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Review

# The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity

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## Abstract

Environmental pollutants such as metals, pesticides, and other organics pose serious risks to many aquatic organisms. Accordingly, a great deal of previous research has characterized physiological mechanisms of toxicity in animals exposed to contaminants. In contrast, effects of contaminants on fish behaviour are less frequently studied. Because behaviour links physiological function with ecological processes, behavioural indicators of toxicity appear ideal for assessing the effects of aquatic pollutants on fish populations. Here we consider the many toxicants that disrupt complex fish behaviours, such as predator avoidance, reproductive, and social behaviours. Toxicant exposure often completely eliminates the performance of behaviours that are essential to fitness and survival in natural ecosystems, frequently after exposures of lesser magnitude than those causing significant mortality. Unfortunately, the behavioural toxicity of many xenobiotics is still unknown, warranting their future study. Physiological effects of toxicants in the literature include disruption of sensory, hormonal, neurological, and metabolic systems, which are likely to have profound implications for many fish behaviours. However, little toxicological research has sought to integrate the behavioural effects of toxicants with physiological processes. Those studies that take this multidisciplinary approach add important insight into possible mechanisms of behavioural alteration. The most commonly observed links with behavioural disruption include cholinesterase (ChE) inhibition, altered brain neurotransmitter levels, sensory deprivation, and impaired gonadal or thyroid hormone levels. Even less frequently studied are the implications of interrelated changes in behaviour and physiology caused by aquatic pollutants for fish populations. We conclude that future integrative, multidisciplinary research is clearly needed to increase the significance and usefulness of behavioural indicators for aquatic toxicology, and aim to highlight specific areas for consideration.

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## 1. Introduction

Environmental pollutants, such as metals, pesticides, and other organics, pose serious risks to many aquatic organisms. A great deal of research has therefore been conducted to understand the effects of toxi-

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cants on the physiology and survival of many animals (e.g. Wood, 2001). As such, regulatory guidelines for aquatic pollutants in natural ecosystems have been traditionally based on acute lethality tests such as the 96 h LC50 (see Table 1; CCME, 1999; USEPA, 2001), although impacts on development, growth, and reproduction have also been considered (Rand and Petrocelli, 1985). The concentrations at which a compound is lethal can depend upon many contributing factors, including species and water quality and some examples of variation in fish are shown in Table 1.

Although useful for generating guidelines to protect against physiological death (i.e. mortality) of aquatic animals, acute lethality tests ignore 'ecological death' that may occur after much lower toxicant exposures; even if animals are not overtly harmed by a contaminant, they may be unable to function in an ecological context if their normal behaviour is altered. Indeed, environmental contamination measured in natural ecosystems often occurs at concentrations well below those causing significant mortality (Jensen and Bro-Rasmussen, 1992; Cabrera et al., 1998; Norris et al., 1999).

Since behaviour serves as the link between physiological and ecological processes, it may be ideal for studying environmental pollutant effects. Fish are an excellent model in this regard, since many ecologically relevant fish behaviours are easily observed and quantified in a controlled setting. Furthermore, a great deal is known about fish physiology, a necessary consideration for integrative studies. Indeed, many researchers have proposed using behavioural indicators in fish for ecologically relevant monitoring of environmental contamination (e.g. Atchison et al., 1987). The performance of normal behaviour by individual fish follows specific physiological sequences, which are triggered by external stimuli acting via neural networks (Weber and Spieler, 1994). Disruption of these sequences before completion is likely to result in detrimental behavioural alterations. Initiation of these sequences is also affected by numerous physiological and environmental influences (Fig. 1). Inappropriate behavioural responses to environmental and physiological stimuli due to toxic effects of aquatic contaminants can have severe implications for survival (Weber and Spieler, 1994).

Although a great deal of literature has considered effects of numerous anthropogenic pollutants on dif-

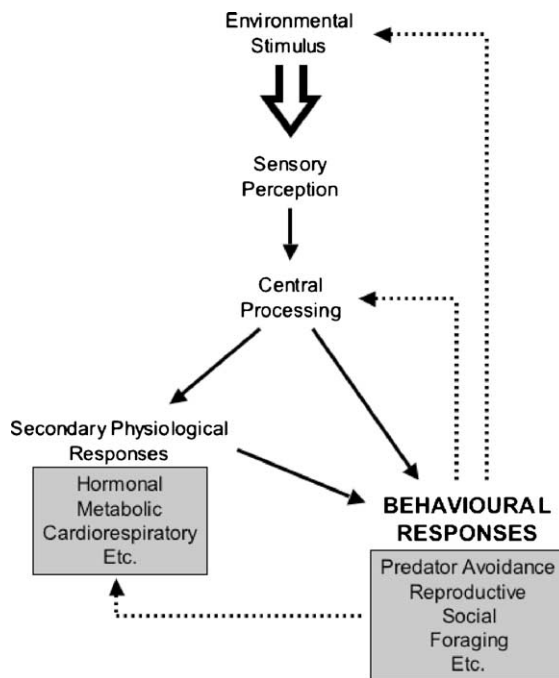


Fig. 1. Many physiological and environmental factors influence the performance of normal behaviour by fish. After appropriate environmental stimuli are perceived, sensory information is generally integrated centrally. Secondary physiological responses may also ensue, and together central and peripheral changes in physiology determine the behavioural responses to stimuli. Behavioural changes may then feedback to influence subsequent physiological processes and environmental stimuli.

ferent fish behaviours (see reviews by Marcucella and Abramson, 1978; Little et al., 1985; Rand, 1985; Atchison et al., 1987; Beitinger, 1990; Little and Finger, 1990; Døving, 1991; Blaxter and Hallers-Tjabbes, 1992; Scherer, 1992; Atchison et al., 1996; Kasumyan, 2001), the majority of research conducted to date has discussed direct behavioural responses of fish to aquatic pollutants, such as preference/avoidance, coughs, and body tremors. More recently, research has begun to focus on the impacts of environmental pollutants on complex fish behaviours that normally occur in the wild. For example, disruption of behaviours associated with foraging, predator avoidance, reproduction, and social hierarchies may be more environmentally relevant than simple behavioural responses to toxicants when considering potential impacts on fish populations.

Table 1  
Examples of acute toxicity for selected aquatic pollutants

Pollutant	LC50 <sup>a</sup> (µg/l)	Duration (h)	Hardness (mg/l as CaCO <sub>3</sub> ) <sup>b</sup>	Fish species <sup>c</sup>	Reference
<b>Metals</b>					
Cadmium	2.53	96	20	rbt	Hollis et al. (2000)
	91	48	20	rbt	Calamari et al. (1980)
	358	48	80	rbt	Calamari et al. (1980)
	<0.5	96	9.2	rbt	Cusimano et al. (1986)
	18	96	140	rbt	Szebedinsky et al. (2001)
	22	96	140	rbt	Hollis et al. (1999)
Copper	300	96	SW	db	Taylor et al. (1985)
	1400	96	SW	gmt	Taylor et al. (1985)
	30.7	96	102	rbt	Howarth and Sprague (1978)
	298	96	361	rbt	Howarth and Sprague (1978)
	31	96	30	rbt	Howarth and Sprague (1978)
	100	96	120	rbt	Taylor et al. (2000)
	2.8	96	9.2	rbt	Cusimano et al. (1986)
Lead	3362	96	44.3	bc	Holcombe et al. (1976)
	1170	96	28	rbt	Davies et al. (1976)
	1000	96	120	rbt	Rogers et al. (2003)
Mercury (inorganic)	168	96	45	fhm	Atchison et al. (1987)
Mercury (methyl)	75	96	45	bc	Atchison et al. (1987)
Nickel	118300	96	SW	gmt	Taylor et al. (1985)
	8100	96	33	rbt	Atchison et al. (1987)
	15000	96	120	rbt	Pane et al. (2003)
	27200	96	–	nti	Alkahem (1994)
Zinc	21500	96	SW	gmt	Taylor et al. (1985)
	103	96	10	rbt	Alsop and Wood (1999)
	66	96	9.2	rbt	Cusimano et al. (1986)
	869	96	120	rbt	Alsop et al. (1999)
	4460	96	386	rbt	Bradley and Sprague (1985)
	170	96	31	rbt	Bradley and Sprague (1985)
<b>Organic pollutants</b>					
Carbaryl	1950	96	–	rbt	Little et al. (1990)
	9400	48	–	md	Carlson et al. (1998)
Chlordane	42	96	–	rbt	Little et al. (1990)
Chlorpyrifos	300	48	–	md	Carlson et al. (1998)
DEF	660	96	–	rbt	Little et al. (1990)
Diazinon	839	96	–	rbt	Scholz et al. (2000)
	2620	96	–	ctt	Scholz et al. (2000)
2,4-DMA	100000	96	–	rbt	Little et al. (1990)
DNP	11000	48	–	md	Carlson et al. (1998)
Endosulfan	1.5	48	–	md	Carlson et al. (1998)
Fenvalerate	1.6	48	–	md	Carlson et al. (1998)
Methyl parathion	7.7	120	–	sf	Welsh and Hanselka (1972)
	3700	96	–	rbt	Little et al. (1990)
1-Octanol	21000	48	–	md	Carlson et al. (1998)
Phenol	42000	96	–	gp	Colgan et al. (1982)
PCP	52	96	–	rbt	Little et al. (1990)

<sup>a</sup> LC50, toxicant concentration that is lethal to 50% of individuals after specific exposure duration.

