

# Effect of the plastic pollutant bisphenol A on the biology of aquatic organisms: A meta-analysis

Nicholas C. Wu, and Frank Seebacher\*

*School of Life and Environmental Sciences A08, The University of Sydney, NSW 2006, Australia*

## Supplementary information

### Data exclusion criteria:

- Non-English literature, books without links to primary source, and non-peer-reviewed articles.
- Dose-response curves and half maximal effective concentration (EC50) with no appropriate controls.
- Response measures taken some time after BPA treatment (e.g. studies with test subjects exposed to BPA for one week, but responses were measured two weeks post-BPA exposure), because BPA degrades relatively quickly under lab conditions.
- Transgenerational effects on F1 and subsequent generations. If presented, relevant data were extracted from the F0 exposed generation only.
- Transcriptome, genomic, proteomic analyses.
- BPA derivatives such as bisphenol S or bisphenol AF, as well as BPA mixed/combined with other chemicals.
- Terrestrial organisms that spend little time in aquatic environments (e.g. freshwater turtles, crocodylians).
- Studies that did not state species, or in which various species were grouped together as a general taxon.
- Non-quantifiable traits or results such as histological sections, electron-microscope images, principal components with arbitrary units.
- Gut microbiota responses were excluded because it is difficult to distinguish direct effect of BPA from indirect effects stemming from other organism responses.
- Mesocosm experiments, because direct and indirect effects are difficult to discern.
- Studies using isolated or cultured cells, as they do not provide ecologically relevant responses.

### Handling of detection limits in BPA data

We compared four different representations of BPA levels below the detection limit to test whether the choice of method affected the outcomes of the analysis. Hence, we included values below the detection limit as 1) the reported detection limit in each study, 2) zero, 3) the detection limit divided

by two, and 4) the detection limit divided by the square root of two. The data from each method were log transformed to achieve normality and compared using an analysis of variance model. There were no significant differences between detection limit methods ( $F_{3,5392} = 0.77$ ,  $P = 0.5$ , and  $F_{3,2472} = 0.23$ ,  $P = 0.9$  respectively for environmental and biota data). The resulting Akaike information criterion (AIC) values from the distribution models was calculated using the ‘fitdist’ function from the ‘fitdistrplus’ package in *R*. The lowest AIC value for both the environment and biota dataset was including values as the detection limit, which we therefore used in the final analysis (**Fig. S2**).

### Publication bias and sensitivity analysis

We observed a significant relationship between the year of publication and effect size (slope estimate = -0.08; 95% CI [-0.11, -0.05];  $P < 0.0001$ ), suggesting time-lag bias (**Fig. S4A**). However, removing one study from (Alexander et al., 1988), eliminated the time-lag bias (slope estimate = -0.005; 95% CI [-0.02, 0.01];  $P = 0.54$ ), thus this study was excluded in the final analysis. The greater variation of effect size in more recent studies may represent increased detectability, or more diverse range of biological traits and taxonomic groups examined.

Egger’s regression testing for the symmetry of the funnel plot of the meta-analytic residuals from the overall model against their precision (inverse s.e.) was not statistically significant ( $t_{2881} = 0.712$ ,  $P = 0.47$ ; **Fig. S4B**). Trim-fill analysis estimated that 16 effect sizes were missing on the left-hand side of the funnel distribution ( $P < 0.0001$ ), however the estimated adjustment was small ( $\ln RR = -0.09$ ; **Fig. S4C**) and does not influence our results qualitatively. Cook’s distance showed five potential outliers that may influence the meta-analysis (**Fig. S4D**). However, upon inspection, we observed those outliers came from reproductive traits (specifically vitellogenin levels) with biologically realistic variation, and thus were kept in the analysis. Overall, it is unlikely that publication bias is driving our main results.

### Supplementary tables

**Table S1.** Raw data descriptors for the meta-analysis

Column name	Description
first_author	Surname of the first author of the paper
effect_size_ID	Unique identifiers for individual effect sizes (observation level)
study_ID	Unique identifiers for each paper
year_published	Year of publication
citation	Annotated citation of the paper.
journal	Name of journal the paper was published
domain	Species grouped into Vertebrate, Invertebrate, and Autotroph

taxa	Generalised grouping of the study species based on phylogenetic relatedness
common_name	Common name for the study species
scientific_name	Binomial nomenclature for species (as <i>Genus latin</i> )
scientific_name_OTL	Scientific name to match Open Tree of Life database ( <a href="https://tree.opentreeoflife.org">https://tree.opentreeoflife.org</a> )
development stage	Developmental stage of the organism (egg, larvae, juvenile, adult, unknown; see <b>Table S2</b> for full description of development stage)
sex	The sex of the study species (male, female, both, unknown meaning not stated in the study)
includes body size	Indicates whether study provided data on body mass (0 = no, 1 = yes)
body_size	Mean mass (g) or length (mm) of the study species for the study
size_unit	Unit of the study species body mass, length
pop_source	Study species were sourced either from the wild or raised in captive settings (past F1) such as farms, aquacultures, laboratories
temperature	Mean temperature (°C) set of the experimental treatments
BPA	Dosage of BPA in the treatment water (or given orally through food pellets)
BPA_unit	Units the paper presented for the BPA treatments
corrected dose	BPA dosage converted to the same unit ( $\mu\text{g l}^{-1}$ )
time	How long the study species were exposed to BPA (or how long the experiments ran for)
time_day	Time of BPA exposure converted to days
trait	Generalised functional trait in groups (see <b>Table S4</b> below for full description of traits)
hierarchy	Biological hierarchy of organisation (see <b>Table S3</b> below for full description of organisation)
response	Specific trait measured in the study (see <b>Table S4</b> for full list of specific response variables)
response_unit	Unit for the response presented in the study
mean_control	Mean response of the control condition (no BPA present, vehicle control, solvent control) relative to the study design
sd_control	The standard deviation of the control response
se_control	The standard error of the control response
n_control	Sample size of the control treatment
mean_treat	Mean response of the BPA treatment relative to the study design
sd_treat	The standard deviation of the treatment response
se_treat	The standard error of the treatment response
n_treat	Sample size of the treatment
mean_c_1	mean_control with addition value of 1 for natural log calculation
mean_t_1	mean_treatment with addition value of 1 for natural log calculation
lnRR	Natural log of response ratio of mean_t_1 and mean_c_1
lnRR_direct	lnRR corrected for direction (see <b>Table S4</b> )
lnRR_variance	lnRR sampling variance calculation

notes	General comments
link	Link to online article

**Table S2.** Description of developmental stages

Development	Description
Embryo	Early developmental period from fertilization to hatching/birth
Juvenile	Organism from birth/hatching to sexually mature adult
Larvae	Juvenile period with a distinct form different from the sexually mature adult e.g. instars for insects, tadpoles for amphibians
Adult	Sexually mature organism
Unknown	Not stated in study, or not applicable e.g. phytoplankton

**Table S3.** Description of the five assigned biological levels with example responses

Hierarchy	Description	Example responses
Organism	Whole-organism response	Growth rate, feeding activity
Organ	Organ-level response	Resting heart rate, gonad mass
Tissue	Tissue-level response	Blood vessel diameter, muscle aberration
Cellular	Cellular-level and subcellular response (activity or abundance)	Cell growth, sperm movement, neutrophil count
Macromolecule	Hormone level, biochemical concentration, enzyme activity, DNA damage	T3 and T4 levels, DNA damage

**Table S4.** Summary of selected biological responses and their assigned functional traits included in the meta-analysis. Negative values or direction relative to the control treatment indicates expected negative/decreased/reduced effect on the organism. An asterisk indicates that positive values would indicate negative effects on organisms (e.g. some abnormality traits) which were corrected for direction towards negative.

