

Assessing variation in the potential susceptibility of fish to pharmaceuticals, considering evolutionary differences in their physiology and ecology

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S1: Life-cycles and Leslie matrix models for model fish species

Supplementary Material Table S1: Fish species with completely sequenced genomes and additional model species representing various taxonomic groups and aquatic environments

Common name	Latin species name	Authority	Family	Order	Infraclass	Class	Super Class	Marine/ Freshwater	Conservation status
Cave fish*	<i>Astyanax mexicanus</i>	De Filippi, 1853	Characidae - charcins & tetras	Characiformes	Teleostei	Actinopterygii – ray-finned fishes	Osteichthyes	Fresh water	IUCN NE Not evaluated
Cod	<i>Gadus morhua</i>	Linnaeus, 1758	Gadidae - codfishes	Gadiformes	Teleostei	Actinopterygii	Osteichthyes	Marine	IUCN VU Vulnerable
Coelacanth	<i>Latimeria chalumnae</i>	Smith, 1939	Coelocanthidae - coelocanths	Coelocanthiformes	-	Saropterygii – lobe-finned fishes	Osteichthyes	Marine	IUCN CR Critically Endangered
Fugu	<i>Takifugu rubripes</i>	Temminck & Schlegel, 1850	Tetraodontidae - pufferfishes	Tetraodontiformes	Teleostei	Actinopterygii	Osteichthyes	Marine	IUCN NE Not evaluated
Medaka	<i>Oryzias latipes</i>	Temminck & Schlegel, 1846	Adrianichthyidae - ricefishes	Beloniformes	Teleostei	Actinopterygii	Osteichthyes	Fresh water	IUCN NE Not evaluated
Platyfish	<i>Xiphophorus maculatus</i>	Günther, 1866	Poeciliidae - live-bearers & swordtails	Cyprodontiformes	Teleostei	Actinopterygii	Osteichthyes	Fresh water	IUCN NE Not evaluated
Sea lamprey	<i>Petromyzon marinus</i>	Linnaeus, 1758	Petromyzontidae - lampreys	Petromyzontiformes	-	Cephalaspidomorphi	Agnatha	Marine – Fresh water	IUCN LC Bern Convention
Spotted gar*	<i>Lepisosteus oculatus</i>	Winchell, 1864	Lepisosteidae – garfishes	Semionotiformes	Holostei	Actinopterygii	Osteichthyes	Marine	IUCN NE Not evaluated
Stickleback	<i>Gasterosteus aculeatus</i>	Linnaeus, 1758	Gasterosteidae - sticklebacks	Gasterosteiformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater - Marine	IUCN LC Least concern
Tetraodon	<i>Tetraodon nigroviridis</i>	Marion de Proce, 1822	Tetraodontidae - pufferfishes	Tetraodontiformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater - Marine	IUCN NE Not evaluated
Tilapia	<i>Oreochromis niloticus</i>	Linnaeus, 1758	Cichlidae - cichlids & tilapias	Perciformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater - Marine	IUCN NE Not evaluated
Zebrafish	<i>Danio rerio</i>	Hamilton, 1822	Cyprinidae - carps & minnows	Cypriniformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater	IUCN LC Least concern
Common carp#	<i>Cyprinus carpio</i>	Linnaeus, 1758	Cyprinidae - carps & minnows	Cypriniformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater	IUCN VU Vulnerable
Fathead minnow#	<i>Pimephales promelas</i>	Rafinesque, 1820	Cyprinidae - carps & minnows	Cypriniformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater	IUCN NE Not evaluated
Rainbow trout#	<i>Oncorhynchus mykiss</i>	Walbaum, 1792	Salmonidae – salmon & trout	Salmoniformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater - Marine	IUCN NE Not evaluated
Rare minnow#	<i>Gobiocypris rarus</i>	Ye & Fu, 1983	Cyprinidae - carp/min	Cypriniformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater	IUCN NE Not evaluated
Roach#	<i>Rutilus rutilus</i>	Linnaeus, 1758	Cyprinidae - carp/min	Cypriniformes	Teleostei	Actinopterygii	Osteichthyes	Freshwater	IUCN LC Least concern

Note * Species fully sequenced, but with incomplete gene builds. # Model species not fully sequenced.