<sup>b</sup> Exposure in freshwater unless otherwise stated; SW: seawater.

<sup>c</sup> Abbreviations: bc, brook charr (*Salvelinus fontinalis*); ctt, cutthroat trout (*Oncorhynchus clarki*); db, dab (*Limanda limanda*); fhm, fathead minnow (*Pimephales promelas*); gmt, grey mullet (*Chelon labrosus*); gp, guppy (*Poecilia reticulata*); mc, mummichog (*Fundulus heteroclitus*); md, medaka (*Oryzias latipes*); nti, nile tilapia (*Oreochromis niloticus*); rbt, rainbow trout (*Oncorhynchus mykiss*); sf, Siamese fighting fish (*Betta splendens*).

Kasumyan (2001) provided an excellent review of the effects of chemical pollutants on foraging behaviours and its corresponding chemoreception physiology, but no comprehensive review of recent literature has discussed the effects of aquatic pollutants on other important complex fish behaviours.

As we strive to further understand the true impacts of aquatic pollution on whole ecosystems, multidisciplinary research becomes increasingly important. Because of its inevitable integration with other levels of biological organization, behaviour needs to be considered not in isolation, but as a predictor and result of other internal and external biological processes. Although we consider behavioural endpoints to be practical, ecologically relevant measures of toxicological effects, we believe that they are benefited by concurrent consideration of both physiological and ecological indicators of toxicity.

This review will integrate behavioural research conducted to date with physiological toxicity mechanisms, and address their potential ecological implications. The review will consist of three parts. Firstly, the existing literature surrounding the effects of aquatic pollutants on predator avoidance, reproductive, and social behaviours will be discussed. Secondly, effects of toxicants on the physiological mechanisms most closely associated with behaviour will be highlighted (Fig. 1). Finally, studies correlating behavioural and physiological aspects of contaminant exposure will be reviewed, and implications for fish populations discussed. The authors hope that this review will stimulate future research to better characterize appropriate behavioural indicators of toxicity and their ecological consequences, and that possible physiological and behavioural interactions will be further explored.

## 2. Behavioural effects of aquatic pollutants

Life histories of fish are intimately associated with numerous interspecific (e.g. predation) and intraspecific (both reproductive and non-reproductive) interactions, which invariably depend on the performance of appropriate behaviours. As such, behavioural interactions associated with predator avoidance, reproductive, and social behaviours form an important part of a successful, adaptive life history strategy. Alteration to normal behavioural patterns caused by expo-

sure to pollutants therefore poses serious risks to the success of fish populations. In this section, the effects of toxicants on fish predator avoidance, reproductive, and social behaviours will be reviewed. Many contaminants disrupt normal fish behaviour after exposures much less severe than those causing significant mortality (compare LC50 data in Table 1 with Tables 2–4 for examples), so behavioural indicators of toxicity are likely to be ideal for assessing sublethal impacts of pollutants. Tables 2–4 include lowest observable effect concentrations (LOEC) of toxicant exposure for all studies discussed. LOECs have previously been employed by researchers considering the behavioural effects of toxicant exposure (Atchison et al., 1987), and may be a good common measure for comparison that allows simple assessment of potential environmental impacts. These data do not, however, indicate threshold concentrations of behavioural disruption, if these indeed exist, since much of the literature examines toxicological effects for only a select number of exposure concentrations.

### 2.1. Predator avoidance behaviour

The effects of predation on prey population dynamics can be complex and unpredictable, largely because population control may also occur through bottom-up (e.g. food supply) and other top-down (e.g. social interactions and disturbance) ecological processes (Kay, 1998; Forrester et al., 1999). Predators often affect prey in a density-dependent fashion, so abundances of predator and prey populations can be tightly linked (Begon et al., 1996). Predators may impact prey species to different extents, depending largely on the ability of prey to avoid predation (Vamosi and Schluter, 2002). Therefore, environmental toxicants that alter the predator avoidance ability of prey could potentially disrupt aquatic communities.

Predator avoidance ability of fish is often altered in response to sublethal toxicant exposure (summarized in Table 2), and the literature contains numerous examples of reduced survival in exposed prey fish subjected to a predator. Indeed, many trace metals and organic toxicants have been shown to increase susceptibility to predation (Hatfield and Anderson, 1972; Kania and O'Hara, 1974; Sullivan et al., 1978; Schneider et al., 1980; Little et al., 1990; Weis and Weis, 1995; Carlson et al., 1998; Zhou and Weis,

Table 2  
Literature summary of the effects of pollutants on fish predator avoidance behaviours

Pollutant	LOEC <sup>a</sup> (µg/l)	Duration	Hardness (mg/l as CaCO <sub>3</sub> ) <sup>b</sup>	Fish species <sup>c</sup>	Disrupted behaviour <sup>d</sup>	Reference
<b>Metals</b>						
Cadmium	375	48 h	41	fhm	Survival	Sullivan et al. (1978)
	25	21 days	349	fhm	Survival	Sullivan et al. (1978)
	2	7 days	120	rbt	AS response	Scott et al. (2003)
Copper	43 <sup>e</sup>	24 h	124	cpm	AS response	Beyers and Farmer (2001)
	56 <sup>e</sup>	96 h	124	cpm	AS response	Beyers and Farmer (2001)
	100	5 h pulse	SW	asi	Schooling	Koltes (1985)
	10	7 days	SW	mc	Survival	Weis and Weis (1995)
Mercury	10	24 h	–	mq	Survival	Kania and O'Hara (1974)
	5	14 days	SW	mc	Schooling	Osozkov and Weis (1996)
	5	≥7 days	50% SW	mc	Survival	Zhou and Weis (1998)
	0.959 <sup>f</sup>	90 days	–	gs	Schooling	Webber and Haines (2003)
<b>Organic pollutants</b>						
Atrazine	5	24 h	–	gf	AS response	Saglio and Trijasse (1998)
Carbaryl	10	96 h	272	rbt	Survival	Little et al. (1990)
	700	24 h	40	md	Survival	Carlson et al. (1998)
Chlordane	2	96 h	272	rbt	Survival	Little et al. (1990)
Chlorpyrifos	270	24 h	40	md	Survival	Carlson et al. (1998)
DDT	1	3 days	–	gf	Schooling	Weis and Weis (1974b)
DEF	50	96 h	272	rbt	Survival	Little et al. (1990)
2,4-DMA	50000	96 h	272	rbt	Survival	Little et al. (1990)
Diazinon	1.0	2 h	65	cs	AS response	Scholz et al. (2000)
DNP	10000	24 h	40	md	Survival	Carlson et al. (1998)
Diuron	5	24 h	–	gf	AS response	Saglio and Trijasse (1998)
Endosulfan	1	24 h	40	md	Survival	Carlson et al. (1998)
Fenvalerate	1	24 h	40	md	Survival	Carlson et al. (1998)
1-Octanol	17800	24 h	40	md	Survival	Carlson et al. (1998)
Parathion	100	96 h	272	rbt	Survival	Little et al. (1990)
PCP	500	1–4 week	–	gp	Survival, pursuit time	Brown et al. (1985)
	0.2	96 h	272	rbt	Survival	Little et al. (1990)
Phenol	25900	24 h	40	md	Survival	Carlson et al. (1998)
	7000	96 h	–	rbt	Survival	Schneider et al. (1980)
Sevin	100	24 h	SW	asi	Schooling	Weis and Weis (1974a)
Sumithion	1000	24 h	–	as	Survival	Hatfield and Anderson (1972)
TBTO	3	Not specified	–	tsb	Visual predator response	Wibe et al. (2001)

<sup>a</sup> Lowest observable effect concentration.

