**Introduction to Special Issue: Disruption of thyroid, sex steroid, and adrenal hormone
systems and their crosstalk in aquatic wildlife**

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"The Greeks call it *lygos*, sometimes *agnos*, because the Athenian matrons, preserving their chastity at the Thesmophoria, strew their beds with its leaves".

Pliny the Elder, referring to leaves of the chasteberry tree (*Vitex agnus- castus*) (Riddle, 2010).

1. Background

There is no precise way of telling how far back in history people have intentionally used various naturally growing herbs, such as chasteberry, for their ability to disrupt normal reproduction and pregnancy. Based on the written works of ancient scholars such as Hippocrates, Dioscorides, and Pliny the Elder (Riddle, 1994) the practice goes back at least a few thousands of years. The idea that we might be unintentionally exposed to chemicals in the environment with similar disruptive potential, however, is relatively recent and can be traced back to the 1930s-1940s. Observations by Walker and Janney (1930), Dodds et al. (1939), and Emmens (1941) indicated that many natural and anthropogenic compounds have estrogenic properties in laboratory animals. Work by these investigators was supplemented by published accounts of livestock exhibiting reproductive abnormalities such as infertility, and the determination that the cause of these observations was the presence of natural xenoestrogens in certain pasture plants (Biggers and Curnow, 1954). Accounts of endocrine disruption caused by anthropogenic chemicals were not restricted only to effects on reproduction or to agriculturally important livestock. In the late 1970s, John Leatherland and colleagues (Moccia et al., 1977) revealed a puzzling increase in thyroid problems of salmonids from the Laurentian Great Lakes, which was

likely due to exposure to still undefined (Carr and Patiño, 2011) environmental endocrine-disrupting chemicals (EDCs).

Despite the earlier published observations, it was not until a conference organized by the World Wildlife Federation in 1991 produced an influential “Consensus Statement” (Bern et al., 1992) that the then loosely-organized research of EDCs was transformed into a well-defined area of scientific inquiry covering health concerns of both humans and wildlife (Matthiessen, 2003). The broader context of research in endocrine disruption is the field of environmental endocrinology, which in turn derives from studies in comparative endocrinology (Denver et al., 2009). Classical comparative endocrinology has been concerned with adaptive and evolutionary features of endocrine mechanisms (Gorbman, 1988) and has been based on either laboratory or field studies, while environmental endocrinology has come to focus on endocrine measurements and mechanisms in nature (Bradshaw, 2007). Research in endocrine disruption grew at a remarkable pace during the first decade after publication of the Consensus Statement, as indicated by the increasing number of published articles that included the terms “endocrine” and “disrupt*” in their titles, abstracts, or keywords (Matthiessen, 2003). We have updated the status of EDC publications using the approach of Matthiessen (2003). The database we used was Web of Science™ Core Collection, which contains articles published since 1981. Although the search terms used may capture some out-of-context articles, results obtained can still be useful for comparative purposes. The first article containing the search terms appeared in 1986, and as of the end of 2014 there was a cumulative total of 15,959 publications. Trend analysis was conducted using results since 1996 because values were low and variable in the earlier period. From 1996 to 2014, the rate of increase was steady at about 88 additional publications year⁻¹ (Fig. 1). Many of these articles, however, describe the results of laboratory studies with limited

ecological applications (Marty et al., 2011). We added the term “population” to the search to estimate the fraction of articles with self-described ecological/population-level implications. The resulting subset was relatively small and showed a rate of increase of about 10 publications year⁻¹ (Fig. 1) but, when expressed as percentage, it remained steady at an average of about 11% of total EDC articles published each year. A closer look at the 198 studies with reference to populations that were published in 2014 revealed that 70 (35%) dealt with wildlife, mostly fish or amphibian species. These observations numerically confirm what is already evident to practitioners, namely, that endocrine disruption has matured as a distinct area of research within environmental endocrinology. They also suggest that while EDC studies with overt population or ecological context are only a small fraction of the total, wildlife is well represented among them.