Grouped trait	Specific responses	Expected effects (*direction correction)
Abnormality & damage	Deformity (whole animal to tissue level changes in morphology)	Increased value* = increased deformity
Abnormality & damage	DNA damage	Increased value* = increased DNA damage
Abnormality & damage	Eye size (including length and width)	Decreased value = smaller eye size
Abnormality & damage	Eye-snout distance	Decreased value = smaller head size
Abnormality & damage	Head-trunk angle	Decreased value = decreased body symmetry

Abnormality & damage	Head width	Decreased value = smaller head size
Abnormality & damage	Interocular distance	Decreased value = smaller distance between eyes
Abnormality & damage	Intramuscular lipids	Decreased value = less lipids in muscles
Abnormality & damage	Pericardial edema	Increased value* = increased edema
Abnormality & damage	Reactive oxygen species (ROS) production (including damage)	Increased value* = increased cellular damage
Abnormality & damage	Sperm damage	Increased value* = increased deformity
Abnormality & damage	Swim bladder area (including inflated swim bladder)	Decreased value = smaller swim bladder
Abnormality & damage	Yolk sac edema	Increased value* = increased edema
Behaviour	Aggression (antagonistic interaction with conspecific)	Decreased value = lower aggression to conspecific
Behaviour	Activity (includes reverse of immobility)	Decreased value = reduced activity
Behaviour	Distance moved (per unit time)	Decreased value = reduced movement
Behaviour	Conspecific interaction (n of approaches, time spent)	Decreased value = lower interaction with conspecific
Behaviour	Defence (protection against external stimulus)	Decreased value = less time defending
Behaviour	Feeding (amount ingested, feeding per day, leaf loss)	Decreased value = less time feeding, or food ingested
Behaviour	Mate choice	Decreased value = less time choosing mates
Development	Emergence (includes ratio and %)	Increased value = longer time before emerging
Development	Hatching success (at specific time)	Decreased value = lower hatching success
Development	Hatching time (includes time to first hatch)	Increased value* = increased time to hatch
Development	Metamorphosis (includes rate, and developmental stage, instar stage)	Increased value = longer time to metamorph
Development	Moulting frequency	Decreased = faster moulting rate
Development	Pupation (number of animals reaching pupation at specific time)	Decreased = less animals pupating
Development	Regeneration of segments (for invertebrates)	Decreased = less segment regenerated at specific time
Development	Yolk sac area	Decreased value = smaller yolk sac
Cardiovascular	Blood cell count (includes RBC and haemocyte count)	Decreased value = less blood cells
Cardiovascular	Blood flow (includes RBC velocity)	Decreased value = reduced blood flow
Cardiovascular	Cardiac output	Decreased value = decrease cardiac output

Cardiovascular	Cardiac SERCA activity	Decreased value = decrease SERCA activity
Cardiovascular	Haematocrit	Decreased value = decrease haematocrit level
Cardiovascular	Haemoglobin	Decreased value = decreased haemoglobin level
Cardiovascular	Heart rate (during resting, active, and dorsal blood vessel pulse rate in inverts)	Decreased value = lower heart rate
Cardiovascular	Vessel diameter	Decreased value = smaller vessel diameter
Energy metabolism	Catalase activity as part of the metabolic process of the antioxidation response in mitochondria (whole-organism or organ-tissue-specific activity)	Decreased value = lower level of catalase activity
Energy metabolism	Hepatosomatic index (HSI; liver size relative to body size)	Decreased value = smaller relative liver size
Energy metabolism	O <sub>2</sub> consumption (includes whole-organism and cellular respiration)	Decreased value = reduced O <sub>2</sub> consumption
Energy metabolism	Pyruvate	Decreased value = less cellular respiration
Energy metabolism	Ventilation	Decreased value = decreased O <sub>2</sub> consumption
Growth	Body size (include length and mass)	Decreased value = smaller organism.
Growth	Cell count (number, density, biomass)	Decreased value = less cells present
Growth	Growth rate (cell growth rate for single-celled organisms and whole animal growth rate for multicellular organisms)	Decreased value = lower growth rate
Growth	Leaf elongation (includes size and rate of growth)	Decreased value = reduced size/growth of leaf
Immune function	Lysozyme activity (whole-organism or organ-tissue-specific activity)	Decreased value = lower level of lysozyme activity
Immune function	Neutrophil count	Decreased value = less circulating neutrophil
Immune function	Phagocytosis activity	Decreased value = lower level of phagocytosis activity
Immune function	White blood cell (WBC) count	Decreased value = less circulating WBCs
Locomotion	Critical swimming speed ( <i>U</i> <sub>crit</sub> )	Decreased value = lower <i>U</i> <sub>crit</sub>
Locomotion	Lipid levels in muscle	Decreased value = less lipids in muscles
Locomotion	Muscle SERCA activity	Decreased value = less SERCA activity
Locomotion	Muscle protein levels	Decreased value = less proteins in muscles
Locomotion	Speed (average, spontaneous, and high speed)	Decreased value = lower speed

Plant-specific	Chlorophyll a (abundance and activity)	Decreased value = less chlorophyll
Plant-specific	Photosynthesis (includes gross primary production)	Decreased value = reduced photosynthesis activity
Survival	Survival (number of organisms or percentage alive in a given treatment). Mortality rates were converted to survival.	Decreased values = fewer individuals in treatment
Reproductive	Atretic follicles	Decreased value = smaller atretic follicles
Reproductive	Brood size	Decreased value = smaller brood
Reproductive	Egg quantity (number of eggs per female)	Decreased value = less eggs produced
Reproductive	Egg size	Decreased value = smaller eggs
Reproductive	Estradiol (E2) level (includes whole-organism, in plasma, or organs)	Decreased value = lower E2 levels
Reproductive	Fertilisation rate	Decreased value = less eggs fertilised
Reproductive	Gonad mass (both testis and ovaries and including gonadosomatic index, relative to body size)	Decreased value = smaller gonad size
Reproductive	Mature oocyte	Decreased value = less mature oocyte at specific time
Reproductive	Offspring output	Decreased value = less offspring produced
Reproductive	11-Ketotestosterone (11-KT) level	Decreased value = less circulating 11-KT level
Reproductive	Sperm concentration (density/volume)	Decreased value = lower sperm concentration
Reproductive	Sperm movement (velocity/flagella oscillation/motility/duration)	Decreased value = slower sperm activity
Reproductive	Testosterone level (includes whole-organism, in plasma, or organs)	Decreased value = lower testosterone levels
Reproductive	Vitellogenin (vtg) level (includes whole-organism, in plasma, or organs)	Increased value = higher Vtg levels
Thyroid response	Triiodothyronine (T3) level	Decreased value = less circulating T3 levels
Thyroid response	Thyroxine (T4) level	Decreased value = less circulating T4 levels
Thyroid response	Thyroid stimulating hormone (TSH)	Decreased value = less circulating TSH levels

**Table S5.** Number of species and observation for each taxonomic group for BPA levels found in the wild.

Taxon	Example organisms	Number of species	Number of observations
Crustaceans	tiger prawn, amphipod, lobster	8	18
Fish	Grey mullet, grass carp, Greenland shark	117	291
Mammals	Harbour seal, pilot whale	2	2
Molluscs	Mudsnail, mussels, clams	33	96
Other invertebrates	Polychaete worm, insects, flatworm	3	4
Plankton	Phytoplankton, zooplankton	Species not specified in study	23
Reptiles	Loggerhead turtle	1	3

**Table S6.** Number of observations of specific responses used in the meta-analysis

Trait	Specific responses	Number of effect size	Number of studies	Number of species	Number of taxa
Abnormality & damage	Deformity	86	13	10	4
	DNA damage	14	5	3	3
	Eye-snout distance	6	1	1	1
	Eye size	27	3	2	1
	Head-trunk angle	6	1	1	1
	Head width	6	1	1	1
	Interocular distance	6	1	1	1
	Intramuscular lipids	2	1	1	1
	Pericardial edema	49	6	1	1
	ROS production	21	5	3	2
	Sperm damage	6	1	1	1
	Swim bladder area	12	2	1	1
	Yolk sac edema	6	2	1	1
Behaviour	Aggression	5	2	1	1
	Activity	89	13	5	4
	Distance moved	19	5	2	1
	Conspecific interaction	20	4	3	2
	Defence	7	1	1	1
	Feeding	29	7	6	3
	Mate choice	4	1	1	1
Development	Emergence	18	4	2	1
	Hatching success	105	16	6	2
	Hatching time	27	7	3	2
	Metamorphosis	16	5	5	3
	Moulting frequency	24	4	2	1
	Pupation	4	2	1	1
	Regeneration of segments	20	2	2	1
	Yolk sac area	12	2	1	1



Cardiovascular	Blood cell count	23	5	5	3
	Blood flow	9	3	1	1
	Cardiac output	7	2	1	1
	Cardiac SERCA activity	8	1	1	1
	Haematocrit	13	2	2	1
	Haemoglobin	13	2	2	1
	Heart rate	52	9	4	3
	Vessel diameter	6	2	1	1
Energy metabolism	Catalase activity	106	15	13	5
	HSI	56	12	9	2
	O <sub>2</sub> consumption	6	2	2	2
	Pyruvate	2	1	1	1
	Ventilation	7	1	1	1
Growth	Body size	381	44	24	6
	Cell count	89	9	8	1
	Growth rate	381	44	24	6
	Leaf elongation	60	1	1	1
Immune function	Lysozyme activity	11	2	2	2
	Neutrophil count	9	2	2	1
	Phagocytosis activity	7	2	2	2
	WBC count	5	1	1	1
Locomotion	Ucrit	10	2	1	1
	Muscle SERCA activity	8	1	1	1
	Muscle protein levels	2	1	1	1
	Speed	25	5	2	1
Plant-specific	Chlorophyll a	112	11	13	2
	Photosynthesis	39	4	5	1
Survival	Survival	383	34	25	6
Reproduction	Atretic follicles	5	2	2	1
	Brood size	15	4	3	2
	Egg quantity	61	16	9	4
	Egg size	4	2	2	1
	E2 level	63	15	11	2
	Fertilisation rate	17	4	3	2
	Gonad mass	99	21	9	1
	Mature oocyte	4	1	1	1
	Offspring output	69	13	7	2
	11-KT level	18	4	2	1
	Sperm concentration	23	8	5	1
	Sperm movement	27	7	4	1
	Testosterone level	56	13	11	3
	Vitellogenin (vtg) level	147	24	13	2
Thyroid response	T3 level	9	3	2	1
	T4 level	15	4	3	2
	TSH	7	2	2	2

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**Table S7.** Total heterogeneities ( $I^2$ ) and heterogeneities at each hierarchical level (fitted as random effects) across different moderators

Data	I2 [species]	I2 [study ID]	I2 [effect size]	I2 [total]
Overall	0.17	0.22	0.61	1.00
Hierarchy	0.16	0.21	0.63	1.00
Traits	0.09	0.18	0.73	1.00
Taxa	0.15	0.23	0.62	1.00
Development	0.07	0.23	0.70	1.00
Sex	0.27	0.26	0.47	1.00

**Table S8.** Summary model output from non-phylogenetic multilevel meta-analyses with continuous moderators (BPA dosage, exposure period, and test temperature). LCI and UCI denotes the lower and upper confidence limits for 95% confidence intervals. Estimates and number of observations (levels) for each random effect were included in the model output. Bolded estimates indicate significance, where the confidence intervals do not overlap with zero. Asterisks indicate level of significance (‘.’  $P < 0.1$ , ‘\*’  $P < 0.05$ , ‘\*\*’  $P < 0.01$ , and ‘\*\*\*’  $P < 0.001$ ).

Continuous moderators						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
Intercept	-0.01	0.29	-0.04	0.96	-0.58	0.55
<b>BPA dosage</b>	<b>-0</b>	<b>0</b>	<b>-9.0</b>	<b>&lt;0.0001</b>	<b>-0</b>	<b>-0</b>
Exposure period	0.0008	0.0007	1.17	0.24	-0	0.002
Temperature	-0.007	0.01	-0.59	0.55	-0.03	0.01
Random effects	Estimate	Levels				
Species	0.16	83				
Study ID	0.2	161				
Effect size	0.49	2741				

**Table S9.** Summary model output from the multilevel meta-analyses (non-phylogenetically corrected) with either sex and hierarchical levels as moderators. LCI and UCI denotes the lower and upper confidence limits for 95% confidence intervals. Estimates and number of observations (levels) for each random effect were included in the model output. Bolded estimates indicate significance, where the confidence intervals do not overlap with zero. Asterisks indicate level of significance (‘.’  $P < 0.1$ , ‘\*’  $P < 0.05$ , ‘\*\*’  $P < 0.01$ , and ‘\*\*\*’  $P < 0.001$ ).

Sex						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
Both	-0.24	0.10	-2.51	<b>0.01</b>	-0.44	-0.05
Female	-0.05	0.07	-0.75	0.46	-0.18	0.08
Male	0.03	0.07	0.45	0.65	-0.10	0.17
Random effects	Estimate	Levels				
Species	0.07	41				
Study ID	0.07	83				
Effect size	0.13	975				
Hierarchical level						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
Macromolecules	0.18	0.09	1.93	0.054	0.00	0.37

<b>Cellular</b>	-0.16	0.07	-2.23	<b>0.026</b>	-0.31	-0.02	*
<b>Tissue</b>	-0.97	0.13	-7.59	<b>&lt;0.0001</b>	-1.22	-0.72	***
<b>Organ</b>	-0.27	0.08	-3.40	<b>0.0007</b>	-0.43	-0.12	***
<b>Organism</b>	-0.28	0.06	-4.35	<b>&lt;0.0001</b>	-0.41	-0.16	***
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.12	90					
Study ID	0.16	172					
Effect size	0.48	2886					

**Table S10.** Summary model output from non-phylogenetic multilevel meta-analyses with the overall functional traits and top five responses for each functional trait [sub-level] as moderators. LCI and UCI denotes the lower and upper confidence limits for 95% confidence intervals. Estimates and number of observations (levels) for each random effect were included in the model output. Bolded estimates indicate significance, where the confidence intervals do not overlap with zero. Asterisks indicate level of significance (‘.’  $P < 0.1$ , ‘\*’  $P < 0.05$ , ‘\*\*’  $P < 0.01$ , and ‘\*\*\*’  $P < 0.001$ ).