<sup>b</sup> Exposure in freshwater unless otherwise stated; SW, seawater.

<sup>c</sup> Abbreviations: as, Atlantic salmon (*Salmo salar*); asi, Atlantic silverside (*Menidia menidia*); cpm, Colorado pikeminnow (*Ptychocheilus lucius*); cs, chinook salmon (*Oncorhynchus tshawytscha*); fhm, fathead minnow (*Pimephales promelas*); gf, goldfish (*Carassius auratus*); gp, guppy (*Poecilia reticulata*); gs, golden shiners (*Notemigonus crysoleucas*); mc, mummichog (*Fundulus heteroclitus*); md, medaka (*Oryzias latipes*); mq, mosquitofish (*Gambusia affinis*); nti, Nile tilapia (*Oreochromis niloticus*); rbt, rainbow trout (*Oncorhynchus mykiss*); tsb, threespine stickleback (*Gasterosteus aculeatus*).

<sup>d</sup> AS: alarm substance.

<sup>e</sup> EC50, concentration estimated to inhibit behaviour in 50% of test organisms, see text.

<sup>f</sup> Dietary exposure, µg/g dry food weight.

Table 3  
Literature summary of the effects of pollutants on fish reproductive behaviours

Pollutant	LOEC ( $\mu\text{g/l}$ )	Duration	Hardness (mg/l as $\text{CaCO}_3$ )	Fish species <sup>a</sup>	Disrupted behaviour	Reference
<b>Metals</b>						
Cadmium	0.5	48 h	30	bko	Homing	Baker and Montgomery (2001)
Copper	22	37 weeks	61	rbt	Homing	Saucier et al. (1991)
	20	40 weeks	61	rbt	Homing	Saucier and Astic (1995)
Lead	500	30 days	130	fhm	Nesting	Weber (1993)
Mercury	0.88 <sup>b</sup>	To sexual maturity	–	fhm	Spawning	Hammerschmidt et al. (2002)
<b>Organic pollutants</b>						
Diazinon	10.0	24 h	65	cs	Migration	Scholz et al. (2000)
<i>p,p'</i> -DDE	0.1 <sup>b</sup>	30 days	–	gp	Courtship	Baatrup and Junge (2001)
Endosulfan	0.6	$\geq 10$ days	291	ci	Courtship/ nest maintenance	Matthiessen and Logan (1984)
Esfenvalerate	1.0	Pulsed	–	bg	Spawning	Tanner and Knuth (1996)
17 $\beta$ -estradiol	1	24–28 days	–	gf	Courtship	Bjerselius et al. (2001)
	10 <sup>b</sup>	24–28 days	–	gf	Courtship	Bjerselius et al. (2001)
	0.05	10 weeks	–	gf	Courtship/ spawning	Schoenfuss et al. (2002)
	3 <sup>b</sup>	14 days	–	md	Courtship/ spawning	Oshima et al. (2003)
Ethynyl estradiol	488	21 days	44–61	md	Fecundity	Seki et al. (2002)
Flutamide	1.0 <sup>b</sup>	30 days	–	gp	Courtship	Baatrup and Junge (2001)
Lindane	1.0	7 days	–	gp	Courtship	Schröder and Peters (1988a,b)
Octylphenol	25	6 months	–	md	Courtship/ success	Gray et al. (1999)
Phenol	10000	48 h	–	gp	Courtship/ spawning	Colgan et al. (1982)
Vinclozolin	1.0 <sup>b</sup>	30 days	–	gp	Courtship	Baatrup and Junge (2001)

<sup>a</sup> Abbreviations: bg, bluegill (*Lepomis macrochirus*); bko, banded kokopu (*Galaxias fasciatus*); ci, cichlid (*Sarotherodon mossambicus*); cs, chinook salmon (*Oncorhynchus tshawytscha*); fhm, fathead minnow (*Pimephales promelas*); gf, goldfish (*Carassius auratus*); gp, guppy (*Poecilia reticulata*); md, medaka (*Oryzias latipes*); rbt, rainbow trout (*Oncorhynchus mykiss*).

<sup>b</sup> Dietary exposure,  $\mu\text{g/g}$  dry food weight.

Table 4  
Literature summary of the effects of pollutants on fish non-reproductive social behaviours

Pollutant	LOEC ( $\mu\text{g/l}$ )	Duration	Hardness (mg/l as $\text{CaCO}_3$ )	Fish species <sup>a</sup>	Disrupted behaviour	Reference
<b>Metals</b>						
Cadmium and zinc	40 and 124	15 days	340	bg	Agonistic	Henry and Atchison (1979a)
	21 and 99	3 days	340	bg	Agonistic	Henry and Atchison (1979b)
Cadmium	3	24 h	120	rbt	Agonistic/dominance	Sloman et al. (2003b)
	2	24 h	120	rbt	Dominance	Sloman et al. (2003c)
Copper	34	96 h	273	bg	Agonistic	Henry and Atchison (1986)
Nickel	1500	96 h	–	nti	Agonistic	Alkalem (1994)
<b>Organic pollutants</b>						
Carbofuran	10	4 h	140	gf	Agonistic	Saglio et al. (1996)
Esfenvalerate	0.1	44 h pulses	283	bg	Agonistic	Little et al. (1993)
Ethynyl estradiol	0.015	Variable	–	tsb	Agonistic	Bell (2001)
Fenitrothion	1000	16 h	13	as	Territoriality	Symons (1973)
Methyl parathion	1000	5 days	–	sf	Agonistic	Welsh and Hanselka (1972)
Prochloraz	10000	15 min	–	gf	Agonistic	Saglio et al. (2001)

<sup>a</sup> Abbreviations: bg, bluegill (*Lepomis macrochirus*); gf, goldfish (*Carassius auratus*); nti, nile tilapia (*Oreochromis niloticus*); rbt, rainbow trout (*Oncorhynchus mykiss*); sf, Siamese fighting fish (*Betta splendens*); tsb, threespine stickleback (*Gasterosteus aculeatus*).

1998, 1999). Although these studies give important insight into toxicant exposures that could have ecological effects, they do not shed light on possible behavioural mechanisms through which pollutants might disrupt predator–prey systems.

Predator avoidance involves a complex set of behaviours. Therefore, to help fully understand the effects of toxicants on predator–prey relations this section will consider several separate antipredator behaviours. The effect of toxicants on predation and foraging behaviours of fish has been the subject of a recent review by Kasumyan (2001), so will not be considered here. Behavioural responses to early warning signs of predation risk (either chemical or visual) may be affected either by a disruption to sensory systems or the motivation to properly respond. Further still, some toxicants have been shown to alter the ability of fish to respond during the later stages of a predation event, by altering the escape abilities of individuals or the schooling behaviours of groups. It is reasonable to propose that such alterations in normal predator avoidance behaviours might alter aquatic predator–prey relations in natural ecosystems, and thus community structure, by increasing the predation susceptibility of prey fish populations. For some chemicals the increased likelihood of a predator being able to catch and consume a contaminated prey fish also increases the chances of contamination passing up through the food chain.

#### 2.1.1. Responses to chemical signals of predation threat

The chemical alarm signalling system in fish (reviewed by Smith, 1992; Chivers and Smith, 1998) is characterized by chemical ‘alarm substance’, which is released from epidermal cells in prey fish skin when attack by a predator causes sufficient damage. Upon detection of alarm substance by olfaction, other individuals or groups of prey fish exhibit stereotypical predator avoidance behaviours that minimize further predation, such as decreased swimming and feeding activities or increased schooling. Disruptions to the detection of and/or proper response to chemical signals of predation threat would likely increase the susceptibility of prey fish to predation. Exposure of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to the organophosphate pesticide, diazinon, for two hours at 1 and 10 µg/l was sufficient to eliminate the be-

havioural responses of individuals to alarm substance (Scholz et al., 2000). Similarly, 1 week exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to 2 µg/l cadmium eliminated the normal behavioural response of individuals to alarm substance (Scott et al., 2003). Behavioural responses to alarm substance by groups of fish are also disrupted by toxicant exposure. Colorado pikeminnow (*Ptychocheilus lucius*) exposed to copper for 24 and 96 h had group response frequencies decreased by 50% at concentrations of 43.3 and 56.0 µg/l (Beyers and Farmer, 2001). Similarly, grouping and/or sheltering behaviour exhibited in response to alarm substance decreased after goldfish (*Carassius auratus*) were exposed to the herbicides atrazine and diuron at 5 µg/l for 24 h (Saglio and Trijasse, 1998).

#### 2.1.2. Responses to visual signals of predation threat

As well as the behavioural responses to chemical signals of predation threat, responses to visual signals associated with predators can be disrupted by toxicant exposure. Acute exposure of threespine sticklebacks (*Gasterosteus aculeatus*) to bis(tributyltin)oxide (TBTO) at both 3 and 9 µg/l (unspecified duration) caused fish to choose more exposed areas of the water column, cease predator avoidance behaviours sooner, and have longer latency times before performing antipredator behaviours when shown a dummy heron bill (Wibe et al., 2001). Similarly, Webber and Haines (2003) found that golden shiners (*Notemigonus crysoleucas*) exposed to dietary methylmercury (0.959 µg/g, but not 0.455 µg/g, for 90 days) showed delayed reforming of groups after being shown a model kingfisher.

#### 2.1.3. Locomotory ability to escape predation

Although toxicant exposure alters the immediate behavioural responses of prey fish to cues of predation risk, either by disrupting sensory perception of a predator or the motivation to properly respond, it has also been shown that some toxicants disrupt the ability of fish to escape from a predator in the later stages of a predation event. For example, exposure of juvenile guppies (*Poecilia reticulata*) to pentachlorophenol for 1–4 weeks at 500 and 700 µg/l decreased the number of necessary strikes performed by largemouth bass (*Micropterus salmoides*) to catch prey, the required number of pursuits, and the pursuit time com-

pared to prey exposed to lower concentrations (Brown et al., 1985). These results are supported by numerous studies showing that toxicants can disrupt startle responses (Carlson et al., 1998) and the swimming performance and activity of prey fish (e.g. Little et al., 1990; Weis and Weis, 1995; Zhou and Weis, 1998, 1999), which has been previously reviewed (Little and Finger, 1990).

#### 2.1.4. Schooling behaviours

Fish schooling decreases prey susceptibility to predation through two interacting mechanisms (Moyle and Cech, 2000). The dilution effect reduces the probability that any one individual in a group will be eaten, and the confusion effect makes it more difficult for a visually oriented predator to capture any one particular fish. If toxicant exposure disrupts the properties of a school, such as by decreasing group cohesion or parallel swimming, or increasing collisions, prey fish within a school may suffer from increased predation. Larval exposure of mummichogs (*Fundulus heteroclitus*) to 10 µg/l methylmercury increased the frequency of collisions within schools of larvae (Ososkov and Weis, 1996). Webber and Haines (2003) showed that golden shiners fed a mercury-contaminated diet (0.959 µg/g for 90 days) were hyperactive and had a reduced group cohesion following visual detection of a predator. Similar results have also been observed after exposure to the organic pollutants, DDT (dichlorodiphenyltrichloroethane) (Weis and Weis, 1974b) and carbaryl (Weis and Weis, 1974a).

Alternatively, toxicants may increase the occurrence of unnecessary schooling, possibly decreasing the time available for other essential activities (e.g. foraging, etc.). Embryonic exposure of mummichogs to 5 and 10 µg/l methylmercury increased parallel swimming in larvae schools (Ososkov and Weis, 1996). Copper exposure in 5 h pulses peaking at approximately 100 µg/l also increased group cohesion in Atlantic silverside (*Menidia menidia*) schools, though they also became hyperactive (Koltes, 1985). The above results suggest that the effects of toxicants on schooling behaviours are variable, possibly depending on fish species, toxicant, and exposure conditions; however, it is clear that some toxicants may decrease the effective function of prey schools as a predation avoidance strategy.

## 2.2. Reproductive behaviour

Successful reproduction in fish requires the performance of several different behaviours. These include spawning site selection, territorial defence of spawning site, nest building, courtship and spawning, and post-fertilization investment (such as nest cleaning, guarding, and fanning behaviours) (reviewed by Potts, 1984). Toxicant exposure could potentially disrupt the effective performance of behaviours associated with all reproductive stages (Table 3), and could thus decrease reproductive success. In particular, alterations in the timing or occurrence of appropriate reproductive behaviours could disrupt mate selection, successful fertilization, or survival of offspring in a natural setting.

#### 2.2.1. Nest building, spawning, and courtship behaviours

Lead and mercury are the only trace metals that so far have been shown to disrupt nest building, spawning, or courtship behaviours of fish. Weber (1993) exposed male-female pairs of fathead minnows (*Pimephales promelas*) for 30 days to 500 µg/l lead and observed longer periods between spawning episodes and less frequent performance of behaviours associated with nest preparation by males than in controls. Similarly, spawning onset was later in minnows exposed to dietary methylmercury (MeHg) until sexual maturity, occurring at concentrations at and above 0.88 µg/g food (Hammerschmidt et al., 2002).

Unlike metals, many organic pollutants influence the reproductive behaviour of fish, some of which are known endocrine disruptors (exogenous chemicals having hormone-like effects). Exposure to 1.0 µg/l esfenvalerate (pyrethroid insecticide) and 0.6 µg/l endosulfan delayed the onset of reproductive behaviours in adult bluegill (*Lepomis macrochirus*) and tropical cichlids (*Sarotherodon mossambicus*), respectively (Matthiessen and Logan, 1984; Tanner and Knuth, 1996), and esfenvalerate subsequently decreased larval offspring survival. Not only do organic pollutants alter the timing of reproductive behaviours, some contaminants have been shown to disrupt the actual performance of reproductive behaviours. For example, Gray et al. (1999) exposed Japanese medaka (*Oryzias latipes*) from one day post-hatch for 6 months to 4-tert-octylphenol, which decreased courtship activity



(approaches and circling of females) in males exposed to at least 25 µg/l, and decreased copulations by males exposed to at least 50 µg/l. Similar results have also been observed after male guppies were exposed to either lindane ( $\gamma$ -HCH), phenol, vinclozolin, *p,p'*-DDE (1,1-dichloro-2,2'-bis(*p*-chlorophenyl)ethane; principal DDT metabolite), flutamide (commercial antiandrogen), or municipal wastewater (Colgan et al., 1982; Schröder and Peters, 1988a,b; Baatrup and Junge, 2001) (see Table 3 for details).

Exogenous estradiols also disrupt reproductive behaviour of fish. Bjerselius et al. (2001) exposed male goldfish to both dietary and waterborne 17 $\beta$ -estradiol for 24–28 days during the spawning period, and observed that at LOECs of 10 µg/g dietary or 1 µg/l waterborne, males performed fewer following, pushing, and spawning behaviours. Similarly, both waterborne and dietary exposure to 17 $\beta$ -estradiol reduced male courtship and spawning behaviours in goldfish and Japanese medaka, respectively (Table 3) (Schoenfuss et al., 2002; Oshima et al., 2003).

### 2.2.2. Spawning site selection and natal homing

A few studies have shown that spawning site selection may also be affected by toxicant exposure. For many fish species, homing to natal streams is an important aspect of their reproductive life histories. Baker and Montgomery (2001) tested attraction of banded kokopu (*Galaxius fasciatus*) to adult migratory pheromones before and 48 h after exposure to cadmium. Attraction was eliminated after cadmium exposure of 0.5 and 1.0 µg/l, compared to fish exposed to 0 and 0.1 µg/l who showed an eight-fold preference for pheromone labelled flows. This disruption was reversible, since 14 days recovery in clean water restored normal behaviour. In two similar studies, copper exposure eliminated rainbow trout preference for rearing water (Table 3), to which unexposed fish showed nearly three-fold preference (Saucier et al., 1991; Saucier and Astic, 1995). Ten weeks post-exposure, normal preference behaviour returned. As well as simple preference/avoidance experiments, Scholz et al. (2000) demonstrated that the homing ability of chinook salmon was disrupted after 24 h diazinon exposure at 10 µg/l. Only 40% of exposed salmon transported 2 km downstream returned to their natal hatchery, whereas nearly 100% of unexposed fish returned. This was unlikely to be a result

of mortality, since this exposure concentration is not lethal to salmonids (see Table 1).