Anthropogenic EDCs are widely dispersed around the globe and also found in places distant from population centers regardless of latitude (AMAP, 2002; Caroli et al., 2001) or altitude (Wang et al., 2006). Their long-range transport can occur not only via physical (e.g., atmospheric and oceanic currents) but also biological vectors (Blais et al., 2007). The ubiquitous presence of EDCs arguably has made their consideration as potential explanatory variables for biological phenomena a requirement of field studies, whether or not their influence on biological condition is a primary target of investigation. The geographic distribution of specific EDCs and their concentrations can vary widely, however, depending on their sources and environmental persistence, thus making knowledge of regional and local EDCs an important variable to consider for proper assessment of corresponding biological impacts. But even when this information is available, the complexity of biotic and abiotic interactions that exist in nature makes the evaluation of endocrine disruption as a mechanism linking EDC exposures to individual or population responses a daunting task. This is especially notable in cases where

EDCs are present at concentrations that are not overtly toxic to organisms. It is not surprising, therefore, that conclusive evidence pointing to endocrine disruption as primary driver of wildlife population declines is limited to a handful of examples (Jobling et al., 2013). Laboratory experimentation under controlled conditions is thus necessary to establish and characterize cause-effect relationships. Exposure concentrations or dosages used in laboratory studies, however, must in turn be justified on the basis of known or anticipated exposure levels in the field. In addition, because EDCs are typically present in the environment as complex mixtures whose effects on organisms may not represent the simple sum of individual EDC effects, laboratory studies should be based on environmentally relevant mixtures and concentrations. Further progress in our understanding of ecologically relevant wildlife endocrine disruption will thus require field and laboratory studies with mutually informed objectives and designs.

While research into disruption of thyroid, reproductive, and adrenal axes in aquatic wildlife has been ongoing for decades, it is only within the last 20+ years that scientists have identified specific targets of EDCs within these endocrine axes. This research has been facilitated by several factors including (1) the development of model systems or assays for quantifying impacts on hormone production, development and reproduction, (2) heterologous expression systems for examining EDC-receptor interactions, and (3) tools for identifying histopathologies in endocrine tissues of non-model animal species (Hontela, 1998; Carr and Patiño, 2011). Studies of EDC effects in aquatic environments present their own set of unique circumstances because, depending upon life history, animals may be directly exposed to (submerged in) EDCs for their entire or large parts of their life cycle and therefore have a correspondingly higher risk of non-dietary exposure to anthropogenic chemicals than most terrestrial species. Some terrestrial species are in close proximity to water and their food chain often begins in, or is closely

integrated with aquatic habitats, and thus can also be exposed to aquatic EDCs via their trophic transfer. Strong interest in particular circumstances surrounding exposures of aquatic animals to EDCs has been driven, in part, by news and media accounts of reproductive abnormalities in fishes (e.g., Blazer et al., 2012) and amphibians (e.g., Skelly et al., 2010), and the mysterious loss of amphibian populations around the globe.

Given the growth in the EDC literature related to wildlife, and the need for scientists, regulators, environmentalists, and other stakeholders to have ready access to research results, it is perhaps surprising that there is not a single peer-reviewed journal dedicated to the comparative study of EDCs. Although there are several environmental toxicology journals that feature studies of EDCs, non-mammalian models, and wildlife, these journals are not focused on and thus do not normally provide a view from the comparative endocrinology perspective. The late Howard Bern, one of the early pioneers in the field of EDCs, was a founding member of the Editorial Board for *General and Comparative Endocrinology* when it launched in April of 1961. *General and Comparative Endocrinology* was the first journal to provide a prominent forum for studies of comparative endocrinology and its first Editor-in-Chief, the late Aubrey Gorbman, authored with Bern the first textbook of comparative endocrinology (Gorbman and Bern, 1962). *General and Comparative Endocrinology* is therefore a fitting venue for a special edition focusing on the impact of EDCs on wildlife endocrine systems. In this special edition we bring together sixteen articles authored by scientists working at the forefront of EDCs and their effects on the thyroid, reproductive, and adrenal axes in aquatic wildlife and other species associated closely with aquatic habitats. Although a major goal for this volume is to publish articles reflecting state-of-the-art research and synthesis in the field of EDCs, we also hope to attract new readers to this journal with interests in the areas of toxicology, environmental science, and regulation but who

may not have been fully aware of the link between comparative endocrinology and the history of EDC research. The collection of papers not only provides a glimpse of how far the area of wildlife endocrine disruption has come since the Consensus Statement was published (Bern et al., 1992), but many of the papers also illustrate how non-mammalian models can provide information to identify new modes of EDC action. In addition, the articles provide a foundation from which to plan future work designed to fill in data gaps and identify research needs for a better understanding of the risk that EDCs pose to the sustainability of aquatic wildlife. While not all articles include the term “population” in their searchable fields, we can assure readers that all have considered the environmental relevance of experimental designs and findings, and three of the articles report observations in the context of exposures of wild populations to complex contaminant mixtures.

2. Special Issue Articles

The consequences of endocrine disruption on organisms and populations reside in the impact that this disruption has on organismal physiology, and ultimately on survival and reproduction. The collection of papers in this volume addresses a number of processes affected by EDC action on the thyroid, reproductive or adrenal axes including iodide homeostasis, somatic growth, metamorphosis, gonadal development and gametogenesis, stress and reproductive physiology, disease resistance, and reproduction.

Amphibian metamorphosis has long been considered a model for studying disruption of the thyroid axis during development (Carr and Patiño, 2011), and its use as an assay for assessing the crosstalk of this axis with other endocrine systems dates back at least to the mid-1940s (Sluzewski and Roth, 1946). In their contribution to this issue, Hammond et al. (2015) show that

the personal care products and EDCs, ibuprofen (a nonsteroidal anti-inflammatory compound) and triclosan (a biocide) disrupt temperature-sensitive, tri-iodothyronine (T₃)-induced changes in gene expression of skin from American Bullfrog [*Rana (Lithobates) catesbeiana*] tadpoles. Carr et al. (2015) report that not only is the sensitivity to the I⁻ transport blocker perchlorate, and environmental EDC, dependent on developmental stage during metamorphosis of the African Clawed Frog (*Xenopus laevis*), but that physiological mechanisms for conserving I⁻ may play a role in this developmental sensitivity.

Amphibians also have been used as models for studying the role of thyroid hormones in the differentiation of gonadal phenotype and, at least in *X. laevis*, thyroid hormones seem to be required for normal testicular development, (Hayes, 1997, 1998; Robertson and Kelley, 1996; Goleman et al., 2002). More recently, von Hippel and colleagues showed that exposure to the thyroid hormone synthesis inhibitor, perchlorate, leads to hermaphroditism in Threespine Stickleback (*Gasterosteus aculeatus*) (Bernhardt et al., 2006), while Patiño and colleagues found that hypo- and hyperthyroidism leads to female- and male-skewed populations of Zebrafish (*Danio rerio*), respectively (Mukhi et al., 2007; Sharma and Patiño, 2013). In this issue, Furin et al. (2015a) extend on this work by reporting that perchlorate has greater and much longer-lasting effects on thyroid and gonadal condition of *G. aculeatus* when exposures occur early during development. In a companion paper, Furin et al. (2015b) report similar findings concerning impacts of perchlorate on bone development of Threespine Stickleback. Consistent with the conclusions of Furin et al. (2015a), Gardell et al. (2015) found that whole-body concentrations of thyroid hormone and 11-ketotestosterone (11KT) in wild-caught adult stickleback were unaffected by perchlorate exposures of up-to-43 d in duration. Working with amphibians, Mathieu-Denoncourt et al. (2015b) present evidence for paradoxical effects of both moderate (11

176 wk) and long term (51 wk) exposure to the plasticizer, monomethyl phthalate during
177 development in Western clawed frogs (*Silurana tropicalis*), whereby it promoted rather than
178 inhibited metamorphosis and also resulted in male-biased populations. While a comparison with
179 Zebrafish was not made in the study of Mathieu-Denoncourt et al. (2015b), it is interesting that
180 their observations with *S. tropicalis* are similar to those with Zebrafish made experimentally
181 hyperthyroid (Mukhi et al., 2007; Sharma and Patiño, 2013).

182 The effects of EDCs on thyroid and reproductive axes and their interactions, and on other
183 physiological functions in aquatic vertebrates are the subject of review articles by Yu et al.
184 (2015) and Mathieu-Denoncourt et al. (2015a). Yu et al. (2015) bring readers up to date on the
185 potential for the flame retardants, polybrominated diphenyl ethers (PBDEs) to adversely affect
186 fish populations based on both field and laboratory studies examining modes of action within the
187 thyroid and reproductive axes. Mathieu-Denoncourt et al. (2015a) provide a general review of
188 plasticizer effects on vertebrates with special emphasis on aquatic species. Their review covers
189 the actions of the better known plasticizers, bisphenol A and phthalates, on development and
190 reproduction via effects on thyroid, growth, and sex steroid hormone systems.

191 A recent emphasis of the fish and amphibian literature has been on identifying endpoints
192 of EDC exposure that can be used to predict impacts to reproductive fitness, so-called “apical
193 endpoints” of exposure. To help determine how apical endpoints may be used in risk assessment,
194 scientists from the U.S. Environmental Protection Agency developed an “adverse outcome
195 pathway” (AOP) framework (Ankley et al., 2010) that links a molecular event initiated by an
196 EDC to an apical endpoint of reproduction with consequences at the level of individuals or
197 populations. This special issue includes four research studies, all of fishes, which consider EDC
198 impacts on reproduction from the wet bench to the field. In their study of Zebrafish, Cosme et al.

(2015) work within the AOP framework to elucidate the effect of the EDC, ethinylestradiol, and the phospholipase A2 inhibitor, quinacrine on spawning and other apical endpoints of reproductive fitness in adult fish. An article by Kennedy and Smyth (2015) presents the kinetics of plasma levels of estradiol-17 β (E2) and testosterone after injection of the polycyclic aromatic hydrocarbon, benzo[a]pyrene, a persistent organic pollutant (POP), in mature male Rainbow Trout (*Oncorhynchus mykiss*). Two articles led by Patiño and Goodbred, respectively, highlight the difficulties of extrapolating results of single or simple-mixture contaminant studies in the laboratory to real world scenarios where fishes may be exposed to hundreds of potential EDCs at a time, both legacy and emerging contaminants. In their study of Common Carp (*Cyprinus carpio*) from Lake Mead National Recreation Area (USA), Patiño et al. (2015) illustrate the value of multivariate/multiple regression approaches for teasing apart the response of natural populations to sublethal, lifetime exposures to complex mixtures of contaminants. In their study of Largemouth Bass (*Micropterus salmoides*) from Lake Mead, Goodbred et al. (2015) found elevated levels of contaminants and reduced IKT and sperm count in male bass from a wastewater effluent/urban runoff-impacted site relative to a reference site, linking by association an apical endpoint of reproductive fitness (sperm count) to relatively high levels of exposure to contaminants. A review article by Senthilkumaran (2015) outlines the current state of knowledge regarding pesticide exposure and the hypothalamus-pituitary-gonadal axis in fishes.

The possibility of transgenerational effects of EDCs in offspring are addressed in a research paper and review article in this volume. Neuman-Lee et al. (2015) exposed pregnant garter snakes (*Thamnophis elegans*; a piscivorous species) to BDE-47, a major PDBE congener in the environment, and examined a number of physiological responses including the response of the hypothalamus-pituitary-interrenal axis and innate immunocompetence in both mothers and

offspring. Schwindt (2015) reviews the evidence for parental transfer of EDC effects as well as the latest data on epigenetic changes that may be caused by EDCs and passed to offspring.

Persistent organic pollutants are by definition stable in the environment, typically lipophilic, and can directly bioaccumulate in organisms and biomagnify through the food chain. Their levels are relatively high in lipid rich organs, especially in top predators. In this volume, Tartu et al. (2015) report that plasma concentrations of the POPs, Hg (measured as total Hg), polychlorinated biphenyls, and organochlorine pesticides were not associated with baseline, stress-related, or dexamethasone-induced changes in plasma concentrations of corticosterone in the piscivorous Black-legged Kittiwake (*Rissa tridactyla*), an Arctic seabird species. Plasma POPs were, however, associated with changes in ACTH-induced corticosterone production, suggesting that exposure to these POPs in the field may lead to differences in ACTH receptor number on adrenal cortical cells. The review article of Mathieu-Denoncourt et al. (2015a) mentioned earlier also covers studies that have addressed the effects of plasticizers, which are also classified as POPs, on the stress axis of animals.

In summary, the sixteen articles in this volume present an update and broad look at the relationships between EDCs and the thyroid, reproductive, and adrenal axes in aquatic wildlife. They also reveal that there is still much more to be learned about these relationships and how laboratory results translate to real populations in the wild. Additional studies are needed, for example, to better characterize linkages between AOPs associated with the various endocrine axes and apical endpoints of exposure, to determine effects of environmentally relevant EDC mixtures and their concentrations under controlled laboratory conditions, and to evaluate statistical approaches for better defining spatiotemporal patterns in environmental and biological

field datasets while accounting for potential analytical issues such as multicollinearity among explanatory variables, et cetera.

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References

- AMAP, 2002. AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xvi +310 pp.
- <http://www.amap.no/documents/doc/amap-assessment-2002-persistent-organic-pollutants-in-the-arctic/96>. Last accessed, 7 April 2015.
- Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrano, J.A., Tietge, J.E., Villeneuve,

266 D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology
 267 research and risk assessment. *Environ. Toxicol. Chem.* 29, 730–741
 268 Bern, H.A., et al., 1992. Consensus statement. In Colborn, T., Clement, C., Eds., Chemically-
 269 induced alterations in sexual and functional development: the wildlife/human connection,
 270 *Advances in Modern Environmental Toxicology*, v. 21, Princeton Scientific Pub. Co.,
 271 Princeton, New Jersey, pp. 1-8.
 272 Bernhardt, R.R., von Hippel, F.A., Cresko, W.A., 2006. Perchlorate induces hermaphroditism in
 273 threespine sticklebacks. *Environ. Toxicol. Chem.* 25, 2087–2096.
 274 Biggers, J.D., Curnow, D.H., 1954. Oestrogenic activity of subterranean clover. 1. The
 275 oestrogenic activity of genistein. *Biochem. J.* 58, 278–282.
 276 Blais, J.M., Macdonald, R.W., Mackay, D., Webster, E., Harvey, C., Smol, J.P., 2007.
 277 Biologically mediated transport of contaminants to aquatic systems. *Environ. Sci. Technol.*
 278 41, 1075–1084.
 279 Blazer, V.S., Iwanowicz, L.R., Henderson, H., Mazik, P.M., Jenkins, J.A., Alvarez, D.A.,
 280 Young, J.A., 2012. Reproductive endocrine disruption in smallmouth bass (*Micropterus*
 281 *dolomieu*) in the Potomac River basin: spatial and temporal comparisons of biological
 282 effects. *Environ. Monit. Assess.* 184, 4309–4334. .
 283 Bradshaw, D., 2007. Environmental endocrinology. *Gen. Comp. Endocrinol.* 152, 125–141.
 284 Caroli, S., Cescon, P., Walton, D.W.H., Eds., 2001. *Environmental Contamination in Antarctica*,
 285 Elsevier Science, Amsterdam, 2001, xiv + 406 p.
 286 Carr, J.A., Patiño, R., 2011. The hypothalamus–pituitary–thyroid axis in teleosts and
 287 amphibians: Endocrine disruption and its consequences to natural populations. *Gen. Comp.*
 288 *Endocrinol.* 170, 299–312.

289 Carr, J.A., Murali, S., Hu, F., Goleman, W.L., Carr, D.L., Smith, E.E., Wages, M..
 290 2015. Changes in gastric sodium-iodide symporter (NIS) activity are associated with
 291 differences in thyroid gland sensitivity to perchlorate during metamorphosis. *Gen. Comp.*
 292 *Endocrinol.* doi: 10.1016/j.ygcen.2014.10.018. [Epub ahead of print].
 293 Cosme, M.M., Lister, A.L., Van Der Kraak, G., 2015. Inhibition of spawning in zebrafish
 294 (*Danio rerio*): Adverse outcome pathways of quinacrine and ethinylestradiol. *Gen. Comp.*
 295 *Endocrinol.* doi: 10.1016/j.ygcen.2015.01.013. [Epub ahead of print]
 296 Denver, R.J., Hopkins, P.M., McCormick, S.D., Propper, C.R., Riddiford, L., Sower, S.A.,
 297 Wingfield, J.C., 2009. Comparative endocrinology in the 21st century. *Integr. Comp. Biol.*
 298 49, 339–348.
 299 Dodds, E.C., Goldberg, L., Lawson, W., Robinson, R., 1939. Synthetic estrogenic compounds
 300 related to stilbene and diphenylethane. Pt. 1. *Proc. Royal Soc. B.* 127, 140–142.
 301 Emmens, C.W., 1941. Precursors of oestrogens. *J. Endocrinol.* 2, 444–58.
 302 Furin, C.G., von Hippel, F.A., Postlethwait, J.H., Buck, C.L., Cresko, W.A., O’Hara, T.M.,
 303 2015a. Developmental timing of sodium perchlorate exposure alters angiogenesis, thyroid
 304 follicle proliferation and sexual maturation in stickleback. *Gen. Comp. Endocrinol.*
 305 doi.org/10.1016/j.ygcen.2015.04.002. [Epub ahead of print].
 306 Furin, C.G., von Hippel, F.A., Postlethwait, J.H., Buck, C.L., Cresko, W.A., O’Hara, T.M.,
 307 2015b. Developmental timing of sodium perchlorate exposure threespine stickleback dermal
 308 bone. *Gen. Comp. Endocrinol.* doi.org/10.1016/j.ygcen.2015.02.016. [Epub ahead of print].
 309 Gardell, A.M., Dillon, D.M., Smayda, L.C., von Hippel, F.A., Cresko, W.A., Postlethwait, J.H.,
 310 Buck, C.L., 2015. Perchlorate exposure does not modulate temporal variation of whole-body

thyroid and androgen hormone content in threespine stickleback. Gen. Comp. Endocrinol. doi:10.1016/j.ygcen.2015.02.014. [Epub ahead of print].

Goleman, W.L., Carr, J.A., Anderson, T.A., 2002. Environmentally relevant concentrations of ammonium perchlorate inhibit thyroid function and alter sex ratios in developing *Xenopus laevis*. Environ. Toxicol. Chem. 21, 590–597.

Goodbred, S.L., Patiño, R., Torres, L., Echols, K.R., Jenkins, J.A., Rosen, M.R., Orsak, E., 2015. Are endocrine and reproductive biomarkers altered in contaminant-exposed wild male Largemouth Bass (*Micropterus salmoides*) of Lake Mead, Nevada/Arizona, USA? Gen. Comp. Endocrinol. doi: 10.1016/j.ygcen.2015.02.015. [Epub ahead of print].

Gorbman, A., 1988. Comparative endocrinology. In McCann, S.M., Ed., Endocrinology: People and Ideas, Springer, New York, pp. 421–441.

Gorbman, A., Bern, H.A., 1962. A Textbook of Comparative Endocrinology. Wiley, New York, xiv + 468 pp.

Hammond, S.A., Veldhoen, N., Helbing, C.C., 2015. Influence of temperature on thyroid hormone signaling and endocrine disruptor action in *Rana* (Lithobates) *catesbeiana* tadpoles. Gen. Comp. Endocrinol. doi: 10.1016/j.ygcen.2014.12.001. [Epub ahead of print].

Hayes, T.B., 1997. Hormonal mechanisms as developmental constraints on evolution: Examples from the anura. Am. Zool 37, 482–490.

Hayes, T.B., 1998. Sex determination and primary sex differentiation in amphibians: genetic and developmental mechanisms. J. Exp. Zool. 281, 373–399.

Hontela, A., 1998. Interrenal dysfunction in fish from contaminated sites: in vivo and in vitro assessment. Environ. Toxicol. Chem. 17, 44–48.

333 Jobling, S., et al., 2013. Evidence for endocrine disruption in humans and wildlife. In: Bergman,
 334 A., Heindel, J.J., Jobling, S., Kidd, K.A., Zoeller, T., (Eds.), State of the Science of
 335 Endocrine Disrupting Chemicals – 2012. World Health Organization and United Nations
 336 Environment Programme, Geneva, Switzerland, pp. 23-188.
 337 <http://www.who.int/ceh/publications/endocrine/en/>. Last accessed, 7 April 2015.
 338 Kennedy, C.J., Smyth, K.R., 2015. Disruption of the rainbow trout reproductive endocrine axis
 339 by the polycyclic aromatic hydrocarbon benzo[a]pyrene. Gen. Comp. Endocrinol., in press.
 340 Marty, M.S., Carney, E.W., Rowlands, J.C., 2011. Endocrine disruption: historical perspectives
 341 and its impact on the future of toxicology testing. Toxicol. Sci. 120, S93–S108.
 342 Mathieu-Denoncourt, J., Wallace, S.J., de Solla, S.R., Langlois, V.S., 2015a. Plasticizer
 343 endocrine disruption: Highlighting developmental and reproductive effects in mammals and
 344 non-mammalian aquatic species. Gen. Comp. Endocrinol. doi: 10.1016/j.ygcen.2014.11.003.
 345 [Epub ahead of print]
 346 Mathieu-Denoncourt, J., de Solla, S.R., Langlois, V.S. 2015b. Chronic exposures to
 347 monomethyl phthalate in Western clawed frogs. Gen. Comp. Endocrinol. doi:
 348 10.1016/j.ygcen.2015.01.019. [Epub ahead of print].
 349 Matthiessen, P., 2003. Historical perspective on endocrine disruption in wildlife. Pure Appl.
 350 Chem. 75, 2197–2206.
 351 Moccia, R.D., Leatherland, J.F., Sonstegard, R.A., 1977. Increasing frequency of thyroid goiters
 352 in Coho salmon (*Oncorhynchus kisutch*) in the Great Lakes. Science 198, 425–426.
 353 Mukhi, S., Torres, L., Patiño, R., 2007. Effects of larval-juvenile treatment with perchlorate and
 354 co-treatment with thyroxine on zebrafish sex ratios. Gen. Comp. Endocrinol. 150, 486–494.

355 Neuman-Lee, L.A., Carr, J., Vaughn, K., French, S.S.. 2015. Physiological effects of
 356 polybrominated diphenyl ether (PBDE-47) on pregnant gartersnakes and resulting offspring.
 357 Gen. Comp. Endocrinol. doi: 10.1016/j.ygcen.2015.03.011. [Epub ahead of print].
 358 Patiño, R., VanLandeghem, M.M., Goodbred, S.L., Orsak, E., Jenkins, J.A., Echols, K., Rosen,
 359 M.R., Torres, L., 2015. Novel associations between contaminant body burdens and
 360 biomarkers of reproductive condition in male Common Carp along multiple gradients of
 361 contaminant exposure in Lake Mead National Recreation Area, USA. Gen. Comp.
 362 Endocrinol. doi: 10.1016/j.ygcen.2014.12.013. [Epub ahead of print].
 363 Riddle, J.M., 1994. Contraception and Abortion from the Ancient World to the Renaissance.
 364 Harvard Univ. Press, Cambridge, MA, USA. 247 pp.
 365 Riddle, J.M., 2010. Goddesses, Elixirs, and Witches: Plants and Sexuality Throughout Human
 366 History. Palgrave Macmillan, p. 122.
 367 Robertson, J.C., Kelley, D.B., 1996. Thyroid hormone controls the onset of androgen sensitivity
 368 in the developing larynx of *Xenopus laevis*. Dev. Biol. 176, 108–123.
 369 Schwindt, A.R., 2015. Parental effects of endocrine disrupting compounds in aquatic wildlife: Is
 370 there evidence of transgenerational inheritance? Gen. Comp. Endocrinol. doi:
 371 10.1016/j.ygcen.2015.01.020. [Epub ahead of print].
 372 Senthilkumaran, B., 2015. Pesticide- and sex steroid analogue-induced endocrine disruption
 373 differentially targets hypothalamo-hypophyseal-gonadal system during gametogenesis in
 374 teleosts - A review. Gen. Comp. Endocrinol. doi: 10.1016/j.ygcen.2015.01.010. [Epub ahead
 375 of print].
 376 Sharma, P., Patiño, R. 2013. Regulation of gonadal sex ratios and pubertal development by the
 377 thyroid endocrine system in zebrafish (*Danio rerio*). Gen. Comp. Endocrinol. 184, 111–119.

378 Skelly, D.K., Bolden, S.R., Dion, K.B., 2010. Intersex frogs concentrated in suburban and urban
 379 landscapes. *EcoHealth* 7, 374–379.

380 Sluczewski, A., Roth, P., 1948. Effects of androgenic and estrogenic compounds on the
 381 experimental metamorphoses of amphibians. *Gynecol. Obstet.* 47, 164–176.

382 Tartu, S., Lendvai, Á.Z., Blévin, P., Herzke, D., Bustamante, P., Moe, B., Gabrielsen, G.W.,
 383 Bustnes, J.O., Chastel, O., 2015. Increased adrenal responsiveness and delayed hatching date
 384 in relation to polychlorinated biphenyl exposure in Arctic-breeding black-legged kittiwakes
 385 (*Rissa tridactyla*). *Gen. Comp. Endocrinol.* doi: 10.1016/j.ygcen.2014.12.018. [Epub ahead
 386 of print].

387 Walker, B.S., Janney, J.C., 1930. Estrogenic substances. II. Analysis of plant sources.
 388 *Endocrinology* 14, 389–392.

389 Wang, X., Yao, T., Cong, Z., Yan, X., Kang, S., Zhang, Y., 2006. Gradient distribution of
 390 persistent organic contaminants along northern slope of central-Himalayas, China. *Sci. Total*
 391 *Environ.* 372, 193–202.

392 Yu, L., Han, Z., Liu, C., 2015. A review on the effects of PBDEs on thyroid and reproduction
 393 systems in fish. *Gen. Comp. Endocrinol.* doi: 10.1016/j.ygcen.2014.12.010. [Epub ahead of
 394 print].

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Figure legends

Figure 1. Time-series plot of the number of published articles in the area of endocrine disruption. The topical search terms initially used were “endocrine” and “disrupt*.” The results obtained were searched again using the term “population.” Both sets of results were analyzed using simple linear regression to estimate the annual rate of growth: 88 and 10 articles year⁻¹, respectively ($r^2 \geq 0.96$, $p < 0.0001$). The cumulative total for the period between 1996 and 2014 is 15,834 articles.

Figure 1 Patino and Carr