# Salinity and sensitivity to endocrine disrupting chemicals: A comparison of reproductive endpoints in small-bodied fish exposed under different salinities

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# Salinity and sensitivity to endocrine disrupting chemicals: A comparison of reproductive endpoints in small-bodied fish exposed under different salinities

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## HIGHLIGHTS

- The influence of salinity was determined in small-bodied fish exposed to EDCs.
- Responses occurred at lower levels under freshwater conditions compared to saline.
- This effect if most pronounced when fish were exposed to estrogenic EDCs.
- Fecundity and female E2 levels were most sensitive to detect impacts of EDCs.

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## ABSTRACT

The influence of salinity on toxicity outcomes has been demonstrated for various contaminants, but has received limited attention for endocrine disrupting chemicals (EDCs). Short-term laboratory tests using small-bodied fish are an important tool for evaluating impacts of EDCs on reproduction. Tests have been developed for both freshwater and estuarine/marine species, providing an opportunity to assess whether concentrations at which small-bodied fish respond to EDCs may be influenced by salinity. We conducted a semi-quantitative review of short-term laboratory tests with small-bodied fish exposed to EDCs, including 59 studies under freshwater conditions (7 species) and 23 studies conducted under saline conditions (5 species). We focused on two model estrogens [17α-ethinylestradiol and 17β-estradiol (E2)], and three androgens (17β-trenbolone, 5α-dihydrotestosterone and 17α-methyltestosterone). The lowest observed adverse effect concentration ($LOAEC_{LOW}$) for key reproductive endpoints was recorded, including sex-steroid and vitellogenin (VTG) levels, fecundity and fertilization. In 65.2% of cases, responses occurred at lower doses under freshwater compared to saline conditions, compared to only 4.3% of cases where fish responded to lower doses under saline conditions. The potential influence of salinity was more pronounced when estrogenic compounds were considered separately, with fish responding to lower doses under fresh compared to saline conditions in 90.5% of cases. Fecundity and E2 level were identified as the most sensitive endpoints for evaluating EDCs regardless of salinity. Interestingly, female VTG levels were a sensitive endpoint under freshwater but not saline conditions. Overall, our results suggest that salinity may be an important factor influencing how small-bodied fish respond to environmental EDCs.



## 1. Introduction

Endocrine disrupting compounds (EDCs) upset hormone pathways in a variety of organisms, potentially negatively influencing reproductive performance (Martin and Voulvoulis, 2009). For this reason, EDCs have attracted significant scientific attention and are a major source of public concern. They are ubiquitous in aquatic

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systems and are found in freshwater, estuarine and marine environments. EDCs enter the aquatic environment through a variety of sources, including agricultural runoff (Gall et al., 2011; Bergman et al., 2013), sewage effluent (Fent et al., 2006; Coleman et al., 2008) and industrial effluents (Parks et al., 2001; Hewitt et al., 2008). Impacts of EDCs have been well documented under both laboratory and field conditions for various aquatic species, with the majority of the research pertaining to fish. Observed reproductive impacts in fish exposed to EDCs include changes in biochemical biomarkers such as vitellogenin (VTG) (Jobling et al., 1998), increased rates of intersex (Jobling et al., 1998; Kidd et al., 2007) and changes in sex ratios (Larsson et al., 2000). Importantly, such lower-level effects can have major ecological significance for fish, since they have the potential to scale up and can ultimately cause population failure (Kidd et al., 2007). It is therefore extremely important to identify factors that may influence the potency of EDCs to fish, so that at-risk populations can be better identified and protected.

Short-term reproductive bioassays using small-bodied fish provide a powerful tool to assess the impacts of EDCs on reproductive endpoints (Ankley and Johnson, 2004). Small-bodied fish species are advantageous because they are often readily available from commercial sources and are easily maintained under laboratory conditions. Reproductive bioassays therefore represent an important testing niche, and widely used protocols have been developed for a variety of species, including fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*). Standardised bioassays using these particular small-bodied species are commonly applied by organizations such as the US EPA (EPA, 2011) and the OECD (OECD) to study impacts of EDCs on fish reproduction. Similar protocols are also frequently applied to improve the ecological relevance of the test species (i.e. tests adapted to local species), for example to investigate effects under specific environmental conditions (e.g., brackish or marine species). Protocols adapted for local small-bodied freshwater species include tests with Chinese rare minnow (*Gobiocypris rarus*; Zha et al., 2008), brook stickleback (*Culaea inconstans*; Muldoon and Hogan, 2016), Rio de la Plata onesided livebearer (*Jenynsia multidentata*; Roggio et al., 2014), and the Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*; Pollino et al., 2007). Protocols for small-bodied brackish and marine species include mummichog (*Fundulus heteroclitus*; Peters et al., 2007; Bosker et al., 2010a), sheepshead minnow (*Cyprinodon variegatus*; Folmar et al., 2000), three-spined stickleback (*Gasterosteus aculeatus*; Allen et al., 2008), sand goby (*Pomatoschistus minutus*; Saaristo et al., 2009) and the brackish medaka (*Oryzias melastigma*; Lee et al., 2014). Regardless the species, the standard approach for such tests involves exposing fish for a relative short-period, ranging from 14 to 28 d depending on the protocol, to either model EDCs (see Dang et al., 2011a) for a summary of studies) or environmental samples, (e.g., municipal, agricultural or industrial effluents). Various reproductive endpoints are subsequently assessed which span different levels of biological organization, most commonly documenting changes in sex-steroid levels, relative gonad size, morphology and broad indicators of fecundity (e.g., egg production and fertilization success).

As indicated, EDCs are ubiquitous globally and occur in a range of aquatic environments. The characteristics of the receiving environment are therefore important to consider for their potential influence on toxicity. Differences in salinity represent an obvious environmental factor that may alter the potency of EDCs to fish, but this has received limited research attention. On a physiological level, salinity is an important variable to consider, since fish living under different salinities have adapted the way in which they osmoregulate (Evans and Claiborne, 1997). Freshwater species are hyperosmotic to their environment and tend to drink very little water, with osmoregulation occurring predominantly through the gills (Evans and Claiborne, 1997). Contrarily, if species are hypoosmotic to their environment they tend to actively drink seawater to maintain their osmotic balance (Evans and Claiborne, 1997). Differences in osmoregulation can therefore result in contaminants entering an organism via different routes, and this in turn can potentially result in toxic effects being realised at different environmental concentrations. The influence of salinity on toxicity outcomes has been documented for various contaminants. For example, a variety of metals (Hall and Anderson, 1995; Wood et al., 2004; Blanchard and Grosell, 2005) and polycyclic aromatic hydrocarbons (PAHs) (Ramachandran et al., 2006; Shukla et al., 2007) have been shown to exhibit differential toxicity in fish exposed under freshwater compared to saline conditions. Considering the global threat that EDCs pose to fish populations, there is a need for research exploring whether salinity might be a factor mediating their toxicity.

A limited number of studies have been conducted directly comparing the impact of EDCs on reproductive parameters in small-bodied fish at different salinities, but the evidence seems to suggest that salinity may be an important factor. For example, Glinka et al. (2015), exposed mummichog to a potent androgen (DHT) under high and low salinity, and found a significant difference in response between freshwater and saline conditions. A direct comparison of the effects of pulp mill effluent, a known source of EDCs, on euryhaline mummichog and freshwater fathead minnow found limited differences between both species (Melvin et al., 2009). However, a comparison of the impacts of the sythetic estrogen EE2 on reproduction showed that in general freshwater fish respond to lower levels of EE2 compared to saline species for a select set of endpoints, including fecundity and VTG levels (Bosker et al., 2016). Freshwater species such as Chinese rare minnow and zebrafish exposed to EE2 exhibited reduced egg production at concentrations as low as 0.2 ng EE2/L (Chinese rare minnow; Zha et al., 2008) and 1 ng EE2/L (zebrafish; Lin and Janz, 2006). In contrast, a study on mummichog under estuarine conditions found reductions in egg production only at exposure concentrations of 100 ng EE2/L (Peters et al., 2007) or no response at all (Bosker et al., 2016). A similar trend of differential sensitivity is apparent for androgens. For example, reduced egg production was observed in sheepshead minnow exposed to 17β-trenbolone (TB; a synthetic androgen used as a growth promoter in the cattle industry) at 5 μg TB/L (Hemmer et al., 2008), whereas fathead minnow responded to the same compound at concentrations 100-fold lower (Ankley et al., 2003). Finally, a review of short-term reproductive tests using three small-bodied freshwater species identified fecundity and gonad histology as two of the most sensitive endpoints to EDCs (Dang et al., 2011a). However, recent studies using the brackish mummichog found no effect of 5α-dihydrotestosterone (DHT) on male and female gonad morphology (Glinka et al., 2015) and no effect of EE2 on fecundity (Bosker et al., 2016). Limited experimental work directly addressing the influence of salinity on EDC potency precludes using purely quantitative techniques (e.g. meta-analysis) to investigate this question. Qualitatively, the existing literature seems to indicate that endpoint sensitivity could differ across species and salinities in fish exposed to EDCs, but given the disparities amongst studies there is a clear need for some form of systematic synthesise of the existing data.

The present study describes a semi-quantitative review of short-term reproductive laboratory bioassays with small-bodied fish. A novel approach to systematically compare endpoint sensitivity was applied to assess whether i) concentrations at which small-bodied fish respond to EDCs differ amongst studies performed under freshwater compared to saline conditions, and ii) whether

sensitivity of specific endpoints differs amongst salinities.

## 2. Materials and methods

### 2.1. Data collection

We performed a systematic review to collect data from short-term reproductive bioassays exposing fish to EDCs. Data was grouped based on the isosmotic point for fish, which is around 30–40% of full saltwater concentration (or approximately 9–13 ppt salinity) (Evans and Claiborne, 1997; Evans, 2008). For example, the isosmotic point for mummichog is estimated to be around 9 ppt (Marshall et al., 1999; Wood and Grosell, 2009). We defined a freshwater exposure as occurring under conditions in which fish were exposed at salinities below the isosmotic point, and saline conditions when the exposure concentration was near or above the isosmotic point.

Only studies in which adult, sexually mature, small-bodied (<150 mm; Environment Canada, 2012) fish were exposed to one of five model EDCs for a timeframe of 14–28 d were included in our analyses. Two EDCs were selected to represent an estrogenic mode of action: 17α-ethinylestradiol (EE2) and 17β-estradiol (E2). Two non-aromatizable androgenic compounds were selected: 17β-trenbolone (TB) and 17α-dihydrotestosterone (DHT), as well as one aromatizable androgen: 17α-methyltestosterone (MT). Studies were identified by searching the Thomson Reuters Web of Science™ database and the OECD website database for short-term reproductive tests. The cut-off date for inclusion in the review was 01 July 2016. Only laboratory experiments in which fish were exposed to at least two concentrations (excluding controls) were included in the analyses. In some cases, multi-generational tests or life-cycle tests were included, but only provided data for the F0 generation was reported for an exposure duration between 14 and 28 d.

Data was collected for a variety of commonly measured reproductive endpoints spanning different levels of biological organization, ranging from biochemical to functional endpoints. The endpoints selected were sex steroid levels [11-ketotestosterone (11KT) and testosterone (T) in males, and T and E2 in females], VTG levels, changes in secondary sex characteristics (SSC), gonadosomatic index (GSI), gonad histology, fecundity, fertilization success and percent hatchability of eggs. Data were collected for both male and female fish whenever available. Measurements of hormone and VTG levels have been conducted using different methodologies in the literature, for example in blood plasma, in vitro (only for hormone levels) or measurements from specific tissues. In addition, there is considerable variation in the histological assessment of gonadal tissue. It is thus important to recognise that differences in protocols and amongst laboratories can influence study outcomes (Hutchinson et al., 2006). However, since various methods are applied under both saline and freshwater conditions, we assume limited impact of these differences on the overall outcomes of our analysis. Differences that are observable despite the inherent variability in methodologies amongst studies could instead add confidence in the conclusions. Nevertheless, for transparency the method of measuring hormone and VTG levels was reported.

The *lowest observed adverse effect concentration* (LOAEC) was recorded for each of the endpoints listed above, when available. If no effect was observed, the highest tested concentration was used since this would be expected to yield a conservative outcome and thus not contribute to erroneous conclusions. The following additional information was recorded for each experiment: data source, test species employed, concentrations at which the fish were exposed, length of the exposure, number of functional replicates, number of fish in each replicate, number of males and females, and the salinity of the water during exposure.

### 2.2. Effect concentrations under different salinities

Results were organized into summary tables presenting the LOAEC values in order to facilitate the identification of possible trends. Data was summarized for both freshwater and saline conditions for each endpoint by providing the lowest LOAEC ($LOAEC_{LOW}$). We defined $LOAEC_{LOW}$ as the absolute lowest concentration at which an effect was observed for an endpoint, across all experiments at either the freshwater or saline conditions. If no effect was observed in any of the experiments we reported the maximum concentration within the concentration range of all experiments as $LOAEC_{LOW}$.

### 2.3. Comparative endpoint sensitivity under different salinities

We assessed whether endpoint sensitivity differed between studies carried out under freshwater compared to saline conditions, for both estrogenic and androgenic EDCs. The approach was adapted from a method recently develop by Dang et al. (2011a, 2011b). For this approach, endpoints from each individual experiment were divided into three categories of effect:

1) If the LOAEC for a specific endpoint was the lowest of all other endpoints measured within that specific experiment, it was grouped in the first category.
2) If a significant effect was observed for an endpoint, but this occurred above the LOAEC for another endpoint in that study, it was grouped in the second category, and;
3) If no effect was observed for any endpoint at the maximum tested concentration the endpoint was grouped in the third category.

The number of observations for each of the three groups (LOAEC, > LOAEC but < no effect, and no observed effect) was summarized separately for estrogenic (E2 and EE2) and androgenic compounds (TB, DHT and MT). To allow for direct comparisons, the relative contribution of each category was calculated as the ratio between the numbers of observations in each category divided by the total number of observations in all three categories.

## 3. Results

Our search of the literature identified 43 publications containing 82 individual experiments that satisfied our criteria for inclusion in the study (Table S1, S2 and S3). Of these, 59 were conducted under freshwater conditions, or at salinities below the isosmotic point of the specific test species. The remaining 23 experiments were conducted under saline conditions, at or above the isosmotic point of the test species, at salinities ranging from 15 to 35 ppt. In all papers fish were labelled either "*sexually-mature*" or "*adult*". We noted whether mature oocytes and/or spermatids were presents, either based on histological assessment, visual inspection or the ability to produce eggs (indication of mature oocyte) and the ability to fertilize eggs (indication of mature male spermatids) (Table S1). Table 1 (estrogens) and Table 2 (androgens) summarize the studies included in our analysis, including the specific endpoints measured within each individual study. Additional information for each study, such as the number of replicate tanks, the number of fish per sex per replicate, and measured concentration of the focal EDCs are presented in Table S1.

The euryhaline mummichog was the only species exposed under a range of salinities. When exposed at salinity below 9 ppt

**Table 1**
Lowest observed adverse effect concentration (LOAEC) for reproductive endpoints in adult fish exposed to either 17α-ethinylestradiol (EE2) or 17β-estradiol (E2) for a duration between 14 and 28 d.

| Condition | | Salinity | Species | Source | Nominal [ ] ng/L | Endpoint LOAEC[a] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Males | | | | | Females | | | | | Other | | | |
| | | | | | | 11KT[b] | T[b] | VTG[c] | HIST | GSI | E2[b] | T[b] | VTG[c] | HIST | GSI | SSC | FEC | FERT | HATCH |
| EE2 | Fresh | FW | *C. inconstans* | Muldoon and Hogan 2016 | 1-10-100 | | | ***100*** | | >100 | | | | | 1 | | | | |
| | | FW | *G. rarus* | Zha et al., 2008 | 5/1/2025 | | | 1 | 5 | 1 | | | 5 | 25 | 5 | | | | |
| | | FW | *P. promelas* | Pawlowski et al., 2004 | 0.1-1-3-10-100 | | | 1 | 3 | 10 | | | 1 | | 100 | 1 | 100 | 10 | |
| | | FW | | Salierno and Kane 2009 | 10-20-40 | 10 | 10 | 10 | 20 | 10 | | | | | | 10 | | | |
| | | FW | | Runnalls et al., 2015 | 0.5-5-25 | 25 | | 0.5 | | 25 | 0.5 | | 25 | | >25 | 25 | 5 | | |
| | | FW | | Armstrong et al., 2016 | 0.5-1.5-4.5 | | >4.5 | 1.5 | | >4.5 | 1.5 | >4.5 | 4.5 | | >4.5 | | 0.5 | >4.5 | |
| | | FW | *O. lapites* | Seki et al., 2002 | 31.3-62.5-125-250-500 | | | **62.5** | 62.5 | | | | | 500 | | | 500 | >500 | |
| | | 1.64‰ | | Tilton et al., 2005 | 0.2-5-500-2000 | | >500 | 500 | | 500 | 5 | >500 | 500 | | 0.2 | | 0.2 | 500 | 500 |
| | | FW | | Miller et al., 2012 | 1–10 | | | | 1000 | | | | | | | | 1000 | 1000 | |
| | | FW | *F. heteroclitus* | Meina et al., 2013 | 50–250 | | >250 | | | >250 | >250 | >250 | | | >250 | | | | |
| | | FW | *J. multidentata* | Roggio et al., 2014 | 10-75-150 | | | | | 75 | | | | | | | | | |
| | | FW | *D. rerio* | Van den Belt et al., 2001 | 5-10-25-50 | | | 5 | | 10 | | | 10 | | 10 | | | | |
| | | FW | | Van den Belt et al., 2002 | 10–25 | | | 10 | 10 | 10 | | | 25 | 25 | 10 | | | | |
| | | FW | | Coe et al., 2008 | 2–10 | 2 | | | | | | | | | | | >10 | | |
| | | FW | | Soffker et al., 2012 | 2–5 | | | ***5*** | | | | | | | | | >5 | 5 | |
| | | FW | | Caspillo et al., 2014 | 5–25 | | | ***5*** | | | | | | | | | | | |
| | | FW | | Xu et al., 2014 | 5–20 | | | 5 | | | | | | | | | | | |
| | Saline | 32‰ | *O. melstigma* | Lee et al., 2014 | 1-10-50-100 | | | | >100 | | | | | | | | 50 | | |
| | | 20‰ | *F. heteroclitus* | Peters et al., 2007 | 0.1-1-10-100 | >100 | >100 | 100 | | 100 | 10 | >100 | >100 | | >100 | | 100 | 100 | |
| | | 16‰ | | Hogan et al., 2010 | 100–500 | | >500 | ***100*** | | 500 | | >500 | | | >500 | | | | |
| | | 16‰ | | Meina et al., 2013 | 50–250 | | >250 | | | >250 | 250 | >250 | | | >250 | | | | |
| | | 32‰ | | Meina et al., 2013 | 50–250 | | >250 | | | >250 | >250 | >250 | | | >250 | | | | |
| | | 16‰ | | Bosker et al., 2016 | 3-30-300-3000 | | | | | 3000 | | | | | >3000 | | >3000 | >3000 | >3000 |
| | | 32‰ | *P. minutus* | Saaristo et al., 2009 | 25–50 | | | ***50*** | | >50 | | | | | | >50 | | | |
| | | 18–21‰ | *C. variegatus* | Folmar et al., 2000 | 20-100-200-500-1000 | | | 100 | | | | | | | | | | | |
| E2 | Fresh | FW | *M. fluviatilis* | Pollino et al., 2007 | 30-100-300-1000 | | >1000 | | >1000 | >1000 | 1000 | >1000 | | | >1000 | | 300 | | >1000 |
| | | FW | *P. promelas* | OECD 2006 LAB1 | 10-32-100 | | | 100 | 100 | >100 | | | 32 | >100 | >100 | | | | |
| | | FW | | OECD 2006 LAB2 | 10-32-100 | | | 10 | >100 | 100 | | | 100 | | 100 | | | | |
| | | FW | | OECD 2006 LAB4 | 10-32-100 | | | 10 | | >100 | | | 100 | >100 | >100 | | | | |
| | | FW | | Shappell et al., 2010 | 9-18-44* | | | 18 | 44 | >44 | | | >44 | >44 | >44 | 44 | | | |
| | | FW | | Dammann et al., 2011 | 5-25-50 | | | >50 | | 50 | | | >50 | | >50 | >50 | | | |
| | | FW | | Dammann et al., 2011 | 5-25-50 | | | >50 | | >50 | | | >50 | | >50 | >50 | | | |
| | | FW | | Seki et al., 2006 | 10-32-100 | | | 100 | | >100 | | | 32 | | >100 | 100 | | | |
| | | FW | *O. lapites* | Kang et al., 2002 | 31.3-62.5-125-250-500 | | | **62.5** | 500 | 500 | | | 500 | | >500 | | 500 | 500 | 500 |
| | | FW | | Seki et al., 2006 | 10-32-100 | | | **10** | | >100 | | | **100** | | >100 | >100 | | | |
| | | FW | | OECD 2006 LAB1 | 10-32-100 | | | 32 | | >100 | | | 100 | >100 | >100 | | | | |
| | | FW | | OECD 2006 LAB2 | 10-32-100 | | | 10 | | >100 | | | 32 | >100 | >100 | | | | |
| | | FW | | OECD 2006 LAB3 | 10-32-100 | | | 32 | | >100 | | | 100 | | >100 | | | | |
| | | FW | | Jukosky et al., 2008 | 76-379-3793 | | ***3793*** | **76** | | >3793 | | ***3793*** | | | >3793 | | 3793 | | |
| | | FW | | Sun et al., 2009 | 5-25-125-625-3125 | | | 125 | | | | | | | | | | | |
| | | FW | *D. rerio* | Van den Belt et al., 2003 | 20–100 | | | 20 | | | | | | | | | | | |
| | | FW | | Brion et al., 2004 | 5-25-100 | | | **25** | >100 | >100 | | | 25 | >100 | 100 | | | | |
| | | FW | | Seki et al., 2006 | 10-32-100 | | | 100 | | >100 | | | 100 | | >100 | | | | |
| | | FW | | OECD 2006 LAB1 | 10-32-100 | | | >100 | >100 | 10 | | | >100 | >100 | >100 | | | | |
| | | FW | | OECD 2006 LAB2 | 10-32-100 | | | 32 | >100 | >100 | | | 32 | >100 | >100 | | | | |
| | | FW | | OECD 2006 LAB3 | 10-32-100 | | | >100 | >100 | 10 | | | 100 | >100 | 10 | | | | |
| | | FW | | OECD 2006 LAB4 | 10-32-100 | | | >100 | 32 | >100 | | | >100 | >100 | >100 | | | | |
| | Saline | 18–21‰ | *C. variegatus* | Folmar et al., 2000 | 20-200-500-1000-2000 | | | 200 | | | | | | | | | | | |
| | | 20‰ | | Cripe et al., 2009 | 10-30-80-200-500 | | | >500 | 200 | >500 | | | >500 | 300 | 500 | | 500 | >500 | |
| | | BW/SW | *G. aculeatus* | Allen et al., 2008 | 10-32-100 | | | 100 | >100 | >100 | | | >100 | >100 | >100 | | | | |
| | | BW/SW | | Allen et al., 2008 | 10-32-100 | | | 100 | >100 | >100 | | | >100 | >100 | >100 | | | | |
| | | BW/SW | | Allen et al., 2008 | 10-32-100 | | | 100 | | >100 | | | >100 | | >100 | | | | |

[a] 11KT: 11-ketotestosterone; T: testosterone; E2: 17β-estradiol; VTG: vitellogenin; SSC: secondary sex characteristics; HIST: gonad histology; GSI: gonadosomatic index; FEC: fecundity; SPAWN: number of spawning events; FERT: fertility; HATCH: hatchability.
[b] ***Bold and italic***: in vitro measurements, **Bold and underscored**: tissue measurement otherwise plasma measurement.
[c] ***Bold and italic***: mRNA measurements, **Bold and underscored**: tissue measurement, otherwise plasma measurement.

**Table 2**
Lowest observed effect concentration (LOAEC) for reproductive endpoints in adult fish exposed to 17β-trenbolone (TB), 5α-dihydrotestosterone (DHT) or methyltestosterone (MT) for a duration between 14 and 28 d. NOTE: nominal concentrations for TB in ng/L, for DHT and MT in μg/L.

| Condition | | Salinity | Species | Source | Nominal [ ] ng/L | Endpoint LOAEC[a] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Males | | | | | Females | | | | | Other | | | |
| | | | | | | 11KT[b] | T[b] | VTG[c] | HIST | GSI | E2[b] | T[b] | VTG[c] | HIST | GSI | SSC | FEC | FERT | HATCH |
| TB | Fresh | FW | *P. promelas* | Ankley et al., 2003 | 5-50-500-5000-50000 | 50,000 | >50,000 | >50,000 | | >50,000 | 500 | 500 | 50 | 50 | >50,000 | 50 | 50 | 500 | 500 |
| | | FW | | Seki et al., 2006 | 50-500-5000 | | | >5000 | | >5000 | | | 5000 | | >5000 | >5000 | | | |
| | | FW | | OECD 2006 LAB1 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 50 | 500 | >5000 | >5000 | | | |
| | | FW | | OECD 2006 LAB2 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 500 | >5000 | >5000 | >5000 | | | |
| | | FW | | OECD 2006 LAB4 | 50-500-5000 | | | 500 | 50 | >5000 | | | 5000 | 500 | >5000 | | | | |
| | | FW | *O. lapites* | Seki et al., 2006 | 50-500-5000 | | | **>5000** | | >5000 | | | **50** | | >5000 | 500 | | | |
| | | FW | | OECD 2006 LAB1 | 50-500-5000 | | | >5000 | | >5000 | | | 50 | 500 | >5000 | 500 | | | |
| | | FW | | OECD 2006 LAB2 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 500 | >5000 | >5000 | 500 | | | |
| | | FW | | OECD 2006 LAB3 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 50 | | 500 | 500 | | | |
| | | FW | | Forsgren et al., 2014 | 10-100-1000 | | | | | | **>1000** | **>1000** | | 10 | | | | | |
| | | FW | *D. rerio* | Seki et al., 2006 | 50-500-5000 | | | >5000 | | >5000 | | | 500 | | 5000 | | | | |
| | | FW | | OECD 2006 LAB1 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 50 | | >5000 | | | | |
| | | FW | | OECD 2006 LAB2 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 50 | >5000 | >5000 | | | | |
| | | FW | | OECD 2006 LAB3 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 50 | 500 | 5000 | | | | |
| | | FW | | OECD 2006 LAB4 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | >5000 | 500 | >5000 | | | | |
| | Saline | 19,1‰ | *C. variegatus* | Hemmer et al., 2008 | 5-50-5000 | | | >5000 | | | | | >5000 | | >5000 | | 5000 | >5000 | >5000 |
| | | 20‰ | | Cripe et al., 2010 | 10-40-200-1000-5000 | | | >5000 | | | | | 1000 | 1000 | 1000 | 200 | 1000 | 1000 | 5000 |
| | | BW/SW | *G. aculeatus* | Allen et al., 2008 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | 50 | >5000 | >5000 | | | | |
| | | BW/SW | | Allen et al., 2008 | 50-500-5000 | | | >5000 | >5000 | >5000 | | | >5000 | >5000 | >5000 | | | | |
| | | BW/SW | | Allen et al., 2008 | 50-500-5000 | | | >5000 | | >5000 | | | 5000 | | >5000 | | | | |
| DHT | Fresh | FW | *P. promelas* | Panter et al., 2004 | 10-32-100 | | | 32 | | >100 | | | 10 | | >100 | 10 | | | |
| | | 2‰ | *F. heteroclitus* | Glinka et al., 2015 | 0.05-0.5-5 | ***5*** | ***>5*** | | >5 | >5 | ***0.5*** | ***0.5*** | | >5 | >5 | | >5 | | |
| | Saline | 16‰ | *F. heteroclitus* | Feswick et al., 2014 | 5–50 | ***50*** | ***>50*** | | >50 | >50 | | | | | | | | | |
| | | 16‰ | | Rutherford et al., 2015 | 10–100 | >10 | 10 | ***100*** | | >100 | 10 | 10 | ***100*** | | >100 | | | | |
| | | 16‰ | | Glinka et al., 2015 | 0.05-0.5-5 | ***5*** | ***>5*** | | >5 | >5 | ***0.5*** | ***0.5*** | | >5 | >5 | | 0.05 | | |
| MT | Fresh | FW | *C. inconstans* | Muldoon and Hogan 2016 | 0.001-0.01-0.1 | | | ***>0.1*** | | >0.1 | | | | | >0.1 | | | | |
| | | FW | *P. promelas* | Pawlowski et al., 2004 | 0.1-1-5-50 | | | 1 | >50 | >50 | | | 50 | 0.1 | 50 | 1 | 5 | 5 | |
| | | FW | *O. lapites* | Kang et al., 2008 | 0.025-0.05-0.1-0.2-0.4 | | | **>0.4** | 0.4 | >0.4 | | | **0.2** | 0.025 | 0.05 | 0.025 | 0.05 | 0.05 | 0.05 |
| | Saline | 15‰ | *F. heteroclitus* | Sharpe et al., 2004 | 0.001-0.01-0.1 | 0.1 | 0.01 | >0.1 | | >0.1 | 0.01 | 0.01 | 0.1 | | >0.1 | | | | |
| | | 16‰ | | Rutherford et al., 2015 | 0.1–1 | >1 | >1 | ***>1*** | | >1 | 1 | >1 | ***>1*** | | >1 | | | | |

[a] 11KT: 11-ketotestosterone; T: testosterone; E2: 17β-estradiol; VTG: vitellogenin; SSC: secondary sex characteristics; HIST: gonad histology; GSI: gonadosomatic index; FEC: fecundity; SPAWN: number of spawning events; FERT: fertility; HATCH: hatchability.
[b] ***Bold and italic***: in vitro measurements, **Bold and underscored**: tissue measurement otherwise plasma measurement.
[c] ***Bold and italic***: mRNA measurements, **Bold and underscored**: tissue measurement, otherwise plasma measurement.

(isosmotic point), it was grouped among the freshwater studies, while if the exposure was conducted above 9 ppt the results were included in the saline studies. Importantly, Japanese medaka, a euryhaline species, was always exposed below the isosmotic point and results were thus interpreted as freshwater, while the euryhyline three-spined stickleback, brackish medaka and sheepshead minnow were always exposed above 13 ppt and thus were included as saline studies.

### 3.1. Difference in observed lowest, median and highest LOEC

The concentration ranges tested under freshwater and saline conditions were comparable for all chemicals, facilitating direct comparison between freshwater and saline conditions (Tables 1–4). However, limited experimental data was available for both DHT and MT, and these results therefore need to be interpreted with caution. It was possible to directly compare freshwater against saline conditions for different endpoints in 47 cases (13 times for EE2, 8 times for E2, 10 times for TB, 11 times for DHT and 4 times for MT).

In 30 out of 46 cases (65.2%) $LOAEC_{LOW}$ was less under freshwater compared to saline conditions (Tables 3 and 4). In contrast, $LOAEC_{LOW}$ was less under saline conditions in only 2 out of 46 (4.3%) cases (Tables 3 and 4). For estrogenic compounds the influence of salinity was most evident, with 19 out of 21 (90.5%) cases reporting the lowest $LOAEC_{LOW}$ under freshwater conditions. Responses for estrogenic EDCs never occurred at lower doses under saline compared to freshwater conditions (Table 3). For androgenic compounds this pattern was not as clear, with $LOAEC_{LOW}$ observed under freshwater conditions in 11 out of 25 (44.0%) of the cases, compared to 2 out of 25 (8.0%) cases for saline conditions (Table 4).

Exposure concentrations at which the $LOAEC_{LOW}$ was observed was considerably less under freshwater conditions compared to saline conditions. On average, for estrogenic compounds, $LOAEC_{LOW}$ was >70-fold lower under freshwater conditions compared to saline conditions. For example, for estrogenic exposures, the lowest observed LOAEC for male VTG induction under freshwater exposures was 0.5 ng/L for EE2 and 10 ng/L for E2. In contrast, this was 50 ng EE2/L and 100 ng E2/L under saline conditions. For female GSI the lowest observed LOAEC was 0.2 ng EE2/L and 10 ng E2/L under freshwater condition (Table 3), whereas no effect was observed at exposure levels up to 100 ng EE2/L or at 500 ng E2/L under saline conditions. On average $LOAEC_{LOW}$ for androgens was >17-fold lower under freshwater conditions compared to saline conditions.

### 3.2. Difference in endpoint sensitivity

Endpoint sensitivity for estrogenic and androgenic EDCs under freshwater and saltwater conditions is reported in Figs. 1 and 2, respectively. For both freshwater and saltwater conditions, endpoints presenting less than 2 observations were excluded. The most sensitive endpoints for estrogenic exposure were male VTG induction, female E2 levels and fecundity, all exhibiting responsiveness in >65% of studies (Fig. 1). The same trend was identified when considering experiments conducted exclusively under freshwater conditions, but the prevalence of responsiveness increased to 80% of studies (Figs. 1 and 2). VTG levels for females were a sensitive endpoint to detect impacts of estrogenic exposure under freshwater conditions, with a significant effect measured in >80% of studies (Fig. 1). Contrarily, when females were exposed to estrogenic EDCs under saline conditions not a single significant difference in VTG-levels was reported (Fig. 1). The least sensitive endpoints for estrogenic exposure included male and female GSI, male and female testosterone levels, as well as histological assessment of gonadal tissue, with <33% of studies reporting significant effects of estrogenic exposure on these endpoints under both freshwater and saline conditions (Fig. 1).

When examining androgenic effects, endpoints measured in males were generally less sensitive compared to endpoints measured in females, with the exception of 11KT levels (Fig. 2). Male testosterone levels, VTG induction, histological assessment of gonad alteration and GSI showed effects in <40% of experiments, regardless of salinity (Fig. 2). The most sensitive endpoints for assessing androgenic effects, again regardless of salinity, were female E2 and T levels, female VTG levels and fecundity (Fig. 2). GSI in females was not a sensitive endpoint to assess androgenic effects. Histological alteration of female gonads was a sensitive endpoint under freshwater conditions (effects observed in nearly 70% of

**Table 3**
The lowest reported LOEC across studies for individual endpoints for fish exposed to 17α-ethinylestradiol (EE2) or 17β-estradiol under either freshwater or saline conditions. Bold indicates under which salinity the lowest $LOAEC_{LOW}$ was observed.

| Contaminant | | Condition | Endpoint LOAEC[a] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Males | | | | | Females | | | | | Other | | | |
| | | | 11KT | T | VTG | HIST | GSI | E2 | T | VTG | HIST | GSI | SSC | FEC | FERT | HATCH |
| EE2 | Concentration range (ng/L) | Fresh | 0.5–25 | 0.2–500 | 0.1–2025 | 0.1–2025 | 0.1–2025 | 0.2–2000 | 0.5–500 | 0.1–2025 | 1–2025 | 0.1–2025 | 0.1–100 | 0.1–2000 | 0.1–2000 | 0.2–2000 |
| | | Saline | 0.1–100 | 0.1–500 | 0.1–1000 | 0.1–100 | 0.1–3000 | 0.1–250 | 0.1–500 | 0.1–100 | 0.1–100 | 0.1–3000 | 25–50 | 0.1–3000 | 0.1–3000 | 3–3000 |
| | Number of experiments | Fresh | 3 | 4 | 13 | 6 | 9 | 4 | 3 | 7 | 3 | 9 | 3 | 8 | 6 | 1 |
| | | Saline | 1 | 4 | 4 | 1 | 6 | 3 | 4 | 1 | 0 | 5 | 1 | 3 | 2 | 1 |
| | LOAECLow | Fresh | **2** | **10** | **0.5** | **3** | **1** | **0.5** | >4.5 | **1** | 25 | **0.2** | **1** | **0.2** | **5** | **500** |
| | | Saline | >100 | >100 | 50 | >100 | 100 | 10 | >100 | >100 | - | >100 | >50 | 50 | 100 | >3000 |
| | | Saline:Fresh | >50 | >10 | 100 | >33 | 100 | 20 | – | >100 | – | >500 | >50 | 250 | 20 | >6 |
| E2 | Concentration range (ng/L) | Fresh | – | 30–3793 | 5–3125 | 10–1000 | 5–3973 | 30–1000 | 30–3793 | 5–500 | 9–100 | 5–3793 | 5–10,000 | 30–3793 | 31.3–500 | 30–1000 |
| | | Saline | – | – | 10–2000 | 10–500 | 10–500 | – | – | 10–500 | 10–500 | 10–500 | – | 10–500 | 10–500 | – |
| | Number of experiments | Fresh | 0 | 2 | 20 | 10 | 18 | 1 | 2 | 17 | 10 | 20 | 5 | 3 | 1 | 2 |
| | | Saline | 0 | 0 | 5 | 3 | 4 | 0 | 0 | 4 | 3 | 4 | 0 | 1 | 1 | 0 |
| | Lowest | Fresh | – | 3793 | **10** | **32** | **10** | 1000 | 3793 | **25** | >44 | **10** | 44 | **300** | **500** | 500 |
| | | Saline | – | – | 100 | 200 | >100 | – | – | >100 | 300 | 500 | – | 500 | >500 | – |
| | | Saline:Fresh | – | – | 10 | 6 | >10 | – | – | >4 | – | 50 | – | 2 | >1 | – |

[a] 11KT: 11-ketotestosterone; T: testosterone; E2: 17β-estradiol; VTG: vitