidance	<i>P. promelas</i>	
	Summer (natural)				
Pesticides					
2,4-D	Herbicide	0.1 mg/L	Avoidance	<i>C. variegatus</i>	Hansen (1969)
		1 mg/L	Avoidance	<i>G. affinis</i>	Hansen et al. (1972)
		1 mg/L	Avoidance	<i>O. mykiss</i>	Folmar (1976)
Acrolein	Algaecide	0.01 mg/L	Avoidance	<i>O. mykiss</i>	Folmar (1976)
Bentazone	Herbicide	0.01 and 10 mg/L	Attraction	<i>C. auratus</i>	Saglio et al. (2001)
Benthocarb	Herbicide	1.7 µg/L	Avoidance	<i>C. carpio</i>	Ishida and Kobayashi (1995)
Dalapon	Herbicide	<50 µM	Attraction	<i>O. mykiss</i>	Folmar (1976)
DDT	Insecticide	0.005 mg/L	Avoidance	<i>C. variegatus</i>	Hansen (1969)
		0.1 mg/L	Avoidance	<i>G. affinis</i>	Hansen et al. (1972)
		10 mg/L	Avoidance	<i>G. affinis (near agriculture)</i>	Kynard (1974)
		10 mg/L	NR	<i>G. affinis (~pristine)</i>	
Dursban (chlorpyrifos)	Insecticide	0.01 mg/L	NR	<i>C. variegatus</i>	Hansen (1969)
		0.1 mg/L	Avoidance	<i>C. variegatus</i>	
		0.1 mg/L	Avoidance	<i>G. affinis</i>	Hansen et al. (1972)
		0.1–0.25 mg/L	Avoidance	<i>C. variegatus</i>	Hansen (1969)
		0.5–1 mg/L	NR	<i>C. variegatus</i>	
		10 mg/L	Avoidance	<i>C. variegatus</i>	
Endrin	Insecticide	0.01 mg/L	NR	<i>G. affinis</i>	Hansen et al. (1972)
		0.25 mg/L	Avoidance	<i>G. affinis (~pristine)</i>	Kynard (1974)
		1 mg/L	Avoidance	<i>G. affinis (near agriculture)</i>	
		0.001 mg/L	Avoidance	<i>C. variegatus</i>	Hansen (1969)
Fenitrothion	Insecticide	10 µg/L	Avoidance	<i>C. auratus</i>	Scherer (1975)
		490 µg/L	Avoidance	<i>C. carpio</i>	Ishida and Kobayashi (1995)
		90 µg/L	Avoidance	<i>O. latipes</i>	Hidaka and Tatsukawa (1989)
Isoprothiolane	Fungicide	6.7 µg/L	Avoidance	<i>C. carpio</i>	Ishida and Kobayashi (1995)
Malathion	Insecticide	0.05 mg/L	Avoidance	<i>G. affinis</i>	Hansen et al. (1972)
		1 mg/L	NR	<i>C. variegatus</i>	Hansen (1969)
Nicosulfuron	Herbicide	1–10 mg/L	Attraction	<i>C. auratus</i>	Saglio et al. (2001)
Parathion	Insecticide	0.2 mg/L	Avoidance	<i>G. affinis (~pristine)</i>	Kynard (1974)
		1 mg/L	Avoidance	<i>G. affinis (near agriculture)</i>	

Table 2 (Continued)

Contaminant		Concentration	Response	Species ^a	Reference	
Prochloraz Roundup®	Fungicide	1–10 mg/L	Attraction	<i>C. auratus</i>	Saglio et al. (2001)	
	Herbicide	10 mg/L (A.I.)	Avoidance	<i>O. mykiss</i>	Tierney et al. (2007c)	
Sevin (carbaryl)	Insecticide	10 mg/L	NR	<i>C. variegatus</i>	Hansen (1969)	
		10 mg/L	Avoidance	<i>G. affinis</i>	Hansen et al. (1972)	
Toxaphene	Insecticide	0.25 mg/L	Avoidance	<i>G. affinis</i> (near agriculture)	Kynard (1974)	
		0.25 mg/L	Avoidance	<i>G. affinis</i> (~pristine)		
Surfactants						
Surfactant	POE-ether	500 µg/L	Avoidance	<i>O. latipes</i>	Hidaka and Tatsukawa (1989)	
	SLS	0.01 µg/L	Avoidance	<i>C. carpio</i>	Ishida and Kobayashi (1995)	
	SLS	10 µg/L	Avoidance	<i>O. latipes</i>	Hidaka and Tatsukawa (1989)	
	Sodium lauryl sulfate (SLS)	0.1 mg/L	Attraction	<i>C. clupeaformis</i>	Hara and Thompson (1978)	
Hydrocarbons						
Hydrocarbon (HC)	Benzene	1.9 mg/L	Avoidance	<i>O. kisutch</i> (parr)	Maynard and Weber (1981)	
		0.01–0.02%	Avoidance	<i>P. pungitius</i>	Jones (1947)	
	Coal distillate	1.7 mg/L	Avoidance	<i>P. promelas</i>	Dauble et al. (1985)	
	Ethanol	1%	Avoidance	<i>P. pungitius</i>	Jones (1947)	
	Formalin	0.1–0.4%	Avoidance	<i>P. pungitius</i>		
	Monocyclic aromatic HCs	1.4 mg/L	Avoidance	<i>O. kisutch</i> (smolt)	Maynard and Weber (1981)	
		3.7 mg/L	Avoidance	<i>O. kisutch</i> (parr)		
	O-xylene	0.2 mg/L	Avoidance	<i>O. kisutch</i> (parr)		
	Toluene	0.9 mg/L	Avoidance	<i>O. kisutch</i> (smolt)		
		1.4 mg/L	Avoidance	<i>O. kisutch</i> (parr)		
	Xylene	1 mg/L	Avoidance	<i>O. mykiss</i>	Folmar (1976)	
Other						
Chloramine		≥70 µg/L	Avoidance	<i>R. atratulus</i>	Fava and Chu-Fa (1978)	
Chlorine	Freshwater	≥70 µg/L	Avoidance	<i>R. atratulus</i>		
	Seawater	10–100 µg/L (16, 20 °C)	Preference	<i>C. aggregata</i>	Stober et al. (1980)	
		175 µg/L (13 °C)	Avoidance	<i>C. aggregata</i>		
		2 µg/L	Avoidance	<i>O. kisutch</i>		
Hydrogen sulfide	H ₂ S	2.2 mg/L (15 °C)	Avoidance	<i>M. saxatilis</i>	Hall et al. (1984)	
		2.3 mg/L (20 °C)	Avoidance	<i>M. saxatilis</i>		
		2.9 mg/L (25 °C)	Avoidance	<i>B. tyrannus</i>		
		3.0 mg/L (25 °C)	Avoidance	<i>M. saxatilis</i>		
		3.0 mg/L (30 °C)	Avoidance	<i>B. tyrannus</i>		
		3.2 mg/L (15 °C)	Avoidance	<i>B. tyrannus</i>		
		3.5 mg/L (30 °C)	Avoidance	<i>M. saxatilis</i>		
		3.6 mg/L (20 °C)	Avoidance	<i>B. tyrannus</i>		
		Not given	Avoidance	<i>C. pallasii</i>	Shelford and Powers (1915)	
PCB	Aroclor (a PCB mix)	0.01 mg/L	Avoidance	<i>G. affinis</i>	Hansen et al. (1974)	
		10 mg/L	NR	<i>C. variegatus</i>		
		10 mg/L	Avoidance	<i>L. rhomboides</i>		
Pulp mill effluent	BKME	0.001%	Avoidance	<i>S. salar</i>	Sprague and Drury (1969)	
		0.1%	Avoidance	<i>L. rhomboides</i>	Lewis and Livingston (1977)	
		0.1%	Avoidance	<i>F. grandis</i>		
		0.13–0.25%	Avoidance	<i>C. albula</i>	Myllyvirta and Vuorinen (1989)	
		12–15%	Avoidance	<i>S. salar</i>	Sprague and McLeese (1968)	
	Humic acid	0.1–0.2 mg/L	Avoidance	<i>C. harengus</i>	Wildish et al. (1977)	
		KME	2.50%	Avoidance	<i>O. tshawytscha</i>	Jones et al. (1956)
			10%	NR	<i>O. kisutch</i>	
			10%	NR	<i>S. canadense</i>	Campbell and Bettoli (1992)
			10%	NR	<i>I. punctatus</i>	
		10%	NR	<i>M. chrysops</i>		
	Sodium lignosulfonate	0.1–0.3 mg/L	Avoidance	<i>C. harengus</i>	Wildish et al. (1977)	

^a Fish key: *B. tyrannus* = Atlantic menhaden, *C. aggregata* = Shiner perch, *C. albula* = vendace, *C. auratus* = Goldfish, *C. carpio* = Carp, *C. clupeaformis* = Lake whitefish, *C. harengus* = Atlantic herring, *C. pallasii* = Pacific herring, *C. variegatus* = Sheepshead, minnow, *F. grandis* = Gulf killifish, *G. affinis* = Mosquitofish, *I. punctatus* = Channel catfish, *L. rhomboides* = Pinfish, *M. chrysops* = striped bass, *M. saxatilis* = striped bass, *N. crysoleucas* = Golden shiner, *O. kisutch* = Coho salmon, *O. latipes* = Medaka, *O. mykiss* = Rainbow trout, *O. tshawytscha* = Chinook salmon, *P. promelas* = fathead minnow, *S. alpinus* = Arctic charr, *S. canadense* = sauger, *S. salar* = Atlantic salmon.

response to pesticides (e.g. Saglio et al., 2001; Table 2). Overall, any attraction or repulsion is likely dependent on how that contaminant is perceived, if it can be perceived at all. If fish cannot avoid exposure or choose to be exposed, contaminants can cause the reduced, altered or eliminated perception of odorants, which can lead to changes in behaviors. Fewer studies have tested behavioral modification following exposure than avoidance of exposure, yet many of the contaminants identified as toxic using EOG/EEG response have also been associated with impaired behavioral responses.

Olfactory-mediated behaviors may be innate or acquired; since both sources rely on olfactory input, either type is amenable to olfactory toxicity testing. Contaminant exposures have been shown to cause reduced food odor attraction and predator scent avoidance, as well as altered alarm response. Changes in attraction to food odors following contaminant exposure has been studied enough to warrant its own review (Kasumyan, 2001). Alarm response has received appreciable toxicological application since it can include many behaviors, such as dashing, freezing and hiding (Berejikian

et al., 1999; Brown and Smith, 1997; Døving et al., 2005; Mirza and Chivers, 2002; Pollock et al., 2003). In general, olfactory-evoked behavioral endpoints bring improved ecological relevance; however, they are not without drawbacks. In some cases, it can be difficult to separate olfactory toxicity from other forms of toxicity.

Behavioral responses potentially integrate many inputs, including other sensory modalities over varying time periods. For example, nervous input regarding environmental chemicals can come from gustatory and solitary chemosensory cells. With potentially wide sensory signal integration, non-olfactory based input may figure into behavioral responses, which may introduce uncertainty when attributing olfactory impairment to altered behavioral responses. For example, should a fish no longer respond to a food cue, it may appear that olfactory impairment is the cause. However, in many cases, food cues are visual. The lack of response to a visual cue is likely due to the inability or unwillingness to respond to the cue, perhaps through systemic neurotoxicity. As another example, consider that through the uptake, distribution, and metabolism of a contaminant, an organism can experience toxic effects in addition to impaired peripheral OSNs. Tierney et al. (2007c) found that juvenile rainbow trout exposed to (1 µg/L) atrazine experienced a decrease in L-histidine preference response and an increase in swimming activity. Such alteration of swimming activity has also been observed in goldfish following (>5 µg/L) carbofuran exposure (Bretaud et al., 2002). Alterations in swimming behavior can clearly have both olfactory and non-olfactory-bases. The following discussion focuses on those contaminants that affect behavior chiefly through olfactory modification, and first provides available information on the potential for fish to avoid exposure before reporting any known exposure effects.