### 2.3. Social behaviour

Many types of social interaction, such as schooling and courtship, have been previously discussed in the context of predator avoidance and reproductive behaviours. The effect of environmental pollutants on other forms of social behaviour have also been studied in fish, in particular those social behaviours associated with territoriality and dominance. Fish dominance hierarchies form primarily through intraspecific competition for finite resources (e.g. food, shelter) (Chapman, 1966), a primary result being that socially dominant animals defend optimal foraging positions and attain the highest growth rates (Li and Brocksen, 1977; Fausch, 1984; Metcalfe, 1986; Grant et al., 1989). In addition, modelled dominance hierarchies have been shown to promote population stability and greater exploitation of food resources provided the rewards of social dominance are not too extreme (Gurney and Nisbet, 1979). Studies of fish social behaviour occur principally in a laboratory setting, where the interactions of pairs or larger groups of fish are observed in surroundings ranging from simple aquaria to lentic or lotic environments simulating natural ecosystems (Sloman and Armstrong, 2002).

#### 2.3.1. Agonistic acts

Perhaps the most simple and frequently measured indicator of altered social relations in fish exposed to toxicants is an altered frequency of agonistic acts, such as threats, nips, or chases (Table 4). Henry and Atchison (1979a; 1979b; 1986) have explored the effects of both copper and combinations of cadmium and zinc on the occurrence of agonistic acts within a dominance hierarchy of bluegill. At concentrations above 40 µg/l cadmium and 124 µg/l zinc in combination for 15 days, the frequency of nips within groups was observed to decrease, while in contrast, 34 µg/l copper for 96 h increased the frequency of agonistic acts. In both cases, there were differential changes between fish of different social status such that agonistic acts by dominant fish became more common at higher toxicant concentrations while those performed by more subordinate fish were less common. Nickel (Alkalem, 1994), prochloraz (an imidazole fungicide)

(Saglio et al., 2001), carbofuran (a carbamate insecticide) (Saglio et al., 1996), esfenvalerate (Little et al., 1993), and methyl parathion (Welsh and Hanselka, 1972) exposure also increased the overall occurrence of total agonistic behaviours amongst groups of fish, though no observation of individuals of different social status was undertaken (Table 4).

Agonistic encounters between pairs of fish are also altered by toxicant exposure. Sloman et al. (2003b) observed the number of agonistic acts between pairs of juvenile rainbow trout during the initial formation of dominance, after 24 h exposure to one of five trace metals (cadmium, copper, nickel, lead, or zinc, at concentrations 15% of their 96 h LC50s). Exposure to 3 µg/l cadmium significantly reduced the total number of attacks, while no similar effect was observed for any of the other metals. Johnsson et al. (2003) observed reduced territorial aggression in newly hatched rainbow trout fry exposed to pulp mill effluent for 4 weeks. Using the response of fry to their own mirror image (mimicking a paired encounter) they demonstrated that fish exposed to 50% pulp mill effluent spent less time interacting towards the mirror and stayed further away from the mirror than control fish.

### 2.3.2. Formation and maintenance of hierarchies

Through alterations to the occurrence of agonistic acts between fish, it is likely that exposure to many toxicants threatens the formation and/or stability of dominance relationships (Table 4). Sloman et al. (2003b) exposed juvenile rainbow trout to 3 µg/l cadmium for 24 h and subsequently observed these fish to be unable to dominate over unexposed juveniles. Furthermore, the inability of cadmium-exposed fish to become dominant persisted for up to 3 days after exposure (Sloman et al., 2003c). Exposure to 2 µg/l cadmium also disrupted the timing of dominance hierarchy formation among groups of trout in a simulated stream environment, with cadmium-exposed social groups forming stable dominance hierarchies faster than controls (Sloman et al., 2003c). In contrast, pre-established dominance hierarchies were unaffected by 7 days cadmium exposure at the same concentration. Seven days of exposure to 30 µg/l copper (Sloman et al., 2002) did not affect pre-established hierarchy stability either. Therefore, the stability of pre-established dominance hierarchies appears more resistant to toxicant exposure than dominance hierarchy formation. This

does not imply, however, that pre-established dominance hierarchies are unaffected by toxicant exposure. Symons (1973) exposed groups of Atlantic salmon (*Salmo salar*) to the organophosphate, fenitrothion, in artificial streams and observed the number of fish holding territories to be reduced for at least 7 days after exposure, indicating that the pre-established social structure was altered (Table 4).

Toxicant exposure disrupts the social relations of fish with agonistic behaviours increasing in many cases, while decreasing in others. Effects of toxicants are likely dependent on many factors, including toxicant, exposure condition, fish species, and whether social structures are pre-established or newly forming. Regardless, disruption to the ability or motivation of fish to perform agonistic behaviours is likely to alter dominance hierarchies. This may result from an inhibited ability of exposed fish to compete with unexposed fish or from disturbances to competitive interactions among exposed groups of fish.

### 3. Possible physiological mechanisms underlying behavioural alteration

The complex behaviours that are necessary for survival are a product of the integration of many physiological systems. Sensory, hormonal, neurological, and metabolic systems all contribute to the performance of these behaviours (see Fig. 1). The effects of aquatic pollutants on complex behaviours of fish are likely caused by interference with a combination of these systems, and so the impacts of pollutants on all of them needs to be considered. Understanding which physiological systems are disturbed during toxicant exposure aids in interpreting behavioural changes. There exists difficulty, however, in correlating the results of separate behavioural and physiological studies, since effects differ as a function of fish species, toxicant, and exposure condition. Therefore, we will not attempt an exhaustive review of this literature and their LOECs, but will merely highlight some areas of previous research most relevant to the discussion of behavioural indicators of toxicity. Toxicological effects on each physiological system deserves more detailed evaluations elsewhere, which in some cases have already been accomplished (e.g. Blaxter and Hallers-Tjabbes, 1992; Hontela, 1998).

### 3.1. Sensory disruption

Chemoreception is an important contributor allowing fish to respond to their environment, and olfaction is believed to be the predominant chemical sense in fish (Hara, 1986); indeed, predator avoidance, reproduction, and dominance behaviours all involve olfaction to some extent (Chivers and Smith, 1993; Moore and Waring, 1996b; Griffiths and Armstrong, 2000). Literature concerning the impacts of toxicants on the olfactory system has been reviewed numerous times (e.g. Rehnberg and Schreck, 1986; Døving, 1991; Blaxter and Hallers-Tjabbes, 1992; Tjälve and Henriksson, 1999), and the majority of previous research has focused on the effects of trace metals. Indeed, many metals have been shown to enter the olfactory system of fish (reviewed by Arvidson, 1994; Tjälve and Henriksson, 1999), where they can potentially cause cell death or disrupt normal olfactory function. Cadmium has been extensively studied in this regard, and readily accumulates throughout the olfactory system of several species (Tjälve and Gottofrey, 1986; Gottofrey and Tjälve, 1991; Evans and Hastings, 1992; Tallkvist et al., 2002; Scott et al., 2003). Studies with cadmium suggest that toxicants often move along olfactory system neurons by axonal transport mechanisms (Gottofrey and Tjälve, 1991; Tallkvist et al., 2002), though the ability to leave primary neurons of the olfactory system, crossing synapses in the olfactory bulb to enter the rest of the brain, is toxicant specific. Cadmium (Evans and Hastings, 1992) and nickel (Tallkvist et al., 1998) are restricted to primary neurons of the olfactory system. Manganese (Rouleau et al., 1995) and mercury (Borg-Neczak and Tjälve, 1996; Rouleau et al., 1999) can enter the rest of the brain either by crossing the olfactory bulb synapses or traversing the blood–brain barrier. Furthermore, some toxicants may not enter olfactory neurons, but other components of the olfactory system. Indeed, copper accumulation in fish has been localized to the melanosomes of the lamina propria (Julliard et al., 1995). After entering the olfactory system, some toxicants cause cell death or sublethal damage of the olfactory system. There appears, however, to be different mechanisms of toxicant-induced cell death, with some toxicant exposures inducing necrotic cell death (Stromberg et al., 1983; Saucier and Astic, 1995) and others causing programmed

apoptotic cell death (Julliard et al., 1996). These two scenarios could result in different functional consequences of toxicant exposure, though this has yet to be verified.

By accumulating in cells of the olfactory system and subsequently causing cell damage or death, toxicants can disrupt electrical transmission of sensory information from the olfactory epithelium to higher levels of the brain. Hara et al. (1976) found that exposure of rainbow trout olfactory pits to copper and mercury decreased bulbar electrical responses to odourants over a 2 h period. Similar results have been observed after acute exposures to copper (Winberg et al., 1992), mercury (Baatrap et al., 1990), and silver (Brown et al., 1982). Over longer durations (1 and 2 weeks), waterborne exposure to cadmium, copper, or mercury reduced bulbar responses by 20–42% in rainbow trout (Brown et al., 1982). In addition to trace metals, several pesticides disrupt electrical transmission within the olfactory system of fish. Moore and Waring (1996a, 1998; Waring and Moore, 1997) exposed the olfactory epithelium of male Atlantic salmon parr to atrazine, diazinon, or carbofuran and measured the electro-olfactogram (EOG) response to the female priming pheromone, prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ). Acute exposure to all three pesticides reduced the EOG response, which correlated with decreased expressible milt and plasma testosterone in exposed males who were subjected to  $PGF_{2\alpha}$ .

### 3.2. Endocrine disruption

Many studies have shown important interrelationships between hormones and behaviour (e.g. Oliveira et al., 1996; Contreras-Sánchez et al., 1998; Pottinger and Carrick, 2001; Sloman et al., 2001; Øverli et al., 2002), so it is likely that disruption of hormonal status will have behavioural consequences. Endocrine disruption can be due to toxicants agonizing or antagonizing endogenous hormones, or disrupting the synthesis or metabolism of endogenous hormones and their receptors (Sonnenschein and Soto, 1998). As discussed above, many chemical pollutants exist that act as agonists or antagonists to naturally-produced hormones in fish, in particular those affecting reproductive hormonal systems. As a consequence, exposure to  $17\beta$ -estradiol, for example, has been shown to reduce milt production and gonadosomal index (Bjerselius et al.,

2001; Chang and Lin, 1998), and decreased fecundity (Oshima et al., 2003). Similarly, flutamide, vinclozolin, and *p,p'*-DDE have been observed to decrease sperm counts and/or gonadosomal index (Baatrap and Junge, 2001). Not surprisingly, exposure to exogenous sex steroids and other steroid mimics have been shown to disturb levels of endogenous reproductive hormones (Singh and Singh, 1992; Trudeau et al., 1993; Chang and Lin, 1998; Tilton et al., 2002), and can alter the activities of enzymes important for endogenous hormone synthesis (Chang and Lin, 1998; Melo and Ramsdell, 2001).

Tollefsen (2002) has provided evidence for a possible mechanism through which hormone agonists/antagonists might disrupt the function of endogenous hormones. Several estrogen mimics were shown to displace radiolabelled estrogen from plasma sex steroid-binding protein in a dose-dependent and competitive manner. Therefore, agonists may interact with excess binding protein to augment normal sex steroid effects, and antagonists may displace endogenous hormone if sufficiently abundant. In addition to agonism/antagonism of endogenous hormones, some toxicants disrupt synthesis of hormone receptors. For example, cadmium decreases the estradiol-stimulated transcriptional activity of the rainbow trout estradiol receptor (Le Guével et al., 2000).

Many metal and organic compounds have been shown to disrupt the hypothalamo-pituitary-interrenal (HPI) axis that controls the cortisol response to stress, which may consequently alter normal fish behaviour. Toxicants have been shown to disrupt plasma cortisol levels either at rest (Fu et al., 1990; Pratap and Wendelaar Bonga, 1990; Tort et al., 1996; Brodeur et al., 1998; Aldegunde et al., 1999) or in response to environmental stressors (Hontela et al., 1995; Brodeur et al., 1997b; Norris et al., 1999; Laflamme et al., 2000; Quabius et al., 2000; Scott et al., 2003). Mechanisms of disruption vary; disruption of steroid synthesis in interrenal cells, those cells responsible for cortisol synthesis and secretion into the blood (Brodeur et al., 1997a,b, 1998; Girard et al., 1998; Leblond and Hontela, 1999; Benguira and Hontela, 2000), and disturbance of signalling systems upstream of cortisol production and secretion (e.g. plasma adrenocorticotrophic hormone mobilization; Quabius et al., 2000) have both been observed.

In addition to their effects on stress hormone physiology, some toxicants have also been shown to disrupt other hormone systems. Growth hormone and thyroid hormone systems both have important implications for fish behaviour (e.g. Comeau et al., 2001; Devlin et al., 2001), so disruption of either physiological system could result in behavioural toxicity. For example, cadmium exposure during development delays growth hormone mRNA expression in rainbow trout until late stages of larval development (Jones et al., 2001). Exposure to numerous pollutants has altered both thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) levels in plasma of several fish species after both acute (endosulfan; Sinha et al., 1991) and chronic exposure (polluted habitats; Hontela et al., 1995; Zhou et al., 1999, 2000). Effects of different contaminants are not consistent, however, indicating that consequences of toxicant exposure may arise from different absolute or relative concentrations of pollutants or species differences, but this remains unclear.

### 3.3. Neurological dysfunction

Brain neurotransmitter levels and enzyme function correlate with behavioural states (Alanärä et al., 1998; Elofsson et al., 2000; Hofmann and Fernald, 2000; Höglund et al., 2001), so it is likely that neurological dysfunction induced by toxicant exposure results in behavioural changes. One of the most commonly observed indicators of altered neural function is brain cholinesterase (ChE) activity, which is frequently used as an indirect measure of acetylcholinesterase (AChE) activity. AChE is located on post-synaptic membranes and is responsible for degrading the neurotransmitter, acetylcholine, to end cholinergic neural transmission. Organophosphates previously shown to inhibit brain ChE activity in fish include azinphosmethyl (Cochran and Burnett, 1996), chlorpyrifos (Phillips et al., 2002; Sandahl and Jenkins, 2002), 2,2-dichlorovinyl dimethyl phosphate (DDVP; Pavlov et al., 1992; Chuiko, 2000), fenitrothion (Sancho et al., 1997a), diazinon, malathion (Beauvais et al., 2000; Brewer et al., 2001), and methidathion (Bálint et al., 1995). Several carbamate pesticides have also been shown to inhibit brain ChE activity, including aldicarb (Perkins and Schlenk, 2000), carbofuran, diuron, nicosulfuron (Bretaud et al., 2000), carbaryl (Beauvais et al., 2001), and thiobencarb (Sancho et al., 2000;

Fernández-Vega et al., 2002). Similar results have been observed in natural ecosystems polluted with organophosphates, carbamates, and other pollutants (Gruber and Munn, 1998; de la Torre et al., 2002). In addition to cholinesterase activity, toxicants can disrupt physiological function at cholinergic synapses by altering cholinergic receptor number. Permethrin, 4-nonylphenol, and copper all decrease muscarinic cholinergic receptor numbers in the brains of several different fish species, though the mechanisms of action remain unclear (Jones et al., 1998).

Brain neurotransmitter levels may also be disrupted by toxicant exposure. Brain serotonin and/or dopamine levels were reduced by copper (De Boeck et al., 1995), mercury (Smith et al., 1995), or lindane (Aldegunde et al., 1999) exposure in several different fish species, while in contrast serotonin and norepinephrine increased after lead exposure in fathead minnows (Weber et al., 1991). Activities of other brain enzymes involved in general cellular functions apart from neurotransmission can also be altered by toxicant exposure. For example, aluminium decreases catalase activity in the brains of murels (*Channa punctatus*) (Jena et al., 2002) and methylmercury alters several anti-oxidant enzyme activities in Atlantic salmon (Berntssen et al., 2003), possibly making the brain more vulnerable to oxidative damage by reactive oxygen species. It has yet to be determined if these effects occur in other organs as well.

Toxicants can affect electrophysiological properties of the brain. Mauthner cells in the hindbrain receive information from several sensory systems and transmit electrical signals through the periphery via motoneurons to lateral white muscle. Carlson et al. (1998) demonstrated that carbaryl and phenol disrupt Mauthner cell to motoneuron transmission, while chlorpyrifos, carbaryl, phenol, and 2,4-dinitrophenol have downstream neuromuscular effects. It is likely that through changes in brain function, including alterations to cholinesterase activity, neurotransmitter levels, enzyme function, or electrophysiological properties, exposure to aquatic pollutants has the potential to alter numerous different behavioural systems of fish.

### 3.4. Metabolic disruption

Metabolism of fish is influenced by many different physiological functions. Metabolic dysfunction

caused by aquatic pollutants could consequently occur due to disruption at many different physiological levels. Furthermore, metabolism is intimately associated with fish behavioural state (e.g. Haller, 1991; Lebedeva et al., 1993; Haller et al., 1996; Alanärä et al., 1998; Sloman et al., 2000), so disruption of normal metabolic processes could have profound influences on normal fish behaviour. Whole organism metabolic disruption has been indicated in a number of different ways, most commonly illustrated by altered resting metabolic rates (i.e. oxygen consumption or ventilation rate) or lowered swim performance (i.e. critical swimming speed,  $U_{crit}$ ). For example, trace metals, copper and cadmium, and the organochlorine pesticide, dieldrin, have all been shown to alter one of these variables (Beyers et al., 1999; McGeer et al., 2000; Rajotte and Couture, 2002). Even if toxicant exposure does not alter metabolic rate, metabolic tradeoffs may exist between detoxification and other normal processes. Handy et al. (1999) chronically exposed rainbow trout to dietary copper and measured metabolic rate and swimming behaviour. Although oxygen consumption, ventilation rates, and serum chemistry (glucose, triglycerides, protein, ions) were unaffected by copper exposure, trout spent significantly less time performing routine swimming activities, suggesting a sequestration of metabolic resources for acclimation to the toxicant.

Several toxicants have been shown to alter levels of metabolic substrates. This is likely to have implications for numerous fish behaviours, since energy availability and requirements influences optimal foraging strategies. The storage or mobilization of metabolic substrates such as glucose, glycogen, lactate, lipid, and protein are disrupted by exposure to several trace metals, including cadmium (Cattani et al., 1996; Soengas et al., 1996; Lyons-Alcantara et al., 1998; Ricard et al., 1998; Almeida et al., 2001; Berntssen and Lundebye, 2001; De Smet and Blust, 2001; Fabbri et al., 2003), manganese (Barnhoorn et al., 1999), nickel (Sreedevi et al., 1992), and metal mixtures in a polluted habitat (Levesque et al., 2002). Similar effects have been observed in fish exposed to pesticides, including endosulfan (Gimeno et al., 1994; Gimeno et al., 1995), carbaryl (Jyothi and Narayan, 1999), carbofuran (Singh and Sharma, 1998), the organophosphates, azinphosmethyl (Oruç and Üner, 1998), fenitrothion (Sancho et al., 1997b; Sancho

et al., 1998), and phorate (Jyothi and Narayan, 1999), the pyrethroid, deltamethrin (Bálint et al., 1995), and the herbicide 2,4-diamin (Oruç and Üner, 1999).

The specific mechanisms by which many of the above toxicants alter metabolic substrate availability are unclear, but may be the result of toxicant effects on metabolic enzyme abundance or activity. Enzymes in carbohydrate metabolism pathways are frequently disrupted by pollutant exposure. In most cases aerobic carbohydrate catabolism increases, possibly to facilitate the added metabolic cost of detoxification. Cadmium exposure affects enzymes regulating carbohydrate metabolism in Atlantic salmon, decreasing glycogen synthetase/phosphorylase activity ratios and increasing phosphofructokinase (PFK) activity in the liver, therefore activating carbohydrate catabolism (Soengas et al., 1996). Increases in the activity of several glycolytic enzymes have also been observed in yellow perch (*Perca flavescens*) from a metal-contaminated habitat (Levesque et al., 2002). However, effects on metabolic enzymes are likely to be toxicant and/or tissue specific; for example, carbofuran exposure decreases lactate dehydrogenase (LDH) activity in the muscle, brain, and liver of catfish (Singh and Sharma, 1998). The effect of cadmium on Nile tilapia muscle enzyme activities was dependent on fibre type. PFK, LDH, and creatine kinase activities decreased in white muscle following cadmium exposure, whereas PFK and LDH activities increased in red muscle (Almeida et al., 2001).

Enzymes involved in protein metabolism are also disrupted by toxicant exposure. Aspartate aminotransferase and alanine aminotransferase activities increase in the gills, liver, and kidney of carp exposed to cadmium (De Smet and Blust, 2001), and in the gills and kidney of carp exposed to sublethal nickel (Sreedevi et al., 1992). Accompanied by increased free amino acids in the above studies, increased aminotransferase activities suggest a shift towards catabolic pathways of protein metabolism, possibly favouring mobilization of energy reserves for detoxification. Exposure of the cichlid, *S. mossambicus*, to carbaryl also increased the activity of aminotransferase enzymes (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) in liver, muscle, and brain, though no measurement of free amino acids was made (Ramaswamy et al., 1999). Toxicant exposure may also favour protein anabolism, however. Sublethal

nickel exposure increased glutamate dehydrogenase activity in the gills and kidney of carp, while decreasing ammonia and urea levels, possibly indicating synthesis of amino acids for protein repair (Sreedevi et al., 1992).

Toxicants disrupt various aspects of metabolism in fish, from whole-organism responses (e.g. metabolic rate and swim performance) to tissue responses (e.g. metabolic substrate availability and enzyme activity). By altering metabolism and thus food requirements and assimilation in fish, aquatic toxicants could alter optimal foraging strategies, which could have potential implications for numerous aspects of fish behaviour.

#### 4. Integrating behavioural and physiological toxicity

There are many ways that behaviour and physiology can interact to affect aquatic toxicology. A few studies have shown correlations between behavioural and physiological indicators of toxicity and have therefore succeeded in eliminating the complicating effects faced when comparing different behavioural and physiological studies. Furthermore, in some cases causative evidence for the underlying physiological mechanisms of behavioural toxicity has been presented. Unfortunately, few studies acknowledge that the behaviour of fish can in turn alter physiological susceptibility to toxicants. Fewer still consider the implications of aquatic toxicology, in the context of behavioural and physiological interactions, for population survival. In this section, we will review studies that have integrated both behavioural and physiological indicators of toxicant exposure, as well as research linking these indicators to ecologically significant fish population effects.

A frequently studied physiological correlate of behavioural change is brain cholinesterase activity. Beauvais et al. (2001) found that exposure of rainbow trout to carbaryl decreased brain ChE activity and this was correlated with decreased swimming behaviour. In the same study, exposure to cadmium did not affect brain ChE activity or swimming speed. Beauvais et al. (2000) and Brewer et al. (2001) also found a correlation between changes in swimming speed and brain ChE activity induced by exposure to diazinon and malathion. A correlation between the interference of the organophosphate, DDVP, on feeding behaviour

and brain acetylcholinesterase was demonstrated in the bream (*Abramis brama*) by Pavlov et al. (1992). Injection of atropine, which is known to counteract the effects of DDVP by antagonizing muscarinic cholinergic receptors, restored feeding behaviour and brain AChE activity. There may, however, exist different behavioural correlates if ChE activity is assayed in different tissues. Roex et al. (2003) observed that whole-body acetylcholinesterase inhibition by parathion correlated to increased food consumption in zebrafish (*Danio rerio*), which the authors attributed to hyperactivity. This effect of parathion was at least partly specific, since another physiological trait, female egg production, was unaffected by exposure. Obviously, behavioural alteration does not always coincide with AChE inhibition; Webber and Haines (2003) demonstrated an effect of methylmercury on predator avoidance in golden shiner which did not appear to be related to brain AChE activity.

Contaminant-induced changes in brain monoamines may link behaviour and physiology. Weber et al. (1991) exposed fathead minnows to lead and found the resulting increase in body lead burden to be accompanied by elevated brain serotonin and epinephrine. The authors hypothesized that these changes in brain neurotransmitters were associated with the changes in socially-facilitated feeding behaviour also recorded in this study. In contradiction, Smith et al. (1995) detected decreased serotonin and its metabolite 5-hydroxyindoleacetic acid in the medulla of mummichogs from a polluted site, in conjunction with a decreased ability to prey on grass shrimp (*Palaemonetes pugio*). It is noted, however, that the links between these two indicators of toxicity may be obscured by the absolute measurement of brain monoamines, rather than rates of turnover and synthesis, which are likely more relevant to fish behaviour. Interestingly, in murrels exposed to carbofuran, disruption of brain dopamine, norepinephrine, and serotonin levels was localized to the region of the brain regulating motor activity and behaviour, suggesting preferential alteration of swimming behaviours (Gopal and Ram, 1995).