Functional traits							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
<b>Abnormality &amp; damage</b>	-0.66	0.09	-7.54	<b>&lt;0.0001</b>	-0.83	-0.49	***
<b>Behaviour</b>	-0.29	0.10	-2.84	<b>0.0045</b>	-0.49	-0.09	**
Cardiovascular	-0.23	0.12	-1.90	<b>0.0574</b>	-0.46	0.01	.
<b>Development</b>	-0.32	0.08	-3.91	<b>&lt;0.0001</b>	-0.48	-0.16	***
Energy metabolism	0.00	0.08	-0.01	0.9933	-0.17	0.16	
Growth	-0.12	0.06	-1.91	0.0556	-0.23	0.0027	.
Immune function	0.19	0.18	1.06	0.2901	-0.16	0.54	
Locomotion	-0.23	0.16	-1.51	0.132	-0.54	0.07	
<b>Plant-specific</b>	-0.33	0.10	-3.30	<b>0.001</b>	-0.53	-0.14	***
Reproduction	-0.06	0.07	-0.89	0.3754	-0.19	0.07	
<b>Survival</b>	-0.57	0.08	-7.30	<b>&lt;0.0001</b>	-0.73	-0.42	***
Thyroid response	-0.19	0.21	-0.93	0.3545	-0.61	0.22	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.07	90					
Study ID	0.12	172					
Effect size	0.50	2883					
Sub-level [Abnormality]							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
<b>Deformity</b>	-1.16	0.25	-4.71	<b>&lt;0.0001</b>	-1.64	-0.67	***
DNA damage	-0.57	0.42	-1.37	0.1712	-1.38	0.25	
<b>Pericardial edema</b>	-1.45	0.29	-5.07	<b>&lt;0.0001</b>	-2.01	-0.89	***
ROS production	-0.12	0.42	-0.29	0.7723	-0.94	0.70	
Swim bladder area	-0.70	0.43	-1.62	0.1044	-1.54	0.14	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.00	15					
Study ID	0.54	26					
Effect size	0.76	194					
Sub-level [Behaviour]							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
<b>Activity</b>	-0.54	0.22	-2.44	<b>0.0147</b>	-0.97	-0.11	*
Distance moved	-0.58	0.43	-1.34	0.1792	-1.42	0.27	

Conspecific interaction	-0.37	0.34	-1.09	0.276	-1.03	0.29	
Feeding	-0.15	0.24	-0.63	0.5305	-0.62	0.32	
<b>Mate choice</b>	-1.59	0.53	-3.01	<b>0.0026</b>	-2.63	-0.56	<b>**</b>
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.21	9					
Study ID	0.00	21					
Effect size	0.63	161					
<b>Sub-level [Cardiovascular]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
Blood cell count	-0.15	0.08	-1.82	0.068	-0.31	0.01	.
Blood flow	0.05	0.11	0.43	0.668	-0.16	0.25	
Haematocrit	-0.18	0.10	-1.86	0.0624	-0.36	0.01	.
<b>Haemoglobin</b>	-0.34	0.09	-3.69	<b>0.0002</b>	-0.52	-0.16	<b>***</b>
Heart rate	0.02	0.08	0.28	0.7791	-0.14	0.18	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.02	8					
Study ID	0.00	13					
Effect size	0.01	110					
<b>Sub-level [Development]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
Emergence	-0.62	0.32	-1.96	0.0505	-1.24	0.00	.
<b>Hatching success</b>	-0.52	0.14	-3.73	<b>0.0002</b>	-0.80	-0.25	<b>***</b>
Hatching time	-0.10	0.21	-0.49	0.6227	-0.51	0.31	
Metamorphosis	-0.25	0.29	-0.87	0.3831	-0.81	0.31	
Moulting frequency	-0.14	0.27	-0.51	0.6095	-0.67	0.39	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.00	17					
Study ID	0.12	32					
Effect size	0.76	190					
<b>Sub-level [Energy metabolism]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
Catalase activity	0.1049	0.0558	1.8812	0.0599	-0.0044	0.2142	.
Hepatosomatic index	0.06	0.06	0.98	0.3259	-0.06	0.17	
O2 consumption	0.01	0.11	0.08	0.9347	-0.21	0.22	
Pyruvate	-0.01	0.19	-0.06	0.9556	-0.38	0.36	
Ventilation	-0.25	0.18	-1.38	0.1679	-0.61	0.11	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.02	24					
Study ID	0.00	30					
Effect size	0.02	177					
<b>Sub-level [Growth]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
Body size	-0.03	0.04	-0.95	0.3419	-0.11	0.04	
<b>Cell count</b>	-0.48	0.06	-8.18	<b>&lt;0.0001</b>	-0.60	-0.37	<b>***</b>
<b>Growth rate</b>	-0.10	0.04	-2.34	<b>0.0194</b>	-0.19	-0.02	<b>*</b>
Leaf elongation	-0.28	0.15	-1.85	0.0638	-0.57	0.02	.
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.02	43					
Study ID	0.00	64					
Effect size	0.06	670					

Sub-level [Immune function]						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
Lysozyme activity	-0.01	0.07	-0.14	0.8886	-0.14	0.12
Neutrophil	0.82	0.14	5.82	<0.0001	0.55	1.10
Phagocytosis	-0.63	0.08	-7.58	<0.0001	-0.79	-0.46
WBC count	0.53	0.09	6.01	<0.0001	0.36	0.70
Random effects	Estimate	Levels				
Species	0.00	6				
Study ID	0.00	6				
Effect size	0.00	32				
Sub-level [Locomotion]						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
Muscle SERCA activity	-0.28	0.21	-1.34	0.1796	-0.70	0.13
Speed	-0.06	0.04	-1.40	0.163	-0.14	0.02
Ucrit	-0.05	0.05	-0.84	0.4021	-0.15	0.06
Random effects	Estimate	Levels				
Species	0	2				
Study ID	0.0042	7				
Effect size	0.0003	43				
Sub-level [Plant-specific]						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
Chlorophyll a	-0.57	0.12	-5.00	<0.0001	-0.80	-0.35
Photosynthesis	-0.44	0.15	-2.92	0.0035	-0.74	-0.14
Random effects	Estimate	Levels				
Species	0.05	14				
Study ID	0.06	12				
Effect size	0.27	151				
Sub-level [Reproduction]						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
E2 level	0.10	0.13	0.78	0.4367	-0.16	0.37
Egg quantity	-0.07	0.17	-0.41	0.684	-0.41	0.27
Gonad mass	-0.10	0.11	-0.96	0.335	-0.32	0.11
Offspring output	-0.52	0.20	-2.62	0.0088	-0.90	-0.13
Vtg level	0.65	0.12	5.59	<0.0001	0.42	0.87
Random effects	Estimate	Levels				
Species	0.07	34				
Study ID	0.06	65				
Effect size	0.23	439				
Sub-level [Thyroid response]						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
T3 level	-0.43	0.18	-2.39	0.017	-0.78	-0.08
T4 level	-0.34	0.18	-1.89	0.0593	-0.69	0.01
TSH level	0.08	0.17	0.45	0.6499	-0.26	0.42
Random effects	Estimate	Levels				
Species	0.09	4				
Study ID	0.02	5				
Effect size	0.0008	31				
Sub-level [Survival]						
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI
	-0.79	0.13	-5.94	<0.0001	-1.05	-0.53

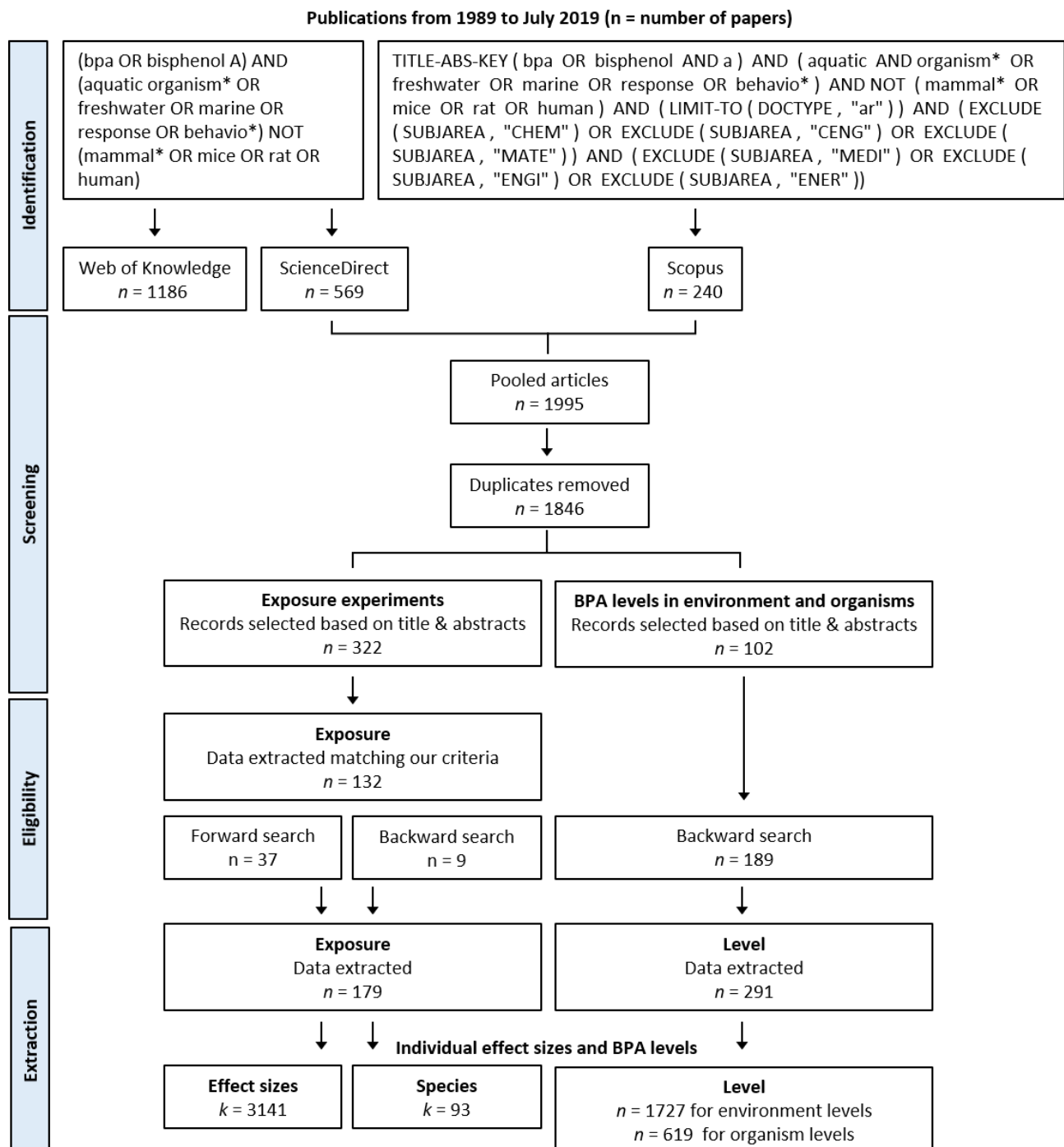
Random effects	Estimate	Levels
Species	0.00	25
Study ID	0.26	33
Effect size	2.12	383

**Table S11.** Summary model output from non-phylogenetic multilevel meta-analyses with either taxa and developmental stage as moderators. LCI and UCI denotes the lower and upper confidence limits for 95% confidence intervals. Sub-level analysis of developmental stage for each taxa were also presented. Estimates and number of observations (levels) for each random effect were included in the model output. Bolded estimates indicate significance, where the confidence intervals do not overlap with zero. Asterisks indicate level of significance (‘.’  $P < 0.1$ , ‘\*’  $P < 0.05$ , ‘\*\*’  $P < 0.01$ , and ‘\*\*\*’  $P < 0.001$ ).

Between taxa							
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI	
Amphibians	-0.53	0.22	-2.46	<b>0.0138</b>	-0.95	-0.11	*
Angiosperms	0.27	0.38	0.71	0.4798	-0.47	1.00	
Crustaceans	-0.01	0.15	-0.09	0.9258	-0.31	0.28	
Fish	-0.13	0.10	-1.25	0.2123	-0.33	0.07	
Insects	-0.25	0.29	-0.85	0.3975	-0.82	0.33	
Macroalgae	-0.57	0.51	-1.11	0.2675	-1.57	0.4348	
Molluscs	-0.57	0.19	-3.00	<b>0.0027</b>	-0.94	-0.20	**
Other invertebrates	-0.41	0.19	-2.14	<b>0.0321</b>	-0.79	-0.04	*
Phytoplankton	-0.07	0.14	-0.52	0.6016	-0.35	0.20	
Random effects	Estimate	Levels					
Species	0.12	90					
Study ID	0.18	172					
Effect size	0.50	2883					
Developmental period							
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI	
Embryo	-0.38	0.10	-3.98	<b>&lt;0.0001</b>	-0.57	-0.19	***
Larvae	-0.41	0.10	-4.04	<b>&lt;0.0001</b>	-0.61	-0.21	***
Juvenile	-0.25	0.09	-2.65	<b>0.0081</b>	-0.43	-0.06	**
Adult	-0.11	0.07	-1.74	0.082	-0.24	0.01	.
Random effects	Estimate	Levels					
Species	0.06	74					
Study ID	0.18	158					
Effect size	0.54	2519					
Sub-level [Angiosperms]							
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI	
Juvenile	-0.26	0.16	-1.61	0.1079	-0.58	0.06	
Adult	-0.08	0.18	-0.43	0.6657	-0.43	0.27	
Random effects	Estimate	Levels					
Species	0.01	2					
Study ID	0.01	2					
Effect size	0.03	65					
Sub-level [Molluscs]							
Fixed effects	Estimate	s.e.	z value	P value	LCI	UCI	
Embryo	-0.71	0.32	-2.21	<b>0.0269</b>	-1.33	-0.08	*
Juvenile	-0.51	0.26	-1.95	0.0509	-1.02	0.00	.