Supplementary Material Table S2: Drug targets orthologs in twelve fully-sequenced fish species with complete gene builds

Supplementary Material Table S3: Sequence similarities (between the human drug targets and their orthologous proteins in twelve fish species) for drugs associated with Anatomical Therapeutic Chemical (ATC) classification codes suggesting direct effects on reproduction

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Supplementary Material Table S4: Multiple sequence alignment of the ligand binding domains of ESR1

	310				δ	δ	\circ	δ	δ		
Human	LTADQMVSA	LDAEPPILYS	EYDPTRPFSE	ASMMGLLTNL	ADRELVHMIN	WAKRVPGFVD					
Tree frog	.E.LI...	ME...V...	.H.S.K.L...	.T...	.K...	.O...					
Zebrafish	MSP..VLLL	.G....AVC.	ROKHS..YT.	IT..S...M	.K....A	.K....O					
Common carp	MPP..VLVL	.G....AVC.	ROKHS..YT.	IT..S...M	.K....A	.K....O					
Roach	MPS..VLVL	.G....AVC.	ROKHSP.YT.	IT..S...M	.K....A	.K....O					
Fathead minnow	MPP..VLVL	.G....AVC.	ROKHSP.YT.	IT..S...M	.K....A	.K....O					
Cavefish	MNPE.VLLQ	.G....T.C.	RQRHS..YT.	VT..T...M	.K....A	.K....OE					
Rainbow trout	MPPE.VLFL	QG...A.C.	RQKVA..YT.	VT..T...SM	.K....A	.K....OE					
Cod	IPP..VLLV	OG....S.	ROKMN..YT.	V...T...SM	.K....A	.K....LE					
Stickleback	MPP..VLLL	QC....T.C.	RQKLN..YT.	VT..T...SM	.K....A	.KL...LQ					
Medaka	IPPE.VLLL	QG....C.	RQKLS..YT.	VT..T...SM	.K....A	.KL...LQ					
Platyfish	.PS..VLLL	OG....C.	ROKMS..YT.	VT..T...SM	.K....A	.KL...LQ					
Tetraodon	IPPE.ILFQ	QG...L.C.	RQKLS..YT.	.T..T...M	.K....A	.KI...Q					
Fugu	.APE.ILFQ	QG...M.C.	RQKLS..YT.	VT..T...M	.K....A	.KI...Q					
Tilapia	MPP..VLLL	QG....S.	ROKMS..YT.	VTI.T...SM	.K....T	.KL...LQ					
Sea lamprey	.E...VI...	.E....TVL.	S...DK.VT.	.L.AA..S.T	.KI...TA					
	370	\wedge	δ	δ	δ	δ	\circ	\wedge			
Human	LTLHDQVHLL	ECAWLEILMI	GLVWRSMHEP	GKLLFAPNLL	LDRNQKGCVE	GMVEIFDMLL					
Tree fro	.S.....O	.SS...V...V	.I...V...	.S.....I	.R.....R	.L.....V					
Zebrafish	.S.....O	.SS...V...	.I...IHS	.I...OD.I	.SE.E...	.A.....A					
Common_carp	.S.....O	.ST...V...	.I...IHS	.I...OD.I	.SE.E...	.A.....A					
Roach	.S.....O	.SS...V...	.I...IHS	.I...OD.I	.E.E...	.A.....A					
Fathead minnow	.S.....O	.SS...V...	.I...IHS	.I...OD.I	.E.E...	.A.....A					
Cavefish	.S.....O	.SS...V...	.I...LHS	.I...OD.I	.E.D...	.A.....A					
Rainbow_trout	.S.....O	.SS...V...	.I...IHC	.I...OD.I	.SE.D...	.A.....A					
Cod	.S.....O	.SS...V...	.I...IHY	.I...OD.I	.TE.D...	.A.....A					