3.4.1. pH

Preference/avoidance response to pH change remains largely untested. Avoidance of acid conditions has been noted for at least one species, as Jones et al. (1985b) found arctic char avoided water flows of pH ≤5.5.

Decreased pH appears capable of altering both preference and avoidance responses. With a 30-min exposure to pH 5.1 (a pH decrease of 2.5 units), Atlantic salmon lost a preference response to L-glycine, and an avoidance response to L-alanine switched to a preference response (Royce-Malmgren and Watson, 1987). With a 14-d exposure to pH 4.5–4.75, Arctic char exhibited decreased attraction to a food odorant (Jones et al., 1985a). Given lowered pH can alter OSN responses in a concentration-dependent manner (Moore, 1994), these data affirm that the perceived concentration of a behaviorally-relevant odorant may determine its preference or avoidance.

3.4.2. Metals

Fish avoid many metals. Specifically, arsenic, cadmium, chromium, cobalt, copper, iron, mercury, nickel, selenium, and zinc are avoided to varying degrees (Table 2). Avoidance thresholds for some of these exist in the µg/L range (e.g. copper and nickel), while others are in the mg/L range (e.g. iron and mercury).

Copper was avoided by chinook salmon (*O. tshawytscha*) and rainbow trout (*O. mykiss*), with chinook exhibiting higher sensitivity (0.7 µg Cu/L vs. 1.6 µg Cu/L) (Hansen et al., 1999a). The avoidance of copper can also be concentration specific, as fish avoided low but not high concentrations (Giattina et al., 1982; Hansen et al., 1999a). Since copper can impair the olfactory epithelium within minutes (Baldwin et al., 2003), conceivably a copper plume could impair neurological detection rapidly enough to prevent an olfactory-mediated behavioral response.

Nickel, as with copper, evokes avoidance/attraction responses that can depend on concentration. In one case, rainbow trout were attracted to low (6 µg/L) but avoided higher (24 µg/L) concentra-

tions (Giattina et al., 1982). With zinc, avoidance responses were noted for rainbow trout at concentrations greater than 5.6 µg/L (Sprague, 1968). Zinc, along with copper, cadmium and lead, were constituents of mixture designed to resemble a river (Clark Fork River, MO, USA) (Hansen et al., 1999c). Here rainbow trout avoided a concentration similar to only 10% (6.6 µg/L) strength river water (Table 2). Rainbow trout exhibited a lower avoidance threshold than brown trout (Hansen et al., 1999c). The major constituents of the mixture were copper and zinc, both of which can evoke avoidance at concentrations similar to or lower than those observed with 10% of the mixture. Fathead minnow (*Pimephales promelas*) also avoided a mixture designed to resemble a river (New River, Virginia, USA), and here the avoidance was found to depend on the season (Hartwell et al., 1987b) (Table 2).

Cobalt is avoided at higher concentrations than either copper or zinc (e.g. 24 µg/L for chinook) (Hansen et al., 1999a) (Table 2). Like copper, species-specific sensitivities exist: for rainbow trout, the threshold was 7.5× greater. Higher (mg/L) concentrations of iron were aversive to coho salmon (Updegraff and Sykora, 1976) (Table 2). Similarly, avoidance of mercuric chloride occurred at a high (272 mg/L) concentration in ninespine stickleback (*Pungitius pungitius*) (Jones, 1947).

One study demonstrated how aversive responses can affect wild fish populations. A mixture of copper and zinc ≥35–43% of LC₅₀ (proportions not given) caused an increase in the number of Atlantic salmon that returned downstream rather than continue upstream during return migration (Saunders and Sprague, 1967). At 80% of LC₅₀, upstream movement was eliminated. The authors noted these 'avoidance' thresholds were higher than other lab-based studies, but pointed out that the lifestage likely provided a motivational force that may have effectively increased the avoidance response threshold.

Available studies show that metal exposure can alter preference/avoidance (Table 3). As would be expected, copper exposure has the capacity to inhibit the avoidance of other substances. For instance, ninespine stickleback exposed to 635 mg/L copper for at least 5 min ceased to avoid chloroform and formalin (Jones, 1947). At lower metal concentrations, adaptation may be possible and this may permit retention of sensory discriminatory abilities. After a lengthy (45-d) exposure to a metal mixture, rainbow trout chose clean water over the metal mixture (Hansen et al., 1999c). Similarly, chronic (3-mo) exposure of coho to iron (1.20 mg Fe/L, to ~4 cm fry) did not alter the subsequent avoidance response to greater (4.25–6.45 mg/L) amounts of iron (Updegraff and Sykora, 1976). In contrast, a study of fathead minnow exposed to a simulated metal-impacted stream found that the preference/avoidance to greater metal mixture concentrations was dependent on the length of exposure, with fish preferring 3× the exposure concentration after 3 mos, avoiding 5× the amount at 6 mos, and losing all response to 10× the amount at 9 mos (Hartwell et al., 1987a).

With alarm response, cadmium and copper exposure have been shown to have impact (Sandahl et al., 2007; Scott et al., 2003). Specifically, exposure to either metal diminished the slowing in speed that alarm cue stereotypically evokes in the salmonid species tested.

3.4.3. Pesticides

With metals, certain environmental concentrations are likely benign or even beneficial, as they serve a variety of roles such as helping to maintain ionic balances across exposed membranes. With pesticides, it is more challenging to conceive of any health benefits from their exposure, and so avoidance should be expected. Indeed, various organophosphates and carbamates do evoke avoidance responses (Table 2). For example, fenitrothion was avoided by goldfish (Scherer, 1975) and medaka (Hidaka and Tatsukawa, 1989) at 10 and 90 µg/L, respectively. Not all OPs and carbamates

Table 3

The alteration of fish behaviors following exposures to various contaminants for varying lengths.

Contaminant	Species ^a	Exposure				Behavior	Reference
		Concentration	Duration	Effect (%)	Odorant		
pH							
pH	<i>S. alpinus</i>	4.5, 4.75	14 d	–	Food extract	Decreased attraction	Jones et al. (1985a)
Metals							
Copper	<i>P. pungitius</i>	0.01 M (635 mg/L)	5–15 min	100%	Chloroform Formalin	Decreased avoidance Decreased avoidance	Jones (1947)
	<i>O. kisutch</i>	2 µg/L 5 µg/L 10 µg/L 20 µg/L	3 h	–50% –80% –80% 20%	Skin extract 10 µg protein	Alarm response	Sandahl et al. (2007)
Iron	<i>O. kisutch</i>	1.2 mg Fe/L	Birth to 4 cm	–	4.25–6.45 mg Fe/L	Avoidance	Updegraff and Sykora (1976)
Metal mix	<i>O. mykiss</i>	12 Cu:1.1 Cd:3.2 Pb:50 Zn 66.3 µg/L	45 d	–	≥4x	Avoidance	Hansen et al. (1999c) Hartwell et al. (1987a)
	<i>P. promelas</i>	1 Cu:0.54 Cr:1.85 Ar:0.38 Se 98 µg/L	3 mo 6 mo 9 mo	– – –	294 µg/L 490 µg/L 980 µg/L	Preference response Avoidance response NR ^b	Hartwell et al. (1987b)
		1 Cu:0.54 Cr:1.85 Ar:0.38 Se 98 µg/L in an artificial stream 98 µg/L in a natural stream	3 mo	– –	1470 µg/L 2940 µg/L	Loss of avoidance Loss of avoidance	
Pesticides							
Atrazine	<i>C. auratus</i>	5 µg/L	24 h	–80% –60%	Skin extract	Decreased sheltering Decreased grouping	Saglio and Trijasse (1998)
Cabofuran	<i>C. auratus</i>	1 µg/L 10 µg/L 100 µg/L 1 µg/L 10 µg/L 100 µg/L 1 µg/L 10 µg/L 100 µg/L	4 h 8 h 12 h	–38% –64% –84% –27% –30% –64% –5% –16% –46%	Food extract Food extract Food extract	Attraction Attraction Attraction	Saglio et al. (1996)
Diazinon	<i>O. tshawytscha</i>	0.1 µg/L 1 µg/L 10 µg/L 0.1 µg/L 1 µg/L 10 µg/L	24 h 2 h	–25% –25% –62% –15% –33% –19%	NA Skin extract	Return migration Alarm response (activity)	Scholz et al. (2000)
Diuron	<i>C. auratus</i>	5 µg/L	24 h	–40%	Skin extract	Decreased grouping	Saglio and Trijasse (1998)
IPBC	<i>O. kisutch</i>	10 µg/L 100 µg/L	30 min	–100% –135%	Skin extract	Alarm response	Tierney et al. (2006b)
Parathion	<i>C. auratus</i>	330 µg/L	24 h	–	Food extract	Attraction	Rand et al. (1975)
Other							
BKME	<i>C. albula</i>	0.13% 2.25% 4.5%	1 wk	– – –	0.13% BKME 0.75–4.5% 0.75–4.5%	Preference Avoidance Avoidance	Myllyvirta and Vuorinen (1989)
Chlorine	<i>S. alpinus</i>	>19 µg/L	6 d	–	Food extract	Decreased attraction	Jones and Hara (1988)

^a Fish key: *C. albula* = Vendace, *C. auratus* = Goldfish, *O. kisutch* = Coho salmon, *O. mykiss* = Rainbow trout, *O. tshawytscha* = Chinook salmon, *P. promelas* = Fathead minnow, *P. pungitius* = Ninespine stickleback, *S. alpinus* = Arctic charr.

^b NR = no response.

evoke avoidance, at least for all species, as sheepshead minnow (*Cyprinodon variegatus*) did not avoid malathion or carbaryl formulations (Hansen, 1969). Mosquito fish (*Gambusia affinis*) avoided a similar suite except for a low (0.01 mg/L) concentration of endrin (Hansen et al., 1972). In another study, a higher concentration of endrin was avoided, as were the pesticides DDT, toxaphene and parathion (Kynard, 1974). In a population of mosquitofish that had been captured near an agricultural area, the avoidance of parathion was reduced from 0.2 to 1 mg/L (Kynard, 1974), suggesting either neuroprotection (adaptation) or persisting damage.

Some pesticides evoke neither avoidance nor attraction. Glyphosate, the active ingredient of Roundup®, was not avoided by rainbow trout even at a concentration of 10 mg/L (Folmar, 1976).

However, another paper found Roundup® was avoided by rainbow trout at the same active ingredient concentration (10 mg/L) (Tierney et al., 2007c). 'Inert' ingredients in Roundup® are known to have included surfactants such as POEA (polyethoxylated tallow amine). Given that surfactants are among the most avoided and toxic chemicals to OSNs (see below), the avoidance response to the formulation is unsurprising.

Perhaps more surprising still than the absence of avoidance, is that some pesticides evoke attraction. Saglio et al. (2001) noted goldfish were attracted to prochloraz and nicosulfuron at concentrations of 1 and 10 mg/L, and bentazone concentrations of 0.01 and 10 mg/L (Table 2). The implication of this finding to an environmental setting is that not only may fish fail to leave an impacted site

when given the choice, but they may choose to occupy areas of pesticide pollution. The ramification of this counterintuitive response to survival is axiomatic.

An ecologically relevant example of an impaired behavioral response following pesticide exposure was for the fidelity of return migrating chinook following diazinon exposure (Scholz et al., 2000). Migration, which can be considered a preference response occurring over distance, was reduced by 20% following a 24-h exposure to 0.1 µg/L diazinon. Greater exposure reduced fidelity further. This work demonstrates that exposure may alter the subsequent perception and behavioral response to an attractant over an extended duration and distance. Typically changes in preference (attraction) responses are measured over shorter distances (Table 3). For goldfish, a 4-h exposure to 1 µg/L carbofuran reduced food odor attraction by 38% (Saglio et al., 1996). Curiously, longer (12-h) exposure caused less impairment. Perhaps with longer exposure, the olfactory tissue had time to adjust and compensate. With brief (30-min) exposures, three other currently-used pesticides altered rainbow trout preference behavior towards an amino acid, L-histidine (Tierney et al., 2007c). Specifically, preference behavior was eliminated by 1 µg/L IPBC and 1 µg/L atrazine, and 100 µg/L AI Roundup®. In the future, longer term exposures may be used to determine whether adaptation is possible to these and other pesticides.

Exposure to an avoided chemical can alter the avoidance of another. For example, the avoidance threshold carp (*C. carpio*) exhibit to the three pesticides was modified by the addition of the SLS (Ishida and Kobayashi, 1995). On their own, avoidance thresholds for fenitrothion and SLS were 490 and 0.01 µg/L, respectively. With 1% SLS in the fenitrothion solution, the fenitrothion avoidance threshold was decreased to 1 µg/L.

Thus far, diuron, atrazine (Saglio and Trijasse, 1998), diazinon (Scholz et al., 2000) and IPBC (Tierney et al., 2006b) have been found to alter alarm behavior (Table 3). Typically, any altered behavior is reported as changes in the freezing portion of the response. With chinook salmon, the freezing was incrementally reduced with exposures in excess of 1 µg/L of diazinon (Scholz et al., 2000). Similar findings were noted for the potent olfactory toxicant IPBC and another salmonid (coho) (Tierney et al., 2006b). An exception is goldfish, where following 24-h exposure to 5 µg/L of either diuron or atrazine, the grouping behavior goldfish perform in response to skin homogenate was decreased (Saglio and Trijasse, 1998). The difference in behavioral response likely reflects variation in alarm response between species. A diminished alarm response suggests fish may not negotiate a predator attack, and may therefore suffer higher mortality.

3.4.4. Other contaminants

Hydrocarbons and some of their constituents can evoke avoidance responses (Blaxter and Hallers-Tjabbes, 1992) (Table 2). For example, coho salmon avoided 3.2 mg/L of PAHs (Weber et al., 1981). The avoidance threshold of hydrocarbons for coho appeared dependent on lifestage. Specifically, coho parr avoided concentrations of 3–4 mg/L of monocyclic hydrocarbons while smolts avoided ≤2 mg/L (Maynard and Weber, 1981). Curiously, three mixture constituents (benzene, toluene and O-xylene) had lower thresholds (Table 2), especially O-xylene (0.2 mg/L). A similar concentration (1.7 mg/L) of coal distillates (total phenols) evoked avoidance in fathead minnow (Dauble et al., 1985). Carbon dioxide also exerts concentration-specific avoidance/attraction in Arctic char, with the avoidance threshold at >50 µM (Jones et al., 1985b). Ninespine stickleback was found to avoid ethanol, chloroform and formalin, but all at fairly high concentrations (Table 2) (Jones, 1947).

Oil spills are a common occurrence, at least in the marine environment (e.g., 54,000 gallons of bunker fuel oil were spilled into San Francisco Bay, January 15, 2008, in the Cosco Busan spill). Exist-

ing behavioral modification data for hydrocarbons are scarce, and with negative findings. Specifically, chinook salmon exposed for 1 h to Prudhoe Bay crude oil under concentrations higher than observed in actual spills returned to the hatchery at the same frequency and time as controls (Brannon et al., 1986). Nevertheless, given the ongoing transport and use of petroleum hydrocarbons in and around aquatic environments, future studies, especially those exploring longer term effects are warranted.

PCBs appear to evoke avoidance, although a considerable species-specific difference exists in the available data. In the avoidance of Aroclor (a PCB mixture), pinfish (*Lagodon rhomboids*) avoided 10 mg/L, mosquitofish avoided 0.01 mg/L, and sheepshead minnows did not respond at all (up to 10 mg/L) (Hansen et al., 1974) (Table 2). Potential issues regarding solubility aside, this interspecies response variation (>1000×) is great, but not as large as observed for the surfactant SLS. With SLS, Ishida and Kobayashi (1995) noted an avoidance response at 0.01 µg/L for carp (*C. carpio*) whereas Hara and Thompson (1978) noted an attraction response at 0.1 mg/L for lake whitefish. This large variation among species highlights the difficulty in predicting avoidance responses across fishes.

The earliest avoidance/preference study to contaminants that the authors are aware of explored the avoidance of hydrogen sulfide by herring (*Clupea pallasii*) (Shelford and Powers, 1915). Unfortunately, methods limited the resolution of concentration. Nevertheless, hydrogen sulfide did appear to evoke an avoidance response. In a more recent study (Hall et al., 1984), striped bass (*Morone saxatilis*) and Atlantic menhaden (*Brevoortia tyrannus*) avoided low mg/L hydrogen sulfite concentrations, and the avoidance threshold appeared to decrease with increasing temperature.

Chlorine on its own, or in other compounds or mixtures, can evoke avoidance responses (Table 2). Both chlorine and chloramine (at concentrations ≥70 µg/L) were avoided by dace (*Rhinichthys atratulus*) (Fava and Chu-Fa, 1978). Similarly, coho and shiner perch (*Cymatogaster aggregate*) avoided chlorine, albeit at a higher concentration (Stober et al., 1980). Intriguingly, with low concentration and elevated temperature, shiner perch exhibited an attraction response (Stober et al., 1980). Both bleached (i.e. with chlorine) an unbleached kraft pulpmill effluent (BKME and KME, respectively) can be aversive at low concentrations (Table 2). Atlantic salmon avoided 0.001% (Sprague and Drury, 1969), while pinfish and gulf killifish (*Fundulus grandis*) avoided 0.1% BKME (Lewis and Livingston, 1977). Two components within pulp mill effluent were avoided by herring (*C. harengus*), albeit in the mg/L rang (Wildish et al., 1977) (Table 2). Given that chlorine is also a BKME constituent, the avoidance responses may be partially due to its presence.

3.5. Integrating neurophysiological, physiological, and behavioral data

Few olfactory toxicological studies have endeavored to relate effects across organizational levels. Nevertheless, those that have can be divided into those that relate changes in electrochemical responses (as measured by EOGs/EEGs) to physiological responses or to behavioral responses, and those that relate olfactory-mediated physiologic responses to behavioral responses. Beyond helping to determine mechanistic relationships between lower order (e.g. biochemical) and higher order (e.g. behavioral) responses, determining relationships across organizational levels may help elucidate differential sensitivities (e.g. is OSN or behavioral response a better indicator of toxicity?) or thresholds (e.g. at what point of OSN impairment does contaminant avoidance fail?); both of which may be used to gauge the usefulness of toxicity data to predicting organismal performance.

Studies continue to suggest cholinesterase impairment as a potential mechanism of olfactory toxicity (e.g. Jarrard et al., 2004;

Table 4 (Continued)

(C) OSN to behavioral impairment relationships				
		Exposure ($\mu\text{g/L}$)	EOG (% pre)	Behavior (% pre)
Sandahl et al. (2007)				
Metal	CuCl ₂	2	45%	50%
Species	<i>O. kisutch</i>	5	35%	20%
EOG	skin extract	10	20%	20%
[stim]	10 μg protein/L	20	15%	–20%
OSN exp.	3 h			
Fish exp.	3 h			
Tierney et al. (2007c)				
Pesticide	Atrazine			
Species	<i>O. mykiss</i>	1	89%	89%
EOG	L-histidine	10	40%	40%
[stim]	10 ^{–7} M	100	14%	14%
OSN exp.	30 min			
Fish exp.	30 min			
Pesticide	IPBC			
Species	<i>O. mykiss</i>	1	36%	29%
EOG	L-histidine	10	30%	0%
[stim]	10 ^{–7} M	100	10%	0%
OSN exp.	30 min			
Fish exp.	30 min			
Pesticide	Roundup®			
Species	<i>O. mykiss</i>	10	67%	100%
EOG	L-histidine	100	32%	3%
[stim]	10 ^{–7} M	1000	19%	2%
OSN exp.	30 min			
Fish exp.	30 min			
(D) Physiological to behavioral impairment relationships				
Alarm response tests			Plasma cortisol	Δ Line crossings (% of control)
Scott et al. (2003)				
Metal	Cadmium			
Species	<i>O. mykiss</i>	0		
OSN exp.	7 d	2	61%	–50%
Fish exp.	15 min	3	30%	100%
Notes: The response after 2 $\mu\text{g/L}$ is negative since fish became active and did not freeze				
Notes: After 3 $\mu\text{g/L}$, the response was the same as control				
Tierney et al. (2006b)				
Pesticide	IPBC	0		Δ freezing
Species	<i>O. kisutch</i>	1	85%	0%
OSN exp.	30 min	10	77%	Reduced 100%
Fish exp.	30 min	100	38%	Reduced 135%

^a Fish key: *O. kisutch* = Coho salmon, *O. mykiss* = Rainbow trout, *S. salar* = Atlantic salmon.

^b 'OSN exp.' is the exposure period for the olfactory rosette tissue.

^c 'Fish exp.' is the exposure period for the whole animal.

Tierney et al., 2007b). The relationship was explored by Jarrard et al. (2004) by recording EOGs and measuring AChE activity in coho rosette tissue following carbofuran exposure (Table 4A). The data suggest such a relationship exists since EOG decreases agreed closely with AChE impairment (i.e. 30-min exposure to 10 $\mu\text{g/L}$ caused 50% decreases in both; 200 $\mu\text{g/L}$ caused ~20%). Sandahl et al. (2005) measured reductions in EOGs, EEGs, and AChE activity in juvenile coho exposures to chlorpyrifos (e.g. ~50% reduction in EOGs and EEGs and 25% reduction in AChE following 7-d exposure to 2.5 $\mu\text{g/L}$). However, debate remains since the presence of AChE in the olfactory epithelium has not been conclusively demonstrated.

Several endocrine responses associated with mating are downstream of and initiated by olfactory neuron responses. Measurements of both OSN and endocrine responses facilitate understanding the ramifications of olfactory impairment to critical behaviors. Furthermore, armed with knowledge of how pesticides alter both responses, the effect pesticide exposure may have on reproductive parameters can be estimated in the future through the use of measurements of OSN function.

With Atlantic salmon, the effects of OSN impairment on priming responses (i.e. milt and hormonal production) of males by female urine have been tested in a series of five papers (Moore and Waring,

1996b, 1998, 2001; Waring and Moore, 1997; Moore and Lower, 2001; Table 4B). In interpreting OSN-physiological relationships in these studies, a consideration is that exposure periods usually differed for each endpoint. Even so, in most cases pesticide exposure was associated with reduced EOG responses and lower levels of plasma testosterone, 11-ketotestosterone (the androgenic hormone of teleosts), 17, 20 β P (a hormone that increases secretion of gonadotropin II (GtH II), Zheng et al., 1997), and expressible milt.

Overall, olfactory-mediated hormonal responses appear to be more sensitive to pesticide exposure than OSN response. In most cases, there is a greater than five-fold difference in sensitivity between EOG reduction and altered testosterone response (Fig. 4). This indicates that there may be a threshold between OSN responses and downstream hormonal responses. For example, following diazinon exposure, the maximum milt reduction in Atlantic salmon occurred at an exposure concentration of 0.8 $\mu\text{g/L}$ (Moore and Waring, 1996b) (Table 4B). In contrast, EOG responses declined in a concentration dependent manner from 1 to 20 $\mu\text{g/L}$. This suggests that small impairments in OSN response may translate to larger declines in milt production.

Although the hormonal system appears more sensitive, the toxic effects may be mediated through alterations not typically associ-

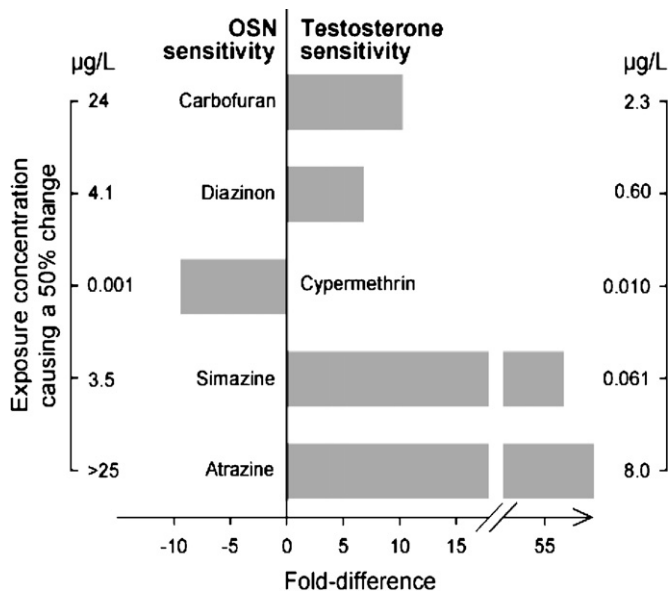


Fig. 4. The relative difference in sensitivity between an olfactory sensory neuron (OSN) and hormonal responses following exposure to various pesticides and priming pheromone. The relative difference was calculated between the pesticide concentration required to cause a 50% change in OSN response (EC_{50} , as measured using electro-olfactogram) and testosterone concentration. The 50% values shown on the y-axis were interpolated from regression models fitted to data in Table 4.

ated with olfaction *per se* (i.e. the effects may occur systemically). For example, with pyrethroid exposure, their mechanism of action would suggest their route of olfactory toxicity would occur through disruption of signal conduction and processing (Fig. 2b and c). Ecologically it is not important where the disconnect in the olfactory signal occurs, the result is that the signal has not evoked the intended or typical response. Across pesticide class, effects appear similar (Fig. 4), and this likely reflects the common mechanism of action of the classes.

Like carbofuran and diazinon, the triazines atrazine and simazine often caused hormonal decreases (Table 4B). For example, in Atlantic salmon exposed to $\geq 6 \mu\text{g/L}$ atrazine for 5 d, the milt and three hormones (testosterone, 11-KT and 17, 20 β P) were not increased to the same extent as unexposed fish (Moore and Waring, 1998). In fact, milt was not increased to the same extent after just $0.04 \mu\text{g/L}$ atrazine exposure. Here, OSN responses were significantly decreased after $2 \mu\text{g/L}$. Again the data suggest that endocrine processes downstream of OSN responses are highly sensitive to OSN impairment.

An important difference occurred with some cases of triazine exposure. For instance, in male Atlantic salmon, plasma testosterone was increased (244% of control) following exposure to $0.5 \mu\text{g/L}$ of simazine (Moore and Lower, 2001) (Table 4B). In contrast, four times this concentration ($2 \mu\text{g/L}$) decreased EOG. This suggests simazine may be causing a non-olfactory-based effect. Supporting this, triazines are known to have anti-androgen properties by inducing aromatase activity, which converts testosterone to estradiol (Hayes et al., 2006). The observed androgen increase indicates testosterone secretion may have been elevated to offset increased plasma estradiol. This could be further investigated by measurements of both hormones.

There are at least two ways in which OSN responses and behavioral response relationships have been established: through correlations between contaminant-evoked EOGs and avoidance/preference responses, and between contaminant-impaired EOG responses (i.e. evoked by another odor) and behavioral responses. Of the few studies that enable comparison of contaminant avoidance and EOG/EEG response, the data indicate that OSN

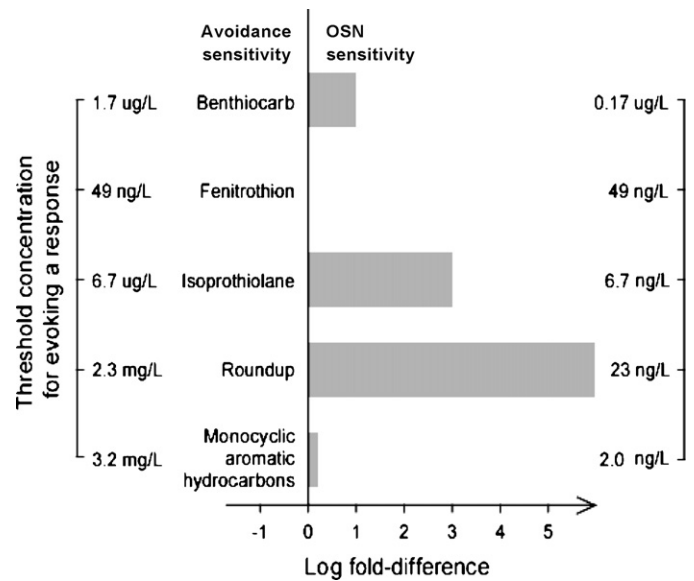


Fig. 5. The relative difference in sensitivity between olfactory sensory neuron (OSN) detection of pesticides and avoidance of pesticides. The y-axis values indicate the threshold concentrations at which OSN and avoidance responses were no longer detected. Benthiocarb, fenitrothion and isoprothiolane values are from Ishida and Kobayashi (1995) and Ishida et al. (1996); Roundup® values are from Tierney et al. (2007c); hydrocarbon values are from Maynard and Weber (1981).

detection typically occurs in advance of avoidance (Fig. 5). For example, the OSN response thresholds for carp to benthiocarb and isoprothiolane were 10 and 1000-fold greater, respectively, than the avoidance thresholds (Ishida and Kobayashi, 1995; Ishida et al., 1996) (Fig. 5). A similar finding was noted with the pesticide formulation Roundup®. Its presence and detection was not sufficient for avoidance (Tierney et al., 2007c). This suggests that responses likely need to be perceived as unpleasant to evoke a behavioral avoidance response. Not all pesticides or their formulations are going to be perceived as such, as Saglio et al. (2001) found pesticide preference responses. These findings together indicate that fish may not avoid pesticide exposure, and subsequent injury, even when given the choice.

For fish exposed to contaminants (i.e. no opportunity for avoidance), behavioral responses may be lost before neurological responses. This was evident in rainbow trout exposed to three pesticides (atrazine, IPBC and Roundup®), as concentration-specific decreases were observed in both preference and OSN responses, yet the behavioral decreases were absent with OSN impairments of 60% or greater (Tierney et al., 2007c) (Table 4C). With copper exposed coho salmon, EOG and alarm response decreases were proportionally similar up to a $10 \mu\text{g/L}$ (Sandahl et al., 2007) (Table 4C). However, at a greater concentration ($20 \mu\text{g/L}$), the fish became somewhat lethargic and the alarm response was completely lost. The implication of these findings to gauging contaminant-mediated toxicity from OSN data alone is that OSN toxicity may be too conservative or inaccurate. Small OSN impairments may cause large and unforeseeable effects on behaviors.

There are many examples above of how stereotypical physiological and behavioral responses do not ensue following contaminant-altered odorant detection. The relationships discussed to this point have focused on neurological responses (EOGs/EEGs), yet other examples of olfactory-based physiological impairments exist. As in the above section, determination of how physiological impairments relate to behavioral alterations has more than basic research value. The relative sensitivity of toxicity endpoints can be determined across biological levels of organiza-

Physical damage to OSNs can impair alarm response behavior. *Beyers and Farmer (2001)* related the OSN ciliary loss that copper causes to changes in the behavioral component of the alarm response in Colorado pikeminnow (*Ptychocheilus lucius*). Unexpectedly, the behavioral alarm responses appeared more affected after 24 h than 96 h for copper exposure concentrations above 66 µg/L. The lack of a concentration-dependent behavioral decrease was theorized by the authors to be from neuroprotective responses, such as mucous generation.

The alarm response is sometimes associated with a stress response, and so certain stress hormones may prove to be good correlates of alarm behavior. *Scott et al. (2003)* monitored changes in rainbow trout alarm behavior and plasma cortisol, a stress hormone that often increases in concentration with alarm response (*Rehnberg and Schreck, 1987*). In unexposed fish, alarm substance caused plasma cortisol to increase 4 fold, whereas in fish exposed to 2 µg/L cadmium, the elevation was only 2 fold. Behaviorally, this alteration corresponded to an impaired alarm response where fish no longer froze. A similar study was conducted with the carbamate fungicide IPBC and the alarm response of coho salmon (*Tierney et al., 2006b*). In this study, the behavioral response was abolished at a lower exposure concentration than the physiological (cortisol) response, which suggests a threshold exists between cortisol secretion and freezing response. Additionally, this study noted IPBC exposure on its own was associated with increased cortisol. For this reason, using some physiological endpoints as correlates of olfactory toxicity is not necessarily prudent.

3.6. Challenges in separating physiologic and olfactory responses

As was discussed previously, separating olfactory-mediated behavioral toxicity from other types of behavioral toxicity (e.g. swimming activity) can be difficult. For instance, decreases in feeding behavior (e.g. with 0.1 ppm fenitrothion exposure, *Bull and McInerney, 1974*) may be due to decreases in the sense of smell and taste, since food can be rejected if unpalatable (e.g. food contaminated with the same pesticide was rejected by *S. salar*, *Symons, 1973*). Additionally, decreased feeding may also be from a decrease in locomotory ability. A parallel example could be drawn with mating responses. Often these responses involve complex displays and other courtship behaviors, many of which are impaired by contaminant exposure (reviewed in *Jones and Reynolds, 1997*). Similarly again, three days following a 7-d exposure to 1 µg/L DDT, goldfish did not regroup into schools as rapidly as controls (*Weis and Weis, 1974*). Such behaviors may or may not include an olfactory component. Nevertheless, from an ecological perspective, such measures of integrated sensory and neurological response may bring increased ability to extrapolate to field conditions and thus improve estimation of environmental impact.

4. Endpoints related to olfactory toxicity

Various measures exist to determine how olfactory tissue can resist or cope with toxicant exposure. These neuroprotective responses include the measurement of inactivation/detoxification (i.e. Phase I and II biotransformation) enzymes within the mucous, globlet (mucous-producing cells), OSN and other cells of the olfactory epithelium. There are several studies of Phase I changes in mammalian tissues. For instance the substituted benzene herbicide dichlobenil induces expression of the cytochrome P450 isozyme 2A5 (CYP2A5) in mouse olfactory tissue (*Piras et al., 2003*). In fish, exploration of CYP induction in fish olfactory cells is rare (*Saucier et al., 1999*). In rainbow trout given a 4-d exposure to the CYP inducer β-naphthoflavone, CYP1A1 was expressed in OSNs, cells in which it had not previously been detected (*Saucier et al., 1999*). Simi-

larly, the polycyclic aromatic hydrocarbon and model carcinogen, benzo[a]pyrene, induced CYP1A1 expression after 48 h in the head-water livebearer (*Poeciliopsis monacha*) (*Smolowitz et al., 1992*).

As with Phase I enzymes, few data exist on the activity of Phase II enzymes in fish olfactory tissue. A recent study noted that a pesticide mixture resembling that found in a salmon-bearing stream caused increases in GST activity in olfactory rosette tissue following 96-h exposure (*Tierney et al., 2008*). As might be expected, the highest GST activity was coincident with normal EOG responses. However, this relationship was observed with the lowest mixture exposure concentration; GST activity did not increase linearly with increasing concentrations, suggesting a limit to its neuroprotective abilities. An earlier study using physical damage found that killing OSNs through severing bulbar innervation caused a rapid decrease in the GST of the olfactory epithelium in rainbow trout (*Starcevic and Zielinski, 1997*). The enzyme activity returned after two months, while full tissue regeneration required three. However, GST activity tells only a portion of the neuroprotective equation: substrate must be present. The substrate glutathione (GSH) had an alternate recovery profile: GSH remained initially high, but dropped to a lower level after 2 wks where it remained to the experiment's conclusion (96 d). Neuroprotective responses may also be measured upstream of enzyme activity through determination of Phase I mRNA (*Gillner et al., 1987*; *Chung-Davidson et al., 2004*), or of expression promoter proteins such as c-fos that are involved with neuroprotection (*Salierno et al., 2006*; *Tierney and Kennedy, 2008*).

Neuronal loss can indicate exposure to a damaging agent, and the regrowth of the olfactory epithelium can be monitored. In mammals suffering complete loss of olfactory tissue, this would necessitate monitoring regeneration using histology or other techniques over at least 4–6 wks (*Schwob, 2005*). In fish, regrowth can be lengthier, as complete regeneration can take up to 7 mos in rainbow trout at 11 °C (*Evans and Hara, 1985*) and ~6.4 mos in goldfish at 18 °C (*Zippel et al., 1997*). During regrowth, the tissue progresses from redevelopment of the apical surfaces of ciliated and microvillar cells to axonal reconnection in the olfactory bulb (*Zippel et al., 1997*).

5. Olfactory toxicity endpoints vs. other toxicity endpoints

Toxicity is related to the level of biological organization measured and whole organism endpoints will likely have higher thresholds than endpoints of specific physiological systems. This review has focused on toxicity to OSNs and the downstream relevance of that toxicity to behavior, since OSNs are exposed and sensitive to contaminants and since the behaviors they support are often critical to survival. However, the significance of measures of olfactory toxicity to organismal survival and beyond remains poorly understood. Furthermore, placing olfactory toxicity endpoints in context of other toxicity endpoints to determine what constitutes a meaningful negative effect is not trivial. In this section, OSN toxicity is considered against other measures of contaminant effects, which helps to address if OSN toxicity is more or less sensitive and meaningful than other measures.

For example, AChE-impairing insecticides inhibit both olfaction (*Sandahl et al., 2004*) and muscle performance (*Tierney et al., 2007a*). For a fish exposed to an anti-AChE agent, it could be difficult to determine whether the ability to escape a predator is more impaired than the ability to smell a predator. Yet if each physiologic system is differentially sensitive, and the representative system may be ranked according to its survival importance, then perhaps risk can be calculated.

With the anti-AChE insecticide chlorpyrifos, three recent studies on coho salmon have been conducted on both olfactory and swim-

ming impairment. OSN responses were reduced by 20% following exposure to 0.72 $\mu\text{g/L}$ (Sandahl et al., 2004) and spontaneous swimming (a correlate of foraging ability) was decreased 27% with 0.6 $\mu\text{g/L}$ (Sandahl et al., 2005). Critical swimming performance (a possible correlate of predator escape ability) was impaired at a higher concentration of $\geq 10 \mu\text{g/L}$ (Tierney et al., 2007a). Lethality values (96-h LC_{50}) are typically higher than OSN or swimming impairment thresholds (e.g. 15 $\mu\text{g/L}$) (Macek et al., 1969). With this example, olfactory ability and spontaneous swimming ability seem approximately equal in sensitivity to chlorpyrifos, and critical swimming ability appears comparatively less so. If an exposure of $\sim 1 \mu\text{g/L}$ were to occur in an ecological setting, perhaps fish would have reduced ability to detect food and forage for it, yet remain capable of escaping predators. In the short-run, such a scenario may not increase the likelihood of death, but in the long-run, may lead to decreased energy reserves and so potentially cause 'ecological death', i.e. not outright mortality, but a much-increased likelihood of death from ecological processes such as predation (Kruzyński and Birtwell, 1994). Ideally, the relevance of any sublethal measure needs to be tied to its potential for enhancing the likelihood of ecological death.

In summary, the impairment of neurological systems is potentially a double-edged sword: not only may contaminant exposure cause incorrect perception of information critical to survival (such as predator scent), but it may also impair the proper response (such as burst swimming). Given the upstream importance of sensory perception, impaired olfaction may in many cases be of more immediate survival concern than other physiological impairments. With the number of contaminants and complexity of some their negative effects, determining the importance of impaired olfaction and other altered physiological conditions can ultimately only be decided on a case-by-case basis and over time, *in situ*.

6. Research directions

In olfactory toxicity work, two aspects representing opposite ends of biological order, remain relatively unexplored: the mechanisms of toxicity of the various contaminants, and the effects of olfactory toxicity on populations. The former can be remedied by molecular studies and more in-depth electrophysiology (e.g. patch clamping), while the latter can be remedied by more behavioral testing, field studies, mesocosm studies, lab studies with field links (such as through the use of pesticide mixtures), and modeling. Ultimately, linking mechanistic effects to altered survivorship will help predict the effects of contaminant exposure on fish populations in the environment. While lab based studies may elucidate toxicity mechanisms, they may lack in simulating the complex behavioral alterations that can occur following exposure to pesticide mixtures. Remedying these will ultimately involve studying olfactory-mediated behavioral (and lower level) effects in a more field setting, and using observed contaminant mixtures.

These research directions are met with several challenges. Determining the mechanism(s) of action for each of the myriad pesticides, inert ingredients and other contaminants is near to impossible. Not only is the number of contaminants huge, but so is the number of fish species ($\sim 30,000$). The variation contaminants evoke in fish responses can be marked, as discussed. Furthermore, many fishes have complex life histories and behaviors that may bring them through various environments, each with their own contaminant concerns. Measuring the impact of single contaminants on behaviors critical to survival is challenge enough—extrapolating to the environment and its contaminant mixtures will be difficult.

Nevertheless, such studies, including those on mixtures, need to be conducted. Two studies have demonstrated that olfac-

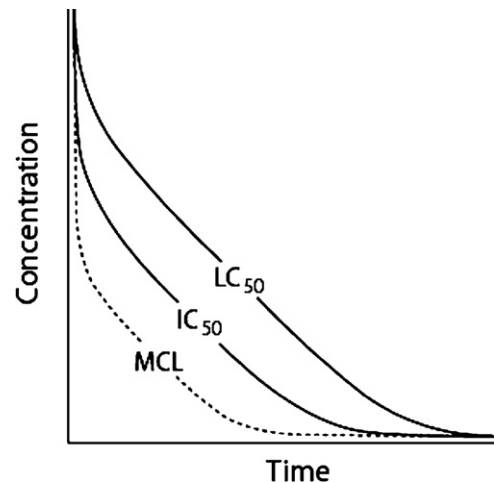


Fig. 6. Various methods exist for measuring toxicity, including median concentration causing lethality (LC_{50}) and median concentration causing (sublethal) inhibition (IC_{50}). Increasing the exposure concentration brings more rapid effect for both endpoints. With olfactory impairment, IC_{50} can be determined from the concentration-dependent decreases pesticides can cause in olfactory sensory neuron response to an odorant. For complete organismal protection (i.e. no observed negative effects), a maximum contaminant level (MCL) would need to be below an olfactory IC_{50} .

tory impairment limited the success of return migrating salmon (Saunders and Sprague, 1967; Scholz et al., 2000). In each case, the contaminant was tied to the environment and to olfactory impairment. With accurate environmental survey data, information on fish movement and olfactory sensitivity, it may be possible to increasingly make mechanistically-based predictions on the impact of contaminants on fish species, even those with complex life histories.

7. Conclusion

Across all levels of olfactory organization discussed above, the recurring theme is that a variety of pesticides, surfactants, metals and hydrocarbons alter the function and structure of olfactory sensory neurons and the higher order behavioral responses they support. Future toxicity studies would benefit by linking the effects of exposure to growth, reproduction or recruitment, which together represent levels of survivorship relevant to population. Relating the importance of a decrease in olfactory neuron response from a short-term pesticide exposure to a population-level impact is a challenge, but not one that is insurmountable.

Hence, olfactory toxicity studies must continue to link toxicity mechanisms to behavioral responses that can be related to changes in survivorship, especially at the ecosystem level. Several studies indicate that thresholds exist between neurological, physiological and behavioral responses. The ramifications for extrapolating neurological and physiological data to behavioral and ecological impacts are straightforward: lower order measures (e.g. EOG) may underestimate the impact of toxicity to higher order biological responses (e.g. mating). Additionally, other toxic effects, such as those independent of olfaction and with possibly unknown mechanisms of action, need to be considered when determining organismal toxicity. Ultimately, regulations will need to be constructed that set contaminant levels lower than where negative effects are observed in olfactory-based responses (Fig. 6). Presently, more than sufficient information exists to indicate that for fishes, olfaction is indispensable and sensitive to contaminants, which makes monitoring its function critical to maintaining fish populations in a changing environment.

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References

- Baatrup, E., Døving, K.B., 1990. Histochemical demonstration of mercury in the olfactory system of salmon (*Salmo salar* L.) following treatments with dietary methylmercuric chloride and dissolved mercuric chloride. *Ecotoxicology and Environmental Safety* 20, 277–289.
- Baatrup, E., Døving, K.B., Winberg, S., 1990. Differential effects of mercurial compounds on the electroolfactogram (EOG) of salmon (*Salmo salar* L.). *Ecotoxicology and Environmental Safety* 20, 269–276.
- Baldwin, D.H., Sandahl, J.F., Labenia, J.S., Scholz, N.L., 2003. Sublethal effects of copper on coho salmon: impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environmental Toxicology and Chemistry* 22, 2266–2274.
- Baldwin, D.H., Scholz, N.L., 2005. The electro-olfactogram: an in vivo measure of peripheral olfactory function and sublethal neurotoxicity in fish. In: Ostrander, G.K. (Ed.), *Techniques in Aquatic Toxicology*. CRC Press, Inc., Boca Raton, FL, pp. 257–276.
- Bardach, J.E., Fujiya, M., Holl, A., 1965. Detergents: effects on the chemical senses of the fish *Ictalurus natalis* (le Sueur). *Science* 148, 1605–1607.
- Belanger, R.M., Corkum, L.D., Li, W., Zielinski, B.S., 2006. Olfactory sensory input increases gill ventilation in male round gobies (*Neogobius melanostomus*) during exposure to steroids. *Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology* 144, 196–202.
- Berejikian, B.A., Smith, R.J.F., Tezak, E.P., Schroder, S.L., Knudsen, C.M., 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 830–838.
- Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases* 22, 25–34.
- Beyers, D.W., Farmer, M.S., 2001. Effects of copper on olfaction of Colorado pikeminnow. *Environmental Toxicology and Chemistry* 20, 907–912.
- Bjerselius, R., Winberg, S., Winberg, Y., Zeipel, K., 1993. Ca²⁺ protects olfactory receptor function against acute Cu(II) toxicity in Atlantic salmon. *Aquatic Toxicology* 25, 125–137.
- Blaxter, J., Hallers-Tjabbes, C., 1992. The effect of pollutants on sensory systems and behaviour of aquatic animals. *Aquatic Ecology* 26, 43–58.
- Brannon, E.L., Quinn, T.P., Whitman, R.P., Nevissi, A.E., Nakatani, R.E., McAuliffe, C.D., 1986. Homing of adult chinook salmon after brief exposure to whole and dispersed crude oil. *Transactions of the American Fisheries Society* 115, 823–827.
- Brethead, S., Saglio, P., Saligaut, C., Auperin, B., 2002. Biochemical and behavioral effects of carbofuran in goldfish (*Carassius auratus*). *Environmental Toxicology and Chemistry* 21, 175–181.
- Brown, G.E., 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries* 4, 227–234.
- Brown, G.E., Smith, R.J.F., 1997. Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Zoology* 75, 1916–1922.
- Bull, C.J., McInerney, J.E., 1974. Behavior of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to sumithion (Fenitrothion), and organophosphate insecticide. *Journal of the Fisheries Research Board of Canada* 31, 1867–1872.
- Campbell, G.J., Bettoli, P.W., 1992. Behavioral reactions of fishes exposed to unbleached kraft mill effluent. *Bulletin of Environmental Contamination and Toxicology* 49, 157–164.
- Canclon, P., 1983. Influence of a detergent on the catfish olfactory mucosa. *Tissue and Cell* 15, 245–258.
- Chung-Davidson, Y.-W., Rees, C.B., Wu, H., Yun, S.-S., Li, W., 2004. (beta)-naphthoflavone induction of CYP1A in brain of juvenile lake trout (*Salvelinus namaycush* Walbaum). *Journal of Experimental Biology* 207, 1533–1542.
- Dauble, D.D., Gray, R.H., Skalski, J.R., Lusty, E.W., Simmons, M.A., 1985. Avoidance of a water-soluble fraction of coal liquid by fathead minnows. *Transactions of the American Fisheries Society* 114, 754–760.
- Døving, K.B., Hamdani, E.H., Høglund, E., Kasumyan, A.O., Tuvikene, A., 2005. A review on the chemical and physiological basis of alarm reactions in cyprinids. In: Reuter, K., Kapoor, B.G. (Eds.), *Fish Chemosenses*. Science Publishers Inc., Enfield, NH, USA and Plymouth, UK, pp. 133–163.
- Dubuc, R., Brocard, F., Antri, M., Fénelon, K., Gariépy, J.-F., Smetana, R., Ménard, A., Le Ray, D., Viana Di Prisco, G., Pearlstein, E., Sirota, M.G., Derjean, D., St-Pierre, M., Zielinski, B., Auclair, F., Veilleux, D., 2008. Initiation of locomotion in lampreys. *Brain Research Reviews* 57, 172–182.
- Dukes, J.P., Deaville, R., Bruford, M.W., Youngson, A.F., Jordan, W.C., 2004. Odorant receptor gene expression changes during the parr–smolt transformation in Atlantic salmon. *Molecular Ecology* 13, 2851–2857.
- Evans, R.E., Hara, T.J., 1985. The characteristics of the electro-olfactogram (EOG): its loss and recovery following olfactory nerve section in rainbow trout (*Salmo gairdneri*). *Brain Research* 330, 65–75.
- Fava, J.A., Chu-Fa, T., 1978. Delayed behavioral responses of the blacknose dace (*Rhinichthys atratulus*) to chloramines and free chlorine. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* 60, 123–128.
- Florea, A.M., Busselberg, D., 2006. Occurrence, use and potential toxic effects of metals and metal compounds. *Biomaterials* 19, 419–427.
- Folmar, L.C., 1976. Overt avoidance reaction of rainbow trout fry to nine herbicides. *Bulletin of Environmental Contamination and Toxicology* 15, 509–514.
- Friedrich, R.W., Korsching, S.L., 1998. Chemotopic, combinatorial, and noncombinatorial odorant representations in the olfactory bulb revealed using a voltage-sensitive axon tracer. *Journal of Neuroscience* 18, 9977–9988.
- Giattina, J.D., Garton, R.R., Stevens, D.G., 1982. Avoidance of copper and nickel by rainbow trout *Salmo gairdneri* as monitored by a computer based data acquisition system. *Transactions of the American Fisheries Society* 111, 491–504.
- Gilbert, P.W., 1977. Two decades of shark research: a review. *BioScience* 27, 670–673.
- Gilliom, R.J., Barbash, J.E., Crawford, C.G., Hamilton, P.A., Martin, J.D., Nakagaki, N., Nowell, L.H., Scott, J.C., Stackelberg, P.E., Thelin, G.P., Wolock, D.M., 2006. The Quality of Our Nation's Waters, Pesticides in the Nation's Streams and Ground Water, 1992–2001. USGS circular 1291, U.S. Geological Survey. 184 pp.
- Gillner, M., Brittebo, E., Brandt, I., Soderkvist, P., Appelgren, L., Gustafsson, J., 1987. Uptake and specific binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the olfactory mucosa of mice and rats. *Cancer Research* 47, 4150–4159.
- Hall, L.W., Burton, D.T., Graves, W.C., Margrey, S.L., 1984. Behavioral modification of estuarine fish exposed to sulfur dioxide. *Journal of Toxicology and Environmental Health* 13, 969–978.
- Hamdani, E.H., Døving, K.B., 2007. The functional organization of the fish olfactory system. *Progress in Neurobiology* 82, 80–86.
- Hansen, D.J., 1969. Avoidance of pesticides by untrained sheepshead minnows. *Transactions of the American Fisheries Society* 98, 426–429.
- Hansen, D.J., Matthews, E., Nall, S.L., Dumas, D.P., 1972. Avoidance of pesticides by untrained mosquitofish, *Gambusia affinis*. *Bulletin of Environmental Contamination and Toxicology* 8, 46–51.
- Hansen, D.J., Schimmel, S.C., Matthews, E., 1974. Avoidance of aroclor® 1254 by shrimp and fishes. *Bulletin of Environmental Contamination and Toxicology* 12, 253–256.
- Hansen, J.A., Marr, J.C.A., Lipton, J., Cacula, D., Bergman, H.L., 1999a. Differences in neurobehavioral responses of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) exposed to copper and cobalt: behavioral avoidance. *Environmental Toxicology and Chemistry* 18, 1972–1978.
- Hansen, J.A., Rose, J.D., Jenkins, R.A., Gerow, K.G., Bergman, H.L., 1999b. Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) exposed to copper: neurophysiological and histological effects on the olfactory system. *Environmental Toxicology and Chemistry* 18, 1979–1991.
- Hansen, J.A., Woodward, D.F., Little, E.E., Delonay, A.J., Bergman, H.L., 1999c. Behavioral avoidance: possible mechanism for explaining abundance and distribution of trout species in a metal-impacted river. *Environmental Toxicology and Chemistry* 18, 313–317.
- Hara, T.J., 1974. Is morpholine an effective olfactory stimulant in fish? *Journal of the Fisheries Research Board of Canada* 31, 1547–1550.
- Hara, T.J., 1975. Olfaction in fish. In: Kerkut, G.A., Phillis, J.W. (Eds.), *Progress in Neurobiology*. Pergamon Press, Oxford, pp. 271–335.
- Hara, T.J., 1992. Mechanisms of olfaction. In: Hara, T.J. (Ed.), *Fish Chemoreception*. Chapman & Hall, London, pp. 150–170.
- Hara, T.J., 1994. The diversity of chemical stimulation in fish olfaction and gustation. *Reviews in Fish Biology and Fisheries* 4, 1–35.
- Hara, T.J., 2006a. Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. *Journal of Fish Biology* 68, 810–825.
- Hara, T.J., 2006b. Olfactory responses to amino acids in rainbow trout: revisited. In: Reutter, K., Kapoor, B.G. (Eds.), *Fish Chemosenses*. Science Publishers, Enfield, pp. 31–64.
- Hara, T.J., Law, Y.M.C., Macdonald, S., 1976. Effects of mercury and copper on the olfactory response in rainbow trout *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* 33, 1568–1573.
- Hara, T.J., Thompson, B.E., 1978. The reaction of whitefish, *Coregonus clupeaformis*, to the anionic detergent sodium lauryl sulphate and its effects on their olfactory responses. *Water Research* 12, 893–897.
- Harden, M.V., Newton, L.A., Lloyd, R.C., Whitlock, K.E., 2006. Olfactory imprinting is correlated with changes in gene expression in the olfactory epithelia of the zebrafish. *Journal of Neurobiology* 66, 1452–1466.
- Harris, K.A., Dangerfield, N., Woudneh, M., Brown, T.G., Verrin, S., Ross, P.S., 2008. Partitioning of current-use and legacy pesticides in salmon habitat in British Columbia, Canada. *Environmental Toxicology and Chemistry* 27, 2253–2262.
- Hartwell, S.I., Cherry, D.S., Cairns, J., 1987a. Avoidance responses of schooling fathead minnows (*Pimephales promelas*) to a blend of metals during a 9-month exposure. *Environmental Toxicology and Chemistry* 6, 177–187.
- Hartwell, S.I., Cherry, D.S., Cairns, J., 1987b. Field validation of avoidance of elevated metals by fathead minnows (*Pimephales promelas*) following in situ acclimation. *Environmental Toxicology and Chemistry* 6, 189–200.
- Hartwell, S.I., Jin, J.H., Cherry, D.S., Cairns, J., 1989. Toxicity versus avoidance response of golden shiner, *Notemigonus crysoleucas*, to five metals. *Journal of Fish Biology* 35, 447–456.
- Hayes, T.B., Stuart, A.A., Mendoza, M., Collins, A., Noriega, N., Vonk, A., Johnston, G., Liu, R., Kpodzo, D., 2006. Characterization of atrazine-induced gonadal malformations in African clawed frogs (*Xenopus laevis*) and comparisons with

- effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17 β -estradiol): support for the demasculinization/feminization hypothesis. *Environmental Health Perspectives* 114, 134–141.
- Hidaka, H., Tatsukawa, R., 1989. Avoidance by olfaction in a fish, medaka (*Oryzias latipes*), to aquatic contaminants. *Environmental Pollution* 56, 299–309.
- Hidaka, I., 1970. The effects of transition metals on the palatal chemoreceptors of the carp. *Japanese Journal of Physiology* 20, 599–609.
- Hubbard, P.C., Barata, E.N., Canário, A.V., 2000. Olfactory sensitivity to changes in environmental [Ca²⁺] in the marine teleost *Sparus aurata*. *Journal of Experimental Biology* 203, 3821–3829.
- Inglis, S.K., Corboz, M.R., Taylor, A.E., Ballard, S.T., 1997. In situ visualization of bronchial submucosal glands and their secretory response to acetylcholine. *American Journal of Physiology—Lung Cellular Molecular Physiology* 272, L203–210.
- Ishida, Y., Kobayashi, H., 1995. Avoidance behavior of carp to pesticides and decrease of the avoidance threshold by addition of sodium lauryl sulfate. *Fisheries Science* 61, 441–446, Tokyo.
- Ishida, Y., Yoshikawa, H., Kobayashi, H., 1996. Electrophysiological responses of three chemosensory systems in the carp to pesticides. *Physiology & Behavior* 60, 633–638.
- Jarrard, H.E., Delaney, K.R., Kennedy, C.J., 2004. Impacts of carbamate pesticides on olfactory neurophysiology and cholinesterase activity in coho salmon (*Oncorhynchus kisutch*). *Aquatic Toxicology* 69, 133–148.
- Jones, B.F., Warren, C.E., Bond, C.E., Doudoroff, P., 1956. Avoidance reactions of salmonid fishes to pulp mill effluents. *Sewage and Industrial Wastes* 28, 1403–1413.
- Jones, J.C., Reynolds, J.D., 1997. Effects of pollution on reproductive behaviour of fishes. *Reviews in Fish Biology and Fisheries* 7, 463–491.
- Jones, J.R.E., 1947. The reactions of *Pygosteus Pungitius* L. to toxic solutions. *Journal of Experimental Biology* 24, 110–122.
- Jones, K.A., Hara, T.J., 1988. Behavioral alterations in arctic char *Salvelinus alpinus* briefly exposed to sublethal chlorine levels. *Canadian Journal of Fisheries and Aquatic Sciences* 45, 749–752.
- Jones, K.A., Hara, T.J., Scherer, E., 1985a. Behavioral modifications in arctic char *Salvelinus alpinus* chronically exposed to sublethal pH. *Physiological Zoology* 58, 400–412.
- Jones, K.A., Hara, T.J., Scherer, E., 1985b. Locomotor response by arctic char *Salvelinus alpinus* to gradients of proton and carbon dioxide. *Physiological Zoology* 58, 413–420.
- Juergensen, L., Busnarda, J., Caux, P.-Y., Kent, R., 2000. Fate, behavior, and aquatic toxicity of the fungicide IPBC in the Canadian environment. *Environmental Toxicology* 15, 201–213.
- Julliard, A.K., Saucier, D., Astic, L., 1996. Time-course of apoptosis in the olfactory epithelium of rainbow trout exposed to a low copper level. *Tissue and Cell* 28, 367–377.
- Kasumyan, A.O., 2001. Effects of chemical pollutants on foraging behavior and sensitivity of fish to food stimuli. *Journal of Ichthyology* 41, 76–87.
- Klaprat, D.A., Brown, S.B., Hara, T.J., 1988. The effect of low pH and aluminum on the olfactory organ of rainbow trout *Salmo gairdneri*. *Environmental Biology of Fishes* 22, 69–78.
- Kruzynski, G.M., Birtwell, I.K., 1994. A predation bioassay to quantify the ecological significance of sublethal responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to the antisepstain fungicide TCMTB. *Canadian Journal of Fisheries and Aquatic Sciences* 51, 1780–1790.
- Kynard, B., 1974. Avoidance behavior of insecticide susceptible and resistant populations of mosquitofish to 4 insecticides. *Transactions of the American Fisheries Society* 103, 557–561.
- Labenia, J.S., Baldwin, D.H., French, B.L., Davis, J.W., Scholz, N.L., 2007. Behavioral impairment and increased predation mortality in cutthroat trout exposed to carbaryl. *Marine Ecology Progress Series* 329, 1–11.
- Laberge, F., Hara, T.J., 2001. Neurobiology of fish olfaction: a review. *Brain Research Reviews* 36, 46–59.
- Laberge, F., Hara, T.J., 2003. Behavioural and electrophysiological responses to F-prostaglandins, putative spawning pheromones, in three salmonid fishes. *Journal of Fish Biology* 62, 206–221.
- Lewis III, F.G., Livingston, R.J., 1977. Avoidance of bleached kraft pulp mill effluent by pinfish *Lagodon-Rhomboides* and gulf killifish *Fundulus-Grandis*. *Journal of the Fisheries Research Board of Canada* 34, 568–570.
- Macek, K.J., Hutchinson, C., Cope, O.B., 1969. The effects of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides. *Bulletin of Environmental Contamination and Toxicology* 4, 174–183.
- Mathison, B.H., Taylor, M.L., Bogdanffy, M.S., 1995. Dimethyl sulfate uptake and methylation of DNA in rat respiratory tissues following acute inhalation. *Fundamental and Applied Toxicology* 28, 255–263.
- Maynard, D.J., Weber, D.D., 1981. Avoidance reactions of juvenile coho salmon *Oncorhynchus kisutch* to mono cyclic aromatics. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 772–778.
- McIntyre, J.K., Baldwin, D.H., Meador, J.P., Scholz, N.L., 2008. Chemosensory deprivation in juvenile coho salmon exposed to dissolved copper under varying water chemistry conditions. *Environmental Science and Technology* 42, 1352–1358.
- McLennan, D.A., 2004. Male brook sticklebacks' (*Culaea inconstans*) response to olfactory cues. *Behavioural Brain Research* 141, 1411–1424.
- Miller, T.G., Mackay, W.C., 1982. Relationship of secreted mucus to copper and acid toxicity in rainbow trout. *Bulletin of Environmental Contamination and Toxicology* 28, 68–74.
- Mirza, R.S., Chivers, D.P., 2002. Brook char (*Salvelinus fontinalis*) can differentiate chemical alarm cues produced by different age/size classes of conspecifics. *Journal of Chemical Ecology* 28, 555–564.
- Mombaerts, P., 1999. Molecular biology of odorant receptors in vertebrates. *Annual Review of Neuroscience* 22, 487–509.
- Moore, A., 1994. An electrophysiological study on the effects of pH on olfaction in mature male Atlantic salmon (*Salmo salar*) parr. *Journal of Fish Biology* 45, 493–502.
- Moore, A., Lower, N., 2001. The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 129, 269–276.
- Moore, A., Waring, C.P., 1996a. Electrophysiological and endocrinological evidence that F-series prostaglandins function as priming pheromones in mature male Atlantic salmon (*Salmo salar*) parr. *Journal of Experimental Biology* 199, 2307–2316.
- Moore, A., Waring, C.P., 1996b. Sublethal effects of the pesticide Diazinon on olfactory function in mature male Atlantic salmon parr. *Journal of Fish Biology* 48, 758–775.
- Moore, A., Waring, C.P., 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. *Pesticide Biochemistry and Physiology* 62, 41–50.
- Moore, A., Waring, C.P., 2001. The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). *Aquatic Toxicology* 52, 1–12.
- Moran, D.T., Rowley III, J.C., Aiken, G.R., Jafek, B.W., 1992. Ultrastructural neurobiology of the olfactory mucosa of the brown trout, *Salmo trutta*. *Microscopy Research and Technique* 23, 28–48.
- Myllyvirta, T.P., Vuorinen, P.J., 1989. Avoidance of bleached kraft mill effluent by pre-exposed *Coregonus albula* L. *Water Research* 23, 1219–1227.
- Narahashi, T., 1996. Insecticides as tools for ion channel studies. *Abstracts of Papers American Chemical Society* 211: AGRO 9.
- Ottoson, D., 1956. Analysis of the electrical activity of the olfactory epithelium. *Acta Physiologica Scandinavica* 35, 1–83.
- Piras, E., Franzen, A., Fernandez, E.L., Bergstrom, U., Raffalli-Mathieu, F., Lang, M., Brittebo, E.B., 2003. Cell-specific expression of CYP2A5 in the mouse respiratory tract: effects of olfactory toxicants. *Journal of Histochemistry and Cytochemistry* 51, 1545–1555.
- Pollock, M.S., Chivers, D.P., Mirza, R.S., Wisenden, B.D., 2003. Fathead minnow, *Pimephales promelas*, learn to recognize chemical alarm cues of introduced brook stickleback, *Culaea inconstans*. *Environmental Biology of Fishes* 66, 313–319.
- Quinn, T.P., Hara, T.J., 1986. Sibling recognition and olfactory sensitivity in juvenile coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Zoology* 64, 921–925.
- Rajakaruna, R.S., Brown, J.A., Kaukinen, K.H., Miller, K.M., 2006. Major histocompatibility complex and kin discrimination in Atlantic salmon and brook trout. *Molecular Ecology* 15, 4569–4575.
- Rand, G., Kleerekoper, H., Matis, J., 1975. Interaction of odor and flow perception and the effects of parathion in the locomotor orientation of the goldfish *Carassius auratus* L. *Journal of Fish Biology* 7, 497–504.
- Rehnberg, B.G., Schreck, C.B., 1987. Chemosensory detection of predators by coho salmon (*Oncorhynchus kisutch*): behavioral reaction and physiological stress response. *Canadian Journal of Zoology* 65, 481–485.
- Royce-Malmgren, C.H., Watson, W.H., 1987. Modification of olfactory-related behavior in juvenile Atlantic salmon by changes in pH. *Journal of Chemical Ecology* 13, 533–546.
- Saglio, P., Olsén, K.H., Bretaud, S., 2001. Behavioral and olfactory responses to prochloraz, bentazone, and nicosulfuron-contaminated flows in goldfish. *Archives of Environmental Contamination and Toxicology* 41, 192–200.
- Saglio, P., Trijasse, S., 1998. Behavioral responses to atrazine and diuron in goldfish. *Archives of Environmental Contamination and Toxicology* 35, 484–491.
- Saglio, P., Trijasse, S., Azam, D., 1996. Behavioral effects of waterborne carbofuran in goldfish. *Archives of Environmental Contamination and Toxicology* 31, 232–238.
- Salierno, J.D., Snyder, N.S., Murphy, A.Z., Poli, M., Hall, S., Baden, D., Kane, A.S., 2006. Harmful algal bloom toxins alter c-Fos protein expression in the brain of killifish, *Fundulus heteroclitus*. *Aquatic Toxicology* 78, 350–357.
- Sandahl, J.F., Baldwin, D.H., Jenkins, J.J., Scholz, N.L., 2004. Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to copper, chlorpyrifos, or esfenvalerate. *Canadian Journal of Fisheries and Aquatic Sciences* 61, 404–413.
- Sandahl, J.F., Baldwin, D.H., Jenkins, J.J., Scholz, N.L., 2005. Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environmental Toxicology and Chemistry* 24, 136–145.
- Sandahl, J.F., Baldwin, D.H., Jenkins, J.J., Scholz, N.L., 2007. A sensory system at the interface between urban stormwater runoff and salmon survival. *Environmental Science and Technology* 41, 2998–3004.
- Sandahl, J.F., Miyasaka, G., Koide, N., Ueda, H., 2006. Olfactory inhibition and recovery in chum salmon (*Oncorhynchus keta*) following copper exposure. *Canadian Journal of Fisheries and Aquatic Sciences* 63, 1840–1847.
- Sato, K., Suzuki, N., 2001. Whole-cell response characteristics of ciliated and microvillous olfactory receptor neurons to amino acids, pheromone candidates and urine in rainbow trout. *Chemical Senses* 26, 1145–1156.
- Sato, Y., Miyasaka, N., Yoshihara, Y., 2007. Hierarchical regulation of odorant receptor gene choice and subsequent axonal projection of olfactory sensory neurons in zebrafish. *Journal of Neuroscience* 27, 1606–1615.
- Saucier, D., Astic, L., 1995. Morpho-functional alterations in the olfactory system of rainbow trout (*Oncorhynchus mykiss*) and possible acclimation in response

- to long-lasting exposure to low copper levels. *Comparative Biochemistry and Physiology Part A: Physiology* 112, 273–284.
- Saucier, D., Julliard, A.K., Monod, G., Bréchar, H.d., Astic, L., 1999. CYP1A1 immunolocalization in the olfactory organ of rainbow trout and its possible induction by β -naphthoflavone: analysis in adults and embryos around hatching. *Fish Physiology and Biochemistry* 21, 179–192.
- Saunders, R.L., Sprague, J.B., 1967. Effects of copper-zinc mining pollution on a spawning migration of atlantic salmon. *Water Research* 1, 419–432.
- Scherer, E., 1975. Avoidance of fenitrothion by goldfish (*Carassius auratus*). *Bulletin of Environmental Contamination and Toxicology* 13, 492–496.
- Schmachtenberg, O., 2006. Histological and electrophysiological properties of crypt cells from the olfactory epithelium of the marine teleost *Trachurus symmetricus*. *The Journal of Comparative Neurology* 495, 113–121.
- Scholz, A., Horrall, R., Cooper, J., Hasler, A., 1976. Imprinting to chemical cues: the basis for home stream selection in salmon. *Science* 192, 1247–1249.
- Scholz, N.L., Truelove, N.K., French, B.L., Berejikian, B.A., Quinn, T.P., Casillas, E., Collier, T.K., 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 57, 1911–1918.
- Schwob, J.E., 2005. Restoring olfaction: a view from the olfactory epithelium. *Chemical Senses* 30, i131–132.
- Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology* 68, 369–392.
- Scott, G.R., Sloman, K.A., Rouleau, C., Wood, C.M., 2003. Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 206, 1779–1790.
- Scott, J.W., Scott-Johnson, P.E., 2002. The electroolfactogram: a review of its history and uses. *Microscopy Research and Technique* 58, 152–160.
- Seegerback, D., Plna, K., Faller, T., Kreuzer, P.E., Hakansson, K., Filser, J.G., Nilsson, R., 1998. Tissue distribution of DNA adducts in male Fischer rats exposed to 500 ppm of propylene oxide: quantitative analysis of 7-(2-hydroxypropyl)guanine by 32P-postlabelling. *Chemico-Biological Interactions* 115, 229–246.
- Shelford, V.E., Powers, E.B., 1915. An experimental study of the movements of herring and other marine fishes. *Biological Bulletin* 28, 315–334.
- Shin, S.-W., Chung, N.-I., Kim, J.-S., Chon, T.-S., Kwon, O.-S., Lee, S.-K., Koh, S.-C., 2001. Effect of diazinon on behavior of Japanese medaka (*Oryzias latipes*) and gene expression of tyrosine hydroxylase as a biomarker. *Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes* 36, 783–795.
- Shoji, T., Yamamoto, Y., Nishikawa, D., Kurihara, K., Ueda, H., 2003. Amino acids in stream water are essential for salmon homing migration. *Fish Physiology and Biochemistry* 28, 249–251.
- Smolowitz, R.M., Schultz, M.E., Stegeman, J.J., 1992. Cytochrome P4501A induction in tissues, including olfactory epithelium, of topminnows (*Poeciliopsis* spp.) by waterborne benzo[a]pyrene. *Carcinogenesis* 13, 2395–2402.
- Solangi, M.A., Overstreet, R.M., 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. *Journal of Fish Diseases* 5, 13–35.
- Sorensen, P.W., 1992. Hormones, pheromones and chemoreception. In: Hara, T.J. (Ed.), *Fish Chemoreception*. Chapman & Hall, Boundary Row, London, UK.
- Sorensen, P.W., Sato, K., 2005. Second messenger systems mediating sex pheromone and amino acid sensitivity in goldfish olfactory receptor neurons. *Chemical Senses* 30, i315–316.
- Sprague, J., 1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. *Water Research* 2, 367–372.
- Sprague, J.B., Drury, R.E., 1969. Avoidance reactions of salmonid fish to representative pollutants. In: Jenkins, S.H. (Ed.), *Advances in Water Pollution Research*. Proceedings of the 4th International Conference Held in Prague. Pergamon Press, New York, pp. 169–186.
- Sprague, J.B., McLeese, D.W., 1968. Toxicity of kraft pulp mill effluent for larval and adult lobsters and juvenile salmon *Salmo-salar* Homarus-americanus. *Water Research* 2, 753–760.
- Starcevic, S.L., Zielinski, B.S., 1997. Glutathione and glutathione S-transferase in the rainbow trout olfactory mucosa during retrograde degeneration and regeneration of the olfactory nerve. *Experimental Neurology* 146, 331–340.
- Stober, Q.J., Dinnel, P.A., Hurlburt, E.F., Dijulio, D.H., 1980. Acute toxicity and behavioral responses of coho salmon ('kisutch) and shiner perch (*Cymatogaster aggregata*) to chlorine in heated sea-water. *Water Research* 14, 347–354.
- Sutterlin, A., Sutterlin, N., Rand, S., 1971. The Influence of Synthetic Surfactants on the Functional Properties of the Olfactory Epithelium of Atlantic Salmon. Fisheries Research Board of Canada Technical Report 287.
- Sutterlin, A.M., Sutterlin, N., 1971. Electrical responses of the olfactory epithelium of atlantic salmon (*Salmo salar*). *Journal of the Fisheries Research Board of Canada* 28, 565–572.
- Symons, P., 1973. Behavior of young atlantic salmon (*Salmo salar*) exposed to or force fed fenitrothion, an organophosphate insecticide. *Journal of the Fisheries Research Board of Canada* 30, 651–655.
- Tallkvist, J., Henriksson, J., d'Argy, R., Tjalve, H., 1998. Transport and subcellular distribution of nickel in the olfactory system of pikes and rats. *Toxicological Sciences* 43, 196–203.
- Thompson, B.E., Hara, T.J., 1977. Chemo sensory bioassay of toxicity of lake waters contaminated with heavy metals from mining effluents. *Water Pollution Research in Canada* 12, 179–189.
- Tierney, K.B., Casselman, M., Takeda, S., Farrell, A.P., Kennedy, C.J., 2007a. The relationship between cholinesterase inhibition and two types of swimming performance in chlorpyrifos-exposed coho salmon (*Oncorhynchus kisutch*). *Environmental Toxicology and Chemistry* 26, 998–1004.
- Tierney, K.B., Kennedy, C.J., 2008. Chapter 6: Background toxicology. In: Walsh, P.J., Smith, S.L., Fleming, L.E., Solo-Gabriele, H., Gerwick, W.H. (Eds.), *Oceans and Human Health: Risks and Remedies from the Seas*. Academic Press/Elsevier, Burlington, MA, pp. 101–120.
- Tierney, K.B., Ross, P.S., Jarrard, H.E., Delaney, K.R., Kennedy, C.J., 2006a. Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides. *Environmental Toxicology and Chemistry* 25, 2809–2817.
- Tierney, K.B., Ross, P.S., Kennedy, C.J., 2007b. Linuron and carbaryl differentially impair baseline amino acid and bile salt olfactory responses in three salmonids. *Toxicology* 231, 175–187.
- Tierney, K.B., Sampson, J.L., Ross, P.S., Sekela, M.A., Kennedy, C.J., 2008. Salmon olfaction is impaired by an environmentally realistic pesticide mixture. *Environmental Science and Technology* 42, 4996–5001.
- Tierney, K.B., Singh, C.R., Ross, P.S., Kennedy, C.J., 2007c. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. *Aquatic Toxicology* 81, 55–64.
- Tierney, K.B., Taylor, A.L., Ross, P.S., Kennedy, C.J., 2006b. The carbamate pesticide IPBC impairs the alarm reaction of juvenile coho salmon. *Aquatic Toxicology* 79, 149–157.
- Tilton, F., Tilton, S.C., Bammler, T.K., Beyer, R., Farin, F., Stapleton, P.L., Gallagher, E.P., 2008. Transcriptional biomarkers and mechanisms of copper-induced olfactory injury in zebrafish. *Environmental Science and Technology* 42, 9404–9411.
- Updegraff, K.F., Sykora, J.L., 1976. Avoidance of lime-neutralized iron hydroxide solutions by coho salmon in the laboratory. *Environmental Science and Technology* 10, 51–54.
- Vilhunen, S., 2006. Repeated antipredator conditioning: a pathway to habituation or to better avoidance? *Journal of Fish Biology* 68, 25–43.
- Waring, C.P., Moore, A., 1997. Sublethal effects of a carbamate pesticide on pheromonal mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. *Fish Physiology and Biochemistry* 17, 203–211.
- Waring, C.P., Moore, A., Scott, A.P., 1996. Milt and endocrine responses of mature male Atlantic salmon (*Salmo salar* L.) parr to water-borne testosterone, 17,20[beta]-dihydroxy-4-pregnen-3-one 20-sulfate, and the urines from adult female and male salmon. *General and Comparative Endocrinology* 103, 142–149.
- Weber, D.D., Maynard, D.J., Gronlund, W.D., Konchin, V., 1981. Avoidance reactions of migrating adult salmon to petroleum hydrocarbons. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 779–781.
- Weis, P., Weis, J.S., 1974. DDT causes changes in activity and schooling behavior in goldfish. *Environmental Research* 7, 68–74.
- Wildish, D.J., Akagi, H., Poole, N.J., 1977. Avoidance by herring of dissolved components in pulp mill effluents. *Bulletin of Environmental Contamination and Toxicology* 18, 521–525.
- Winberg, S., Bjerselius, R., Baatrup, E., Døving, K.B., 1992. The effect of Cu(II) on the electro-olfactogram (EOG) of the Atlantic salmon (*Salmo salar* L.) in artificial freshwater of varying inorganic carbon concentrations. *Ecotoxicology and Environmental Safety* 24, 167–178.
- Xue, W., Warshawsky, D., 2005. Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. *Toxicology and Applied Pharmacology* 206, 73–93.
- Zhainazarov, A.B., Ache, B.W., 1995. Odor-induced currents in *Xenopus* olfactory receptor cells measured with perforated-patch recording. *Journal of Neurophysiology* 74, 479–483.
- Zhang, C., Brown, S.B., Hara, T.J., 2001. Biochemical and physiological evidence that bile acids produced and released by lake char (*Salvelinus namaycush*) function as chemical signals. *Journal of Comparative Physiology B* 171, 161–171.
- Zheng, W., Strobeck, C., Stacey, N., 1997. The steroid pheromone 4-pregnen-17,20 β -diol-3-one increases fertility and paternity in goldfish. *Journal of Experimental Biology* 200, 2833–2840.
- Zielinski, B.S., Hara, T.J., 2001. The neurobiology of fish olfaction. In: Kapoor, B.G., Hara, T.J. (Eds.), *Sensory Biology of Jawed Fishes: New Insights*. Science Publishers, Enfield, pp. 347–366.
- Zielinski, B.S., Hara, T.J., Olfaction 2007. In: Zielinski, B.S., Hara, T.J. (Eds.), *Fish Physiology*. Elsevier/Academic Press, NY, New York, pp. 1–43.
- Zippel, H.P., 1993. Regeneration in the peripheral and the central olfactory system: a review of morphological, physiological and behavioral aspects. *Journal für Hirnforsch* 34, 207–229.
- Zippel, H.P., Hansen, A., Caprio, J., 1997. Renewing olfactory receptor neurons in goldfish do not require contact with the olfactory bulb to develop normal chemical responsiveness. *Journal of Comparative Physiology* 181A, 425–437.