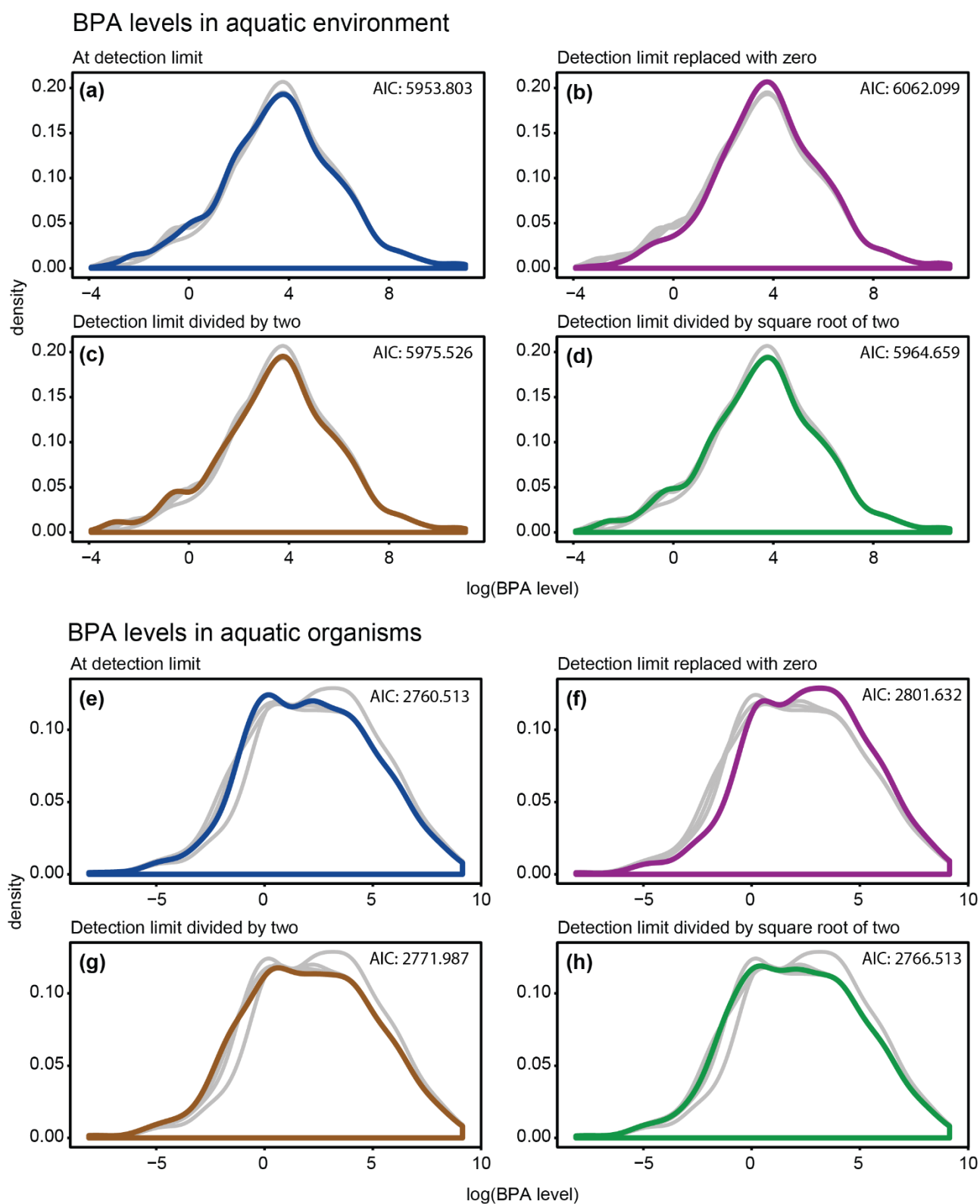
Adult	-0.38	0.23	-1.66	0.0977	-0.84	0.07	.
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.00	9					
Study ID	0.48	13					
Effect size	0.58	200					
<b>Sub-level [Other invertebrates]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
<b>Embryo</b>	-0.62	0.27	-2.30	<b>0.0213</b>	-1.14	-0.09	*
Larvae	-0.30	0.29	-1.04	0.2967	-0.86	0.26	
<b>Juvenile</b>	-0.91	0.30	-3.08	<b>0.0021</b>	-1.50	-0.33	**
<b>Adult</b>	-0.47	0.17	-2.78	<b>0.0055</b>	-0.80	-0.14	**
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.04	9					
Study ID	0.00	10					
Effect size	0.74	102					
<b>Sub-level [Insects]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
<b>Larvae</b>	-0.45	0.16	-2.79	<b>0.0053</b>	-0.77	-0.13	**
Adult	-0.15	0.36	-0.41	0.6783	-0.86	0.56	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.00	2					
Study ID	0.08	9					
Effect size	0.85	77					
<b>Sub-level [Crustacean]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
Larvae	0.00	0.30	0.01	0.9919	-0.58	0.58	
<b>Juvenile</b>	-0.51	0.15	-3.30	<b>0.001</b>	-0.81	-0.21	***
Adult	-0.10	0.12	-0.89	0.3719	-0.33	0.13	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.03	13					
Study ID	0.09	22					
Effect size	0.45	376					
<b>Sub-level [Amphibian]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
Embryo	-0.08	0.45	-0.18	0.8558	-0.96	0.79	
<b>Larvae</b>	-0.93	0.39	-2.35	<b>0.0189</b>	-1.70	-0.15	*
Adult	-0.03	0.83	-0.03	0.9746	-1.66	1.61	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.00	11					
Study ID	0.75	12					
Effect size	2.21	113					
<b>Sub-level [Fish]</b>							
<b>Fixed effects</b>	<b>Estimate</b>	<b>s.e.</b>	<b>z value</b>	<b>P value</b>	<b>LCI</b>	<b>UCI</b>	
<b>embryo</b>	-0.33	0.11	-3.15	<b>0.0017</b>	-0.54	-0.13	**
larvae	-0.21	0.14	-1.50	0.1338	-0.49	0.06	
juvenile	-0.07	0.13	-0.52	0.6065	-0.31	0.18	
adult	-0.05	0.07	-0.65	0.5145	-0.19	0.10	
<b>Random effects</b>	<b>Estimate</b>	<b>Levels</b>					
Species	0.01	27					
Study ID	0.18	99					

## Supplementary figures

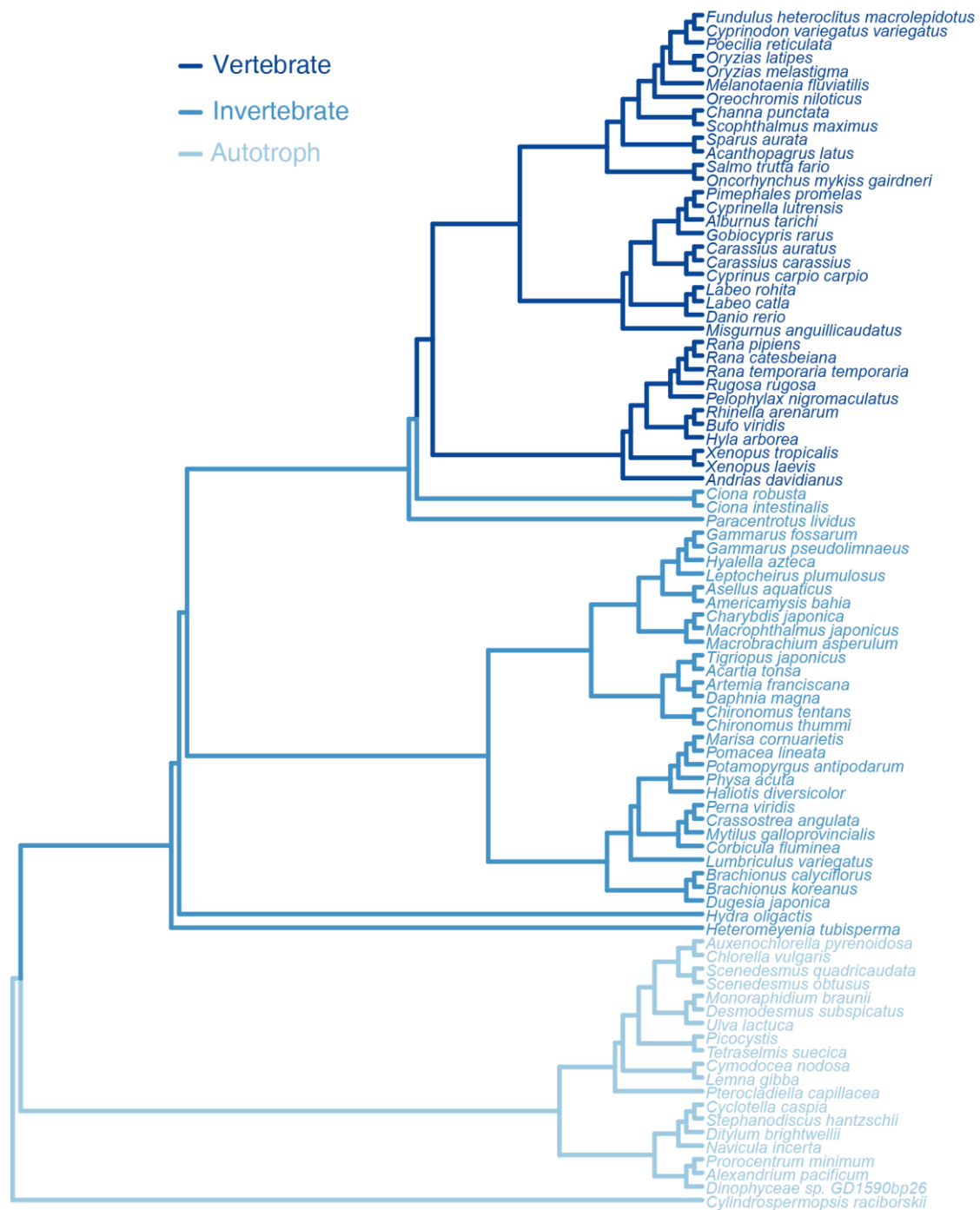


**Fig. S1. PRISMA flow diagram for the systematic data-collection process.** Search terms for each online database were included. *n* = number of papers remaining after each stage of selection. *K* = number of effect size after processing individual data, and *n* is number of observations after processing data. All studies on exposure experiments were obtained under laboratory conditions, and BPA levels in aquatic organisms were obtained from field studies.

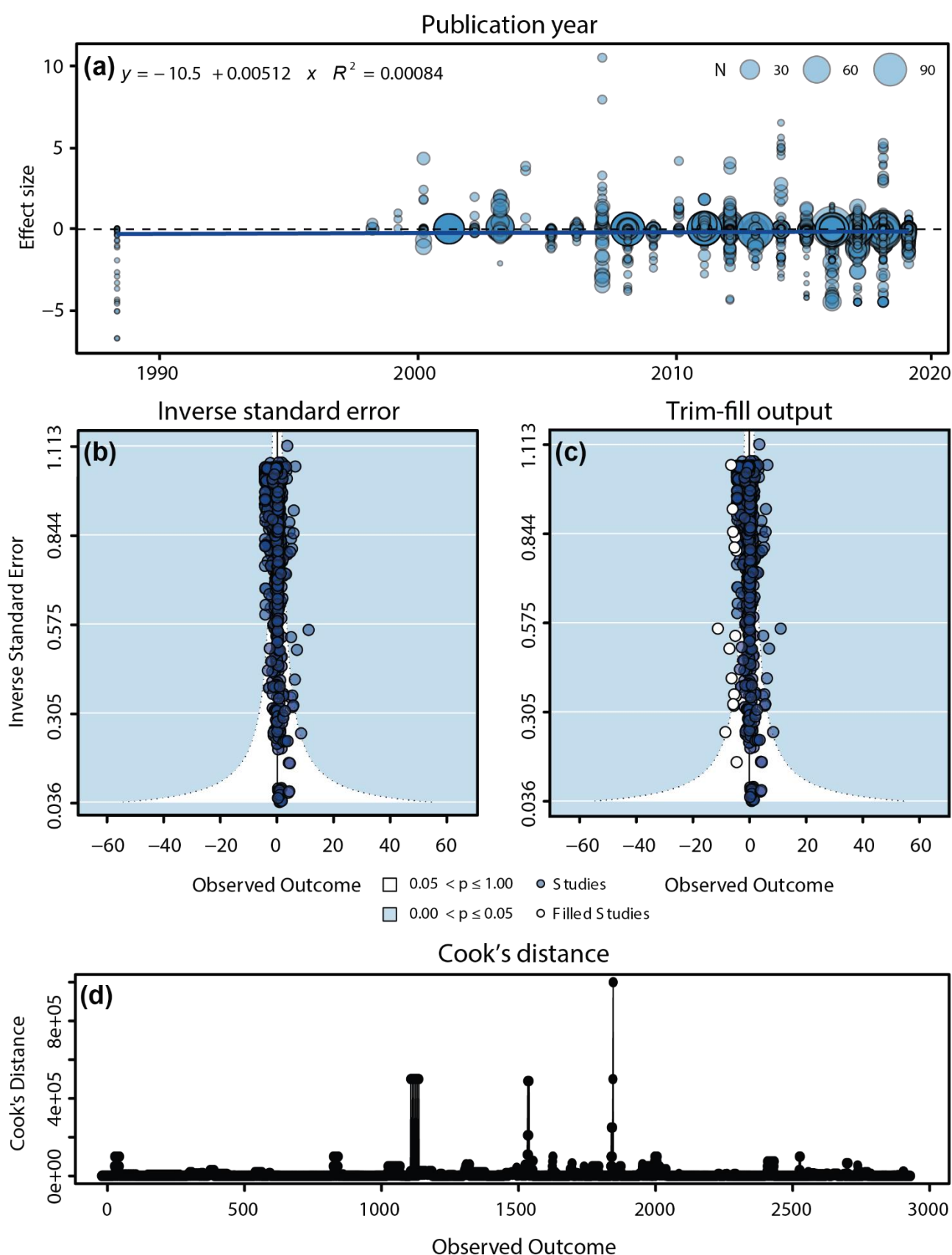




**Fig. S2. Distribution of BPA levels from the aquatic environment (a–d), and in aquatic organisms (e–h) using different methods of detection limit handling. Akaike information criterion (AIC) output presented on the top right corner of each figure.**

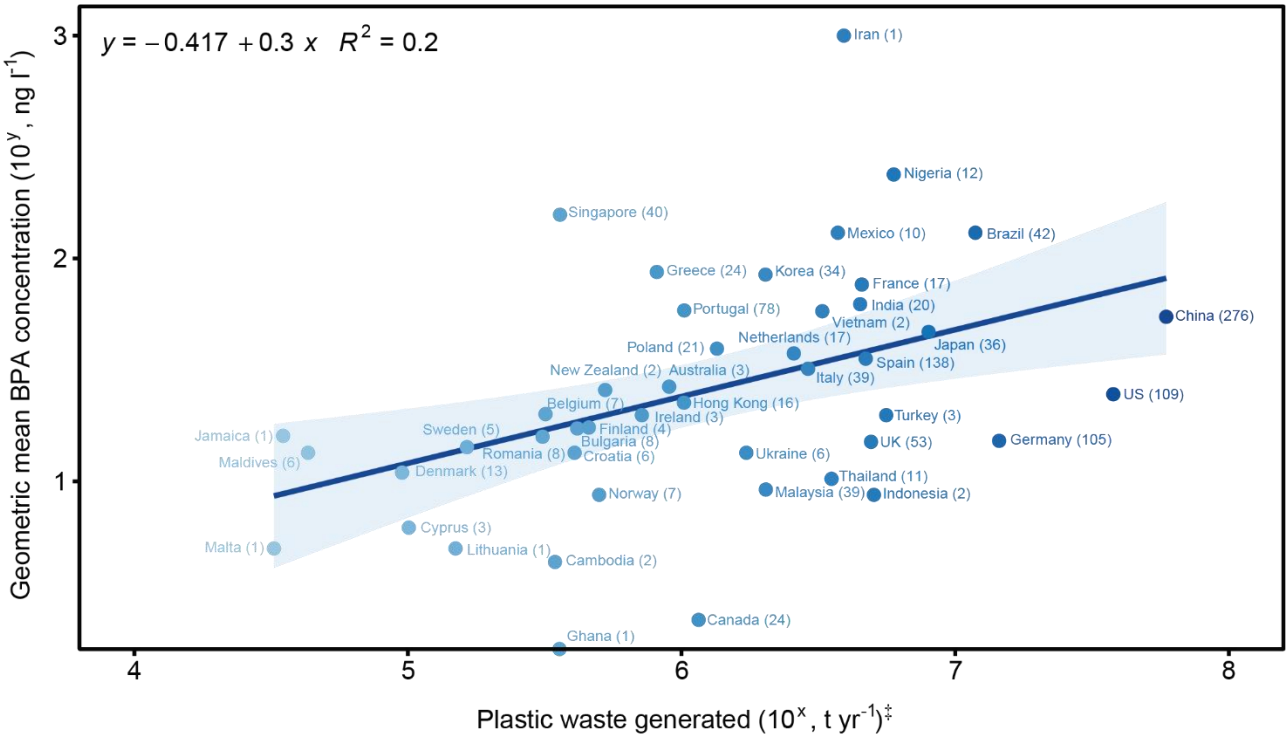


**Fig. S3. Estimated phylogenetic reconstruction of all species included in the analysis based on the Open Tree of Life (<https://tree.opentreeoflife.org/>). The 88 species that were used to produce a correlation matrix for the phylogenetic multilevel model are shown.**

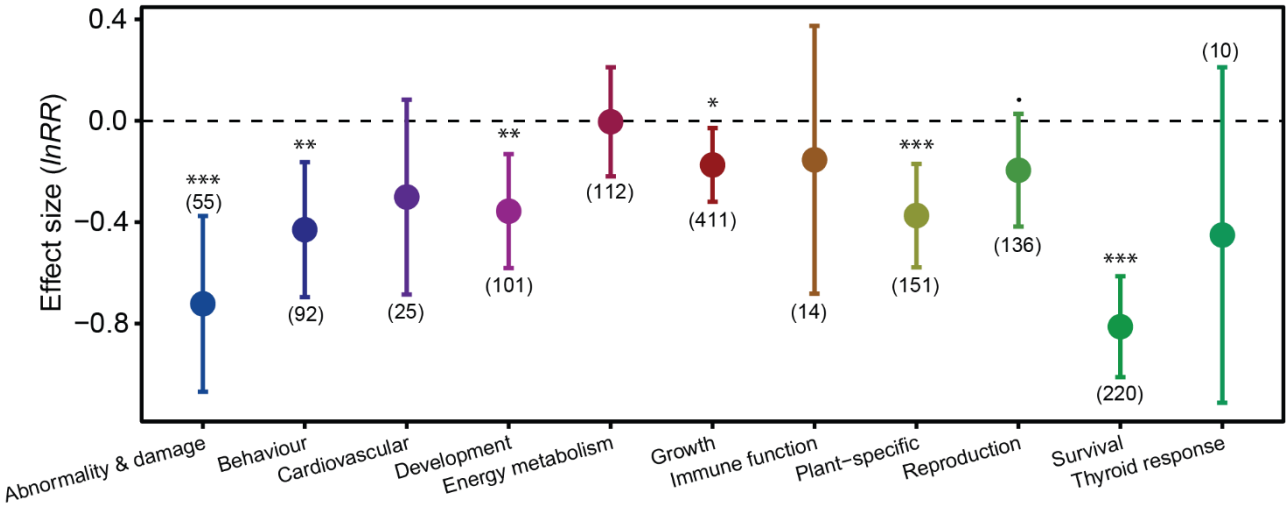


**Fig. S4. Publication bias outcomes.** (a) Relationship between effect size ( $\ln RRR$ ) and the year of publication to examine evidence of time-bias. The dashed line represents the meta-analytic mean from the overall non-phylogenetic model, and the solid line represents model prediction.  $N$  = sample size of each effect calculated. (b) Funnel plot of the observed outcome residuals (filled dots) from the overall non-phylogenetic model against the inverse standard error (precision). (c) Funnel plot with the trim-filled model (empty dots) for the estimated number of studies missing from the meta-analysis.

Dotted lines represent the 95% pseudo CI. (d) Cooks distance output of the outcome residuals from the overall non-phylogenetic model.



**Fig. S5. Relationship between mean bisphenol A (BPA) concentrations (ng g<sup>-1</sup>) in natural surface waters with the total plastic waste generated (t yr<sup>-1</sup>) per country.** Data presented as individual data points (geometric mean BPA levels per country with sample size) on a log-to-log scale (10<sup>x</sup> or <sup>y</sup>). Regression line represent model fit ( $\pm$  95% confidence interval shading), and equation in the top-left corners depict the linear model fit. ‡ obtained from Jambeck et al. (2015) for the year 2010.



**Fig. S6. Overall effect sizes of BPA exposure on the 12 traits categories with the exclusion of fish.** Data are presented as mean effect sizes (log response ratio; *lnRR*)  $\pm$  95 % CI, and numbers within parentheses represent the number of effect

sizes in the sample. Horizontal dashed line at zero indicates no effect, and negative values represent negative effects on biological functions. Effect sizes were considered significant when 95 % CI do not overlap with the zero line as denoted by asterisks ('.'  $P < 0.1$ , '\*'  $P < 0.05$ , '\*\*'  $P < 0.01$ , and '\*\*\*'  $P < 0.001$ ).

## References

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- Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R. and Law, K. L.** (2015). Plastic waste inputs from land into the ocean. *Science* **347**, 768-771.