Stickleback	.G.....Q	.SS...V...	.I...IHC	.I...OD.I	.E.D...	.A.....A					
Medaka	.S.....L	.SS...V...	.I...IHC	.I...OD.I	.E.D...	.T.....T					
Platyfish	.S.....L	.SS...V...	.I...IHC	.I...OD.I	.E.D...	.A.....A					
Tetraodon	.S.....K	.SS...V...	.I...IHY	.I...D.I	.E.D...	.A.....A					
Fugu	.S.....K	.SS...V...	.I...IHY	.I...D.I	.E.D...	.A.....A					
Tilapia	.S.....L	.SS...V...	.I...IQC	.I...OD.I	.E.T...	.A.....A					
Sea lamprey	IG.S...Q	.C.....IV	.I...IDR	.Q.H....I	.G.EDARN	.LDM....					
	430			457							
Human	ATSSRFMRMN	LOGEEFVCLK	SIILLNS---	-GVYTFLSST	LKSLEEKDHI	HRVLDKITDT					
Frog	T.AT....R	R....I...			.E...DT.L	.I.....I					
Zebrafish	.VA...SLK	.KL.....A	.I.....I	.AFS.C.P	VEP.MDNFMV	OCM.N..A					
Common_carp	.VT...NLK	.KL.....A	.I.....I	.AFS.C.Q	VEP.MDSFMV	OCM.N..A					
Roach	.VA...SLK	.KL.....A	.I.....I	.AFS.C.P	VEP.MDSFMV	OCM.N..A					
Fathead minnow	.VA.L.SLK	.KL.....A	.I.....I	.AFS.C.P	VEP.MDSFMV	OCM.N..A					
Cavefish	.VA...ALK	.KS.....A	.VRK	L.AFS.CC.TP	GEP.RDSYV	OCM.N..A					
Rainbow_trout	.V...L.K	.KP.....A		.AFS.C.NS	VE.HNSSAV	ESM.N..A					
Cod	.V...LQ	.NP.....A		.AFS.CT.	MEA.HDSLAV	OGM.T..A					
Stickleback	.A...LLK	.KP.....A		.AFS.CTG.	MEP.HDTAAV	QHM.T...					
Medaka	.A...VLK	.KP.....A		.AFS.CTG.	MEP.HNSAAV	QSM.T..A					
Platyfish	.T...LQ	.KP.....A		.AFS.CTG.	MEP.HNSAAV	QSM.T..A					
Tetraodon	.T...LQ	.KP.....A	.F.....	.AFS.CTG.	MEP.HNSAAV	QNI.NN.M.A					
Fugu	.T...L.K	.KP.....A		.AFS.CTGS	MEP.HNSAAV	QNI.NN.M.A					
Tilapia	.A...VLK	.KP.....A		.AFS.CTG.	MEP.HNSAAV	QHM.T..A					
Sea lamprey	V.V...ELH	.RR..Y...	AM.....	-----	-----	-----					
	486			δ	δ	\wedge					
Human	LIHLMAKAGL	TLQQQHQRLA	QLLLILSHIR	HMSNKGMEHL	YSMKCKNVVP	LYDLLLEMLD					
Frog	.V.F...S	S...QR...									
Zebrafish	.Y.CI.S.S.A	S.L.SR.Q	.L.....		.R....R						
Common_carp	F.YGIS.S.A	S.L.SR.Q	.L.....		.R....R						
Roach	.YGIS.S.A	S.L.SR.Q	.L.....		.H....R						
Fathead minnow	.YGIS.S.A	S.L.SR.Q	.L.....		.H....R						
Cavefish	.YISQS.I	SV.L.CR.Q	.L.....	RF						
Rainbow_trout	.HISHS.A	SV...PR.Q	.L.....		.I...K.						
Cod	.HISQS.C	SI...SR.Q	.L.....	K.						
Stickleback	.HIGQS.C	SV...SR.Q	.L.....	K.						
Medaka	.YISQS.Y	LA.E.AR.Q	.L.....	K.						
Platyfish	.HISQS.Y	SA...AR.Q	.L.....	K.						
Tetraodon	.HISQS.F	SA...FR.Q	.L.....	.Q.....R.						
Fugu	.HIGQS.F	SA...SR.Q	.L.....	.T.....R.						
Tilapia	.FHISHL.C	SA...SR.Q	.L.....	K.						
Sea lamprey	-----	-----	-----	-----	-----	-----					