Links between behaviour and electrophysiology were addressed by Carlson et al. (1998) on medaka. They found that medaka appeared to be more susceptible to predation (measured as startle and escape response) after exposure to chlorpyrifos, carbaryl,

fenvalerate, endosulfan, phenol, 1-octanol and 2,4-dinitrophenol. By externally measuring biopotentials created by small juveniles near microelectrode grids, time to onset of neural and muscular activity could be inferred from the recorded electrical activity. Carbaryl and phenol affected Mauthner cell to motoneuron transmission while chlorpyrifos, carbaryl, phenol and 2,4-dinitrophenol showed neuromuscular effects, and both neurological mechanisms could explain the changes in predation susceptibility; behavioural and neurophysiological endpoints used had similar LOECs.

Some studies have aimed to link toxicant-induced behavioural changes to changes in endogenous hormone levels. In a suite of studies, Zhou and colleagues examined the behaviour and physiology of mummichogs from a site polluted with a mixture of organic and inorganic compounds. One month old larval mummichogs from this polluted site were more vulnerable to predation (Zhou and Weis, 1999) and adults had larger thyroid follicles, greater follicle epithelia cell heights and increased plasma T<sub>4</sub> levels (Zhou et al., 1999). It is hypothesised that the impairment of thyroid function induced by environmental contamination is in part responsible for behavioural differences. However, no experimental manipulations were carried out to investigate the strength of this correlation.

Bell (2001) found a correlation between plasma gonadal steroids and behaviour, demonstrating a negative correlation in male three-spined sticklebacks between nesting behaviour and 11-ketotestosterone and a positive correlation between courtship behaviour and estradiol. Based on these correlations between behaviour and hormonal status, the effects of exogenous ethynyl estradiol on aggressive behaviour were assumed to be elicited by changes in endogenous hormone levels. Bjerselius et al. (2001) exposed male goldfish to exogenous 17 $\beta$ -estradiol, which inhibited courting frequency and spawning acts for concentrations at and above 10  $\mu$ g/g dietary and 1  $\mu$ g/l waterborne. Furthermore, at the same concentrations, this behavioural alteration correlated with depressed gonadosomatic index and elevated plasma estradiol (two to eight-fold above controls). The authors suggest that the altered sexual behaviour seen in male goldfish is a result of decreased androgen production caused by effects of estradiol on the testes.

The putative link between behavioural changes in response to toxicants and impaired olfactory ability has been addressed by a few researchers by measuring both behavioural disruptions and physiological changes in the olfactory system. [Rehnberg and Schreck \(1986\)](#) showed a depressed avoidance of the amino acid L-serine, a potent odour to fish, by coho salmon exposed to copper and mercury. While they found no inhibitory effects of copper on the binding of serine to the olfactory epithelium, mercury clearly inhibited serine binding. [Beyers and Farmer \(2001\)](#) demonstrated a disruption of the behavioural response of Colorado pike minnow to alarm substance by waterborne copper. The authors also provided correlative evidence that behavioural alteration is due to olfactory impairment as the occurrence of ciliated olfactory receptor cells was reduced during copper exposure. [Scott et al. \(2003\)](#) found that exposure to 2 µg/l cadmium for 7 days impaired the normal antipredator behaviour of juvenile rainbow trout exhibited in response to alarm substance, which was accompanied by a significant accumulation of cadmium in the olfactory system during waterborne exposure. To further investigate the potential link between cadmium accumulation in the olfactory system and changes in behaviour, [Scott et al. \(2003\)](#) demonstrated that behavioural impairment was not associated with dietary cadmium exposure (at the same whole-body cadmium burden as waterborne exposure). During dietary exposure, cadmium would not be able to enter the olfactory system, further suggesting a direct effect of cadmium on olfactory function.

The behaviour of fish may also influence their susceptibility to toxicants, mediated by the effects of behaviour on physiology. Social behaviour, as discussed above, can be altered by aquatic toxicants. In addition, social status of fish can impact upon the physiological condition of individuals ([Sloman and Armstrong, 2002](#)). Recently, [Sloman et al. \(2003a\)](#) demonstrated that the increased sodium uptake rates induced in subordinate fish as a result of their submissive behavioural status results in an increased uptake of both copper and silver during waterborne exposure. It is also possible that more dominant fish would be exposed to a greater concentration of dietary contaminants as they would consume a larger proportion of available food.

Perhaps the most important, and arguably the hardest, concept to address is the implications of these alterations in interrelated changes in behaviour and

physiology, brought about by aquatic pollutants, for fish populations. Few studies take this step. The easiest predictor of population effect to measure is reproductive success (reviewed by [Jones and Reynolds, 1997](#)). [Weber \(1993\)](#) measured behavioural and physiological changes in fathead minnows exposed to lead in relation to their reproductive success, and observed increased lead levels in testes and ova, correlated to suppressed spermatocyte production and retarded ovarian development. As a result, decreased numbers of eggs were oviposited, longer durations existed between spawning events, and embryo development was suppressed. These changes in the integration of specific behavioural acts that normally help ensure reproductive success, as well as alterations in the physiology of the fish, together resulted in decreased reproductive fitness and thus threatened population stability.

Fathead minnows fed dietary methylmercury displayed delayed spawning where days to spawning was positively correlated with concentration of total carcass mercury ([Hammerschmidt et al., 2002](#)). Gonadosomatic index and reproductive effort of female fish were inversely correlated with carcass mercury but development and hatching success of embryos and larvae were unrelated to parental mercury burden. Delayed spawning has consequences for population survival as many fishes reproduce at a set time per year. Disruption of the timing of endogenous to exogenous feeding of young can result in decreased growth and increased susceptibility of young-of-the-year fish to predation. In a more detailed study, [Matta et al. \(2001\)](#) investigated the effect of dietary methylmercury on the reproduction and survival of exposed killifish, the survival of offspring and their subsequent reproduction, and the hatching and survival of the second generation. By altering male behaviour, increasing aggression in some fish and lethargy in others, mercury resulted in a greater mortality of male fish. Offspring of exposed fish were less able to reproduce successfully and displayed altered sex ratios. Combined physiological and behavioural changes resulted in adverse effects on transgenerational reproduction that had the potential to impact fish populations.

Similar studies on the population implications for alterations in predator/prey interactions and dominance hierarchies are to our knowledge lacking. Evidence suggests that alterations in these behaviours, coupled with changes in physiology would alter pop-



ulation stability. Even a small variation in mortality can have a major impact on recruitment (Houde, 1987) so increased susceptibility to predation induced by aquatic toxicants could affect recruitment of prey fish populations. Dominance hierarchies are believed to add stability to populations (Gurney and Nisbet, 1979) and therefore alterations in these structures could also have population effects. However, it is becoming increasingly obvious that future studies are needed to address interactions between behaviour, physiology and toxicology and the implications of these interactions for population survival.

## 5. Conclusions

The implications of the disruption of complex behaviours by aquatic pollutants for fish populations are only beginning to be realized. Through analysis of sensitive and appropriate behavioural indicators of toxicant exposure, it is our hope that environmental pollutant exposures will be minimized to levels that protect the viability of fish populations. Not only does this involve eliminating mortality, but also preventing alteration to the complex behaviours that provide the foundation for fish population structure and aquatic communities. Indeed, the literature contains many examples illustrating disruption to predator avoidance, reproductive, and social behaviours caused by toxicant exposure, often at concentrations well below those that cause significant mortality. Although research is beginning to address the impacts of toxicants on complex fish behaviours, future studies should strive to broaden our scope of knowledge. Many common aquatic pollutants are extremely understudied and there is a need for continued exploration of toxicant effects. Furthermore, more research is required concerning the impact of chronic exposure to low toxicant concentrations on fish behaviours. Although difficult to perform, these studies are more environmentally realistic than the acute exposure conditions that have dominated previous research.

Behavioural indicators provide insight into various levels of biological organization, being a result and determinant of molecular, physiological, and ecological aspects of toxicology. Arguably, a better understanding of toxicological effects in natural systems can be attained by integrating behavioural indicators of toxic-

ity with those of other levels. Importantly, studies are beginning to correlate physiological changes induced by toxicant exposure with behavioural disruption, thus providing ecological relevance to physiological measures of toxicity. In particular, neurological, endocrine, and olfactory dysfunction have been correlated with alterations to fish behaviour. Normal differences in behaviour have also been shown to influence physiological aspects of toxicity, such as is the case for fish within a dominance hierarchy. Unfortunately, few studies have sought to correlate integrated changes in behaviour and physiology to fish population effects, and this appears to be where the field of behavioural toxicology is most lacking. Future research should strive towards greater integration of knowledge. As an applied field of biology it is essential that toxicology be multidisciplinary, focussing less on isolated aspects of toxicant exposure.

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