The amino-acid positions indicated on the first row are based on the human ESR1. Dots represent amino acids that are identical to those at corresponding positions in human ESR1. Residues highlighted in grey in the human ESR1 sequence correspond to the set of amino-acid residues that have been experimentally shown to participate in ligand binding in at least one of the co-crystal complexes, available through pocketome.org, accessed in April 2014. \circ and/or \wedge above the human ESR1 sequence show the set of amino acids that have direct contact with the co-crystallized ligand, E2 (\circ) or DES (\wedge) in at least one experimentally determined complex structure (pocketome.org).

Supplementary Material Table S5: Life-history trait values used to calculate indices of ecological susceptibility for all selected fish species

S5a: Calculation of Population Survivorship Index focusing on reproductive and developmental ecology (after Spromberg and Birge, 2005)

Common name	Spawnings (per yr)	Spawning index	Parental behaviour index	Lifespan mean (yrs)	Lifespan index	Recruitment (M)	Recruitment index	Replacement ratio (R ₀)	Replacement ratio index	Eco niche (stages)	Survivorship	
											Recruitment	Replacement
Cave fish*	20	10	1	1.7	3	2.44	3	28226	3	1	0.58	0.61
Cod	15	10	1	18	1	0.19	1	46	1	10	0.74	0.74
Coelacanth	1	2	4	38	1	0.1	1	203	1	1	0.29	0.32
Fugu	1	2	1	10	1	0.26	2	142	1	5	0.35	0.35
Medaka	80	10	3	1	3	6.9	4	974×10 ⁹	4	5	0.81	0.81
Nile tilapia	12	10	4	5	3	0.95	2	143	2	5	0.77	0.77
Southern platyfish	6	10	4	2	3	4.18	4	51534	4	5	0.84	0.84
Sea lamprey	<1	1	1	6	2	0.14	1	106	1	5	0.32	0.35
Spotted gar*	1	4	1	6	2	0.22	2	73	1	5	0.45	0.42
Threespine stickleback	6	10	4	1.6	3	2.96	3	864	3	5	0.81	0.81
Tetraodon	1	4	4	4.1	3	1.44	3	5136	3	5	0.61	0.65
Zebrafish	60	10	1	1	3	3.2	3	7.3×10 ⁷	4	1	0.71	0.74
Common carp#	4	8	1	6	2	0.73	2	96	1	5	0.58	0.55
Fathead minnow#	26	10	4	2	3	1.76	3	44802	3	5	0.81	0.84
Rainbow trout#	0.3	2	3	6	2	0.81	2	42	2	5	0.45	0.42
Rare minnow#	50	10	1	2	3	4.75	4	873270	4	5	0.74	0.74
Roach#	1	4	1	9	2	0.47	2	85	1	5	0.45	0.42

Population survivorship is calculated from the sum of 5 indices: spawning; parental behavior; lifespan; recruitment (or replacement); eco niche divided by 31 (max score).

Note * Species fully sequenced, but with incomplete gene builds. # Model species not fully sequenced.

Spawning index: 1 = 1 spawn/life-time; 2 = >1 spawn/life-time, not annually; 4 = 1 spawn/yr; 6 = 2 spawns/yr; 8 = 3-4 spawns/yr; 10 = >4 spawns/yr

Lifespan index: 1 >= 10 years; 2 >5<10 years; 3 <=5 years

Recruitment index (based on natural mortality M calculated using FishBase's Life-history Tool): 1 <=0.2M; 2 >0.2<1M; 3 >=1<4M; 4 >=4M

Replacement ratio index: 1 <100 R₀; 2 =100<400; 3 =400<2000; 4 >=2000

Ecological niche index: 1 = Specialist, large niche overlap between stages; 5 = Medium specialist, some niche overlap between stages; 10 = Generalist with little or no niche overlap between stages

S5b1: Calculation of Ecological Vulnerability Index (after De Lange et al., 2009)

Trait values

Common name	External exposure							Internal Exposure					Ecotox Ecotox cmpnd specific	Population resilience					
	* Habit at pref ^a (cat)	* Max life- span (mnths)	* Log home range (est.) ^b	# Food pref ^c (cat)	* Food need (Q/B)	# Season depen ^d (cat)	Home range <spill zone	* Metab' (log Q/B)	# Season depen ^d (cat)	Store (y/n)	Excret' (y/n)	Detox (y/n)		*# Repro age (mnths)	*# Log off- spring log no.	* Recruit ment (M)	# Disp- ersal ^e (cat)	# Patchi- ness	# Terri- torial- ity ^f (cat)
Cave fish	3	42	2	3	10.8	2	y	1.03	2	y	n	n		12	4.27	2.44	4	y	1
Cod	3	276	5	4	2.58	2	n	0.41	2	y	n	n		48	8.91	0.19	1	n	1
Coelacanth	3	576	0.33	4	1.4	2	y	0.15	2	y	n	n		324	2.11	0.1	5	y	3
Fugu	3	228	1	3	3.4	2	n	0.53	2	y	n	n		48	2.48	0.26	4	y	3
Medaka	3	24	1.5	3	20	2	n	1.30	2	y	n	n		3.6	3.51	6.9	3	n	2
Nile tilapia	3	108	1	3	17	4	n	1.23	4	y	n	n		12	3.18	0.95	3	y	2
Southern platyfish	4	48	0.5	3	22	3	n	1.34	3	y	n	n		6	3.16	4.18	5	y	2
Sea lamprey	5	132	2	4	1.9	2	y	0.28	2	y	n	n		60	5.33	0.14	4	y	2
Spotted gar	3	216	1.5	4	2.5	3	n	0.40	3	y	n	n		36	4.17	0.22	2	y	2
Threespine stickleback	4	30	1.5	3	3.85	1	n	0.59	1	y	n	n		12	3.18	2.96	4	y	2
Tetraodon	4	72	1.5	3	14	2	n	1.15	2	y	n	n		14.4	4.16	1.44	4	y	2
Zebrafish	4	26.4	1	3	34.9	2	y	1.54	2	y	n	n		3.6	3.43	3.2	3	y	1
Carp	1	240	2	3	17.6	2	n	1.25	2	y	n	n		36	6.30	0.73	2	y	1
Fathead minnow	4	36	0.5	3	40.6	1	n	1.61	2	y	n	n		6	3.24	1.76	3	y	2
Rainbow trout	4	132	1	4	3.7	1	y	0.57	1	y	n	n		36	3.50	0.81	2	y	2
Rare minnow	4	36	0	3	11.3	1	n	1.05	3	y	n	n		6	4.65	4.75	3	y	2
Roach	1	168	2	3	13.6	2	n	1.13	2	y	n	n		36	5.19	0.47	2	y	1

Increasing trait values indicate increasing vulnerability. Increasing trait values indicate decreasing vulnerability.

Data source: * FishBase (version 12/2013, Froese and Pauly, 2013); # FishTraits (version 2, Xie et al., 2013)

^aHabitat preference categories: 1 vegetation; 2 on soil; 3 in water; 4 in soil; 5 in sediment

^bLog home range estimated from territoriality^f classification

^cFood preference categories: 1 vegetables, nectar, seeds fruit; 2 soil, detritus, waste material; 3 insect, soil organism, vertebrate herbivores; 4 vertebrate carnivores

^dSeasonal dependence categories: 1 winter; 2 summer; 3 summer and some winter; 4 year-round presence

^eDispersal capacity categories: 1 free distribution; 5 minimal dispersal. ^fTerritorial behavior categories: 1 never; 2 sometimes; 3 always

S5b2: Calculation of Ecological Vulnerability Index (after De Lange et al., 2009)

Standardised scores

	External exposure							Internal Exposure					Ecotox	Population resilience					
Common name	Habit at pref ^a (cat)	Max life-span (mnths)	Log home range (m)	Food pref ^b (cat)	Food need (Q/B)	Season depen ^c (cat)	Home range <spill zone	Metab' (log Q/B)	Season depen ^c (cat)	Store (y/n)	Excret' (y/n)	Detox (y/n)	Ecotox cmpnd specific	Repro age (mnths)	Log off-spring log no.	Recruit ment (M)	Disp-ersal ^d (cat)	Patchi-ness	Terri-torial-ity ^e (cat)
Cave fish	0.6	0.07	0.6	0.75	0.27	0.5	1	0.36	0.5	0	1	1		0.04	0.52	0.65	0.2	1	0.3333
Cod	0.6	0.48	0	1	0.06	0.5	0	0.74	0.5	0	1	1		0.15	-3E-05	0.97	0.8	0	0.3333
Coelacanth	0.6	1	0.93	1	0.03	0.5	1	0.91	0.5	0	1	1		1	0.76	0.99	0	1	1
Fugu	0.6	0.39	0.8	0.75	0.08	0.5	0	0.67	0.5	0	1	1		0.15	0.72	0.96	0.2	1	1
Medaka	0.6	0.31	0.5	0.75	0.04	0.5	0	0.87	0.5	0	1	1		0.22	0.80	0.98	0.4	0	0.6667
Nile tilapia	0.6	0.04	0.7	0.75	0.49	0.5	0	0.19	0.5	0	1	1		0.01	0.61	0	0.4	1	0.6667
Southern platyfish	0.6	0.18	0.8	0.75	0.42	1	0	0.24	0	0	1	1		0.04	0.64	0.86	0.4	1	0.6667
Sea lamprey	0.8	0.08	0.9	0.75	0.54	0.75	0	0.17	0.25	0	1	1		0.02	0.65	0.39	0	1	0.6667
Spotted gar	1	0.23	0.6	1	0.05	0.5	1	0.83	0.5	0	1	1		0.2	0.40	0.98	0.2	1	0.6667
Threespine stickleback	0.6	0.38	0.7	1	0.06	0.75	0	0.75	0.25	0	1	1		0.11	0.53	0.97	0.6	1	0.6667
Tetraodon	0.8	0.05	0.7	0.75	0.09	0.25	0	0.64	0.75	0	1	1		0.04	0.64	0.57	0.2	1	0.6667
Zebrafish	0.8	0.13	0.7	0.75	0.34	0.5	0	0.29	0.5	0	1	1		0.04	0.53	0.79	0.2	1	0.6667
Common carp	0.2	0.42	0.6	0.75	0.43	0.5	1	0.23	0.5	0	1	1		0.11	0.29	0.89	0.6	1	0.3333
Fathead minnow	0.8	0.23	0.8	1	0.09	0.25	1	0.65	0.75	0	1	1		0.1	0.61	0.88	0.6	1	2
Rainbow trout	0.8	0.06	0.9	0.75	1	0.25	0	0.001	0.5	0	1	1		0.02	0.64	0.74	0.4	1	2
Rare minnow	0.8	0.06	1	0.75	0.28	0.25	0	0.35	0.25	0	1	1		0.019	0.48	0.31	0.4	1	0.67
Roach	0.2	0.29	0.6	0.75	0.33	0.5	1	0.30	0.5	0	1	1		0.11	0.42	0.93	0.6	1	0.33

S5b3: Calculation of Ecological Vulnerability Index (after De Lange et al., 2009)

Standardised scores \times weight factors

Common name	External exposure							Internal Exposure					Ecotox Ecotox cmpnd specific	Population resilience					
	Habit at pref ^a (cat)	Max life- span (mnths)	Log home range (m)	Food pref ^b (cat)	Food need (Q/B)	Season depen ^c (cat)	Home range <spill zone	Metab' (log Q/B)	Season depen ^c (cat)	Store (y/n)	Excret' (y/n)	Detox (y/n)		Repro age (mnths)	Log off- spring log no.	Recruit ment (M)	Disp- ersal ^d (cat)	Patchi- ness	Terri- torial- ity ^e (cat)
Cave fish	0.014	0.005	0.029	0.032	0.006	0.021	0.000	0.043	0.000	0.000	0.061	0.121		0.002	0.031	0.038	0.020	0.039	0.007
Cod	0.014	0.034	0.000	0.043	0.001	0.125	0.000	0.090	0.000	0.000	0.061	0.121		0.009	0.000	0.057	0.078	0.000	0.007
Coelacanth	0.014	0.071	0.044	0.043	0.001	0.021	0.000	0.110	0.000	0.000	0.061	0.121		0.059	0.045	0.058	0.000	0.039	0.020
Fugu	0.014	0.028	0.038	0.032	0.002	0.021	0.000	0.081	0.000	0.000	0.061	0.121		0.009	0.042	0.056	0.020	0.039	0.020
Medaka	0.014	0.022	0.024	0.032	0.001	0.021	0.000	0.106	0.000	0.000	0.061	0.121		0.013	0.047	0.057	0.039	0.000	0.013
Nile tilapia	0.014	0.003	0.033	0.032	0.011	0.021	0.000	0.023	0.000	0.000	0.061	0.121		0.001	0.036	0.000	0.039	0.039	0.013
Southern platyfish	0.014	0.013	0.038	0.032	0.009	0.043	0.000	0.029	0.000	0.000	0.061	0.121		0.002	0.038	0.051	0.039	0.039	0.013
Sea lamprey	0.019	0.006	0.043	0.032	0.012	0.032	0.000	0.020	0.000	0.000	0.061	0.121		0.001	0.038	0.023	0.000	0.039	0.013
Spotted gar	0.024	0.016	0.029	0.043	0.001	0.021	0.000	0.100	0.000	0.000	0.061	0.121		0.011	0.024	0.057	0.020	0.039	0.013
Threespine stickleback	0.014	0.027	0.033	0.043	0.001	0.032	0.000	0.091	0.000	0.000	0.061	0.121		0.007	0.031	0.057	0.059	0.039	0.013
Tetraodon	0.019	0.004	0.033	0.032	0.002	0.011	0.000	0.077	0.000	0.000	0.061	0.121		0.002	0.038	0.033	0.020	0.039	0.013
Zebrafish	0.019	0.009	0.033	0.032	0.007	0.021	0.000	0.035	0.000	0.000	0.061	0.121		0.003	0.031	0.046	0.020	0.039	0.013
Common carp	0.005	0.030	0.029	0.032	0.009	0.021	0.000	0.027	0.000	0.000	0.061	0.121		0.007	0.017	0.052	0.059	0.039	0.007
Fathead minnow	0.019	0.016	0.038	0.043	0.002	0.011	0.000	0.078	0.000	0.000	0.061	0.121		0.007	0.036	0.052	0.059	0.039	0.013
Rainbow trout	0.019	0.004	0.043	0.032	0.022	0.011	0.000	0.000	0.000	0.000	0.061	0.121		0.001	0.037	0.044	0.039	0.039	0.013
Rare minnow	0.019	0.004	0.048	0.032	0.006	0.011	0.000	0.042	0.000	0.000	0.061	0.121		0.001	0.028	0.018	0.039	0.039	0.013
Roach	0.005	0.021	0.029	0.032	0.007	0.021	0.000	0.036	0.000	0.000	0.061	0.121		0.007	0.024	0.055	0.059	0.039	0.007

Weight factors for generic veterinary pharmaceutical ivermectin (from De Lange et al., 2009)

In category	0.07	0.21	0.14	0.13	0.07	0.13	0.00	0.36	0.00	0.09	0.18	0.36	1.00	0.18	0.18	0.18	0.29	0.12	0.06
Category	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.00	0.33	0.33	0.33	0.33	0.33	0.33
Overall	0.02	0.07	0.05	0.04	0.02	0.04	0.00	0.12	0.00	0.03	0.06	0.12	0.00	0.06	0.06	0.06	0.10	0.04	0.02

S5b4: Calculation of Ecological Vulnerability Index (after De Lange et al., 2009)

Weighted scores summed over all traits

Common name	Vulnerability Index
Cave fish	0.46862
Cod	0.64025
Coelacanth	0.70711
Fugu	0.58493
Elephant shark	0.57209
Medaka	0.44768
Nile tilapia	0.54225
Southern platyfish	0.46025
Sea lamprey	0.57981
Spotted gar	0.62938
Threespine stickleback	0.50519
Tetraodon	0.49126
Zebrafish	0.47259
Common carp	0.516
Fathead minnow	0.48634
Rainbow trout	0.59423
Rare minnow	0.48262
Roach	0.52288

Supplementary Material Table S6: Vital rates for control and exposure scenarios used in life-table response experiments for model fish species

Age class	Parameter	No-chemical control (wild fish)		Exposure treatment (1 ng/L EE2)	
		Vital rate	SD	Vital rate	SD
Medaka					
0	Fertilisation success	1 ^a	0.015 ^a	0.746 ^b	0.055 ^b
	Proportion of females	0.5 ^a	-	0.5 ^b	-
	Proportion of viable females	0.5 ^a	0.015 ^a	0.373 ^b	0.055 ^b
0+	Survivorship (sx)	0.0365 ^a	0.00365 ^a	0.0365 ^a	0.00365 ^a
1+	Survivorship (sx)	0.0147 ^a	0.00147 ^a	0.0147 ^a	0.00147 ^a
0+	Fecundity (mx)	300 ^a	78 ^a	219 ^c	105 ^c
1+	Fecundity (mx)	720 ^a	187 ^a	526 ^c	251 ^c
Zebrafish					
0	Fertilisation success	0.85 ^d	0.015 ^d	0.545 ^e	0.05 ^e
	Proportion of females	0.5 ^d	0.1 ^d	0.57 ^e	0.1 ^e
	Proportion of viable females	0.425 ^d	0.1 ^d	0.264 ^e	0.1 ^e
0+	Survivorship (sx)	0.0126 ^d	0.00126 ^d	0.0126 ^{e,f}	0.00126 ^{e,f}
1+	Survivorship (sx)	0.019 ^d	0.0019 ^d	0.019 ^{e,f}	0.0019 ^{e,f}
0+	Fecundity (mx)	300 ^d	60 ^d	228 ^e	96 ^e
1+	Fecundity (mx)	3000 ^d	600 ^d	2280 ^e	960 ^e
Fathead minnow					
0	Fertilisation success	0.93 ^g	0.015 ^g	0.65 ^h	0.3 ^h
	Proportion of females	0.5 ^g	-	0.65 ^h	-
	Proportion of viable females	0.465 ^g	0.015 ^g	0.4225 ^h	0.2 ^h
0+	Survivorship (sx)	0.001 ^g	0.0001 ^g	0.001 ^h	0.0001 ^h
1+	Survivorship (sx)	0.00039 ^g	-	0.00039 ^h	-
2+	Survivorship (sx)	0.0001521 ^g	-	0.0001521 ^h	-
0+	Fecundity (mx)	750 ^g	150 ^g	398 ^h	179 ^h
1+	Fecundity (mx)	1500 ^g	300 ^g	795 ^h	358 ^h
2+	Fecundity (mx)	3000 ^g	600 ^g	1590 ^h	716 ^h

Proportion of viable females = fertilisation success × proportion of females

Vital rates reduced in treatments are highlighted in red.

References in main manuscript: a) Meng et al., 2006; b) Balch et al., 2004; c) Scholz and Gutzeit, 2000; d) Hazelerigg et al., 2013; e) Schaefers et al., 2007; f) Nash et al., 2004; g) Miller and Ankley, 2004; h) Länge et al., 2001

Supplementary Material Table S7: Life-table data for wild fish representing ‘controls’ in life-table response experiments

S6a) Medaka

Stage (x)	Survivorship to end of stage (sx)	SD (sx)	Cumulative survivorship to each stage (lx)	SD (lx)	Eggs produced per surviving individual at each stage (mx)	SD (mx)	Eggs produced per original individual in each stage (lxmx)
Female eggs (viable, fertilised)	0.5	0.015	0.5	0.015	0		0
Age 0+	0.0365	0.00365	0.01825	0.001825	300	78	5.475
Age 1+	0.0147	0.001470747	0.000268411	2.68411E-05	720	187	0.193256103

Medaka life-table data from Meng et al. (2006). Reference in main manuscript.

S6b) Zebrafish

Stage (x)	Survivorship to end of stage (sx)	SD (sx)	Cumulative survivorship to each stage (lx)	SD (lx)	Eggs produced per surviving individual at each stage (mx)	SD (mx)	Eggs produced per original individual in each stage (lxmx)
Female eggs (viable, fertilised)	0.425	0.015	0.425	0.015	0		0
Age 0+	0.0126	0.00126	0.005355	0.0005355	300	60	1.6065
Age 1+	0.019	0.0019	0.000101745	1.01745E-05	3000	600	0.305235

Zebrafish life-table data from Hazlerigg et al. (2014). Reference in main manuscript.

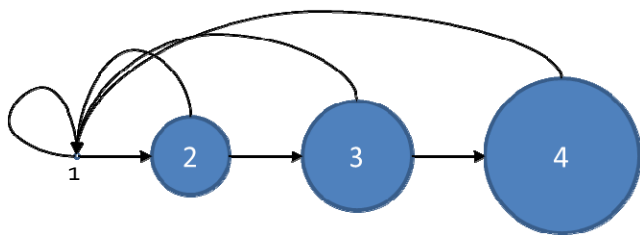
S6c) Fathead minnow

Stage (x)	Survivorship to end of stage (sx)	SD (sx)	Cumulative survivorship to each stage (lx)	SD (lx)	Eggs produced per surviving individual at each stage (mx)	SD (mx)	Eggs produced per original individual in each stage (lxmx)
Female eggs (viable, fertilised)	0.5	0.015	0.5	0.015	0		0
Age 0+	0.001	0.0001	0.0005	0.00005	750	150	0.375
Age 1+	0.00039	0.000039	0.000000195	1.95E-08	1500	300	0.0002925
Age 2+	0.0001521	0.00001521	2.96595E-11	2.96595E-12	3000	600	8.89785E-08

Fathead minnow life-table data from Miller and Ankley (2004). Reference in main manuscript.

Supplementary Material Figure S1: Life-cycles and Leslie matrix models for model fish species

Fathead minnow life-cycle showing life-stages and their relative fecundity (scaled circles)



Stage 1 = Age 0 (viable females at 0 days post hatch = proportion of female eggs × fertilisation success), Stage 2 = Age 0+ juveniles (to end of 1st year of life), Stage 3 = Age 1+ adults (to end of 2nd year of life), Stage 4 = Age 2+ adults (adults (to end of 3rd year of life).

Fathead Minnow Leslie matrix model for unexposed (control) population

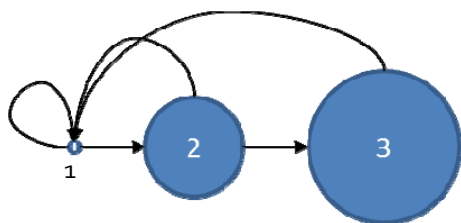
	Age0	Age 0+	Age 1+	Age 2+
Age0	0.465	750	1500	3000
Age 0+	0.001	0	0	0
Age 1+	0	0.00039	0	0
Age 2+	0	0	0.0001521	0

Fecundity = number of eggs per individual female (only females modelled)

Survivorship = probability of survival

Both vital rates were allowed to vary stochastically within their reported standard deviations for n=100 projections

Medaka life-cycle showing life-stages and their relative fecundity (scaled circles)



Stage 1 = Age 0 (viable females at 0 days post hatch = proportion of female eggs × fertilisation success), Stage 2 = Age 0+ juveniles (to end of 1st year of life), Stage 3 = Age 1+ adults (to end of 2nd year of life)

Medaka Leslie matrix model for unexposed (control) population

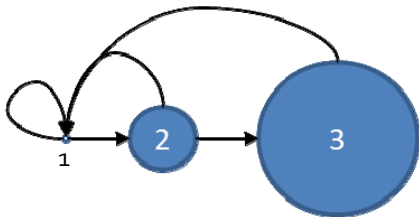
	Age0	Age 0+	Age 1+
Age0	0.5	300	720
Age0+	0.0365	0	0
Age1+	0	0.0147	0

Fecundity = number of eggs per individual female (only females modelled)

Survivorship = probability of survival

Both vital rates were allowed to vary stochastically within their reported standard deviations for n=100 projections

Zebrafish life-cycle showing life-stages and their relative fecundity (scaled circles)



Stage 1 = Age 0 (viable females at 0 days post hatch = proportion of female eggs \times fertilisation success), Stage 2 = Age 0+ juveniles (to end of 1st year of life), Stage 3 = Age 1+ adults (to end of 2nd year of life)

Zebrafish Leslie matrix model for unexposed (control) population

	Age0	Age 0+	Age 1+
Age0	0.425	300	3000
Age 0+	0.0126	0	0
Age 1+	0	0.19	0

Fecundity = number of eggs per individual female (only females modelled)

Survivorship = probability of survival

Both vital rates were allowed to vary stochastically within their reported standard deviations for n=100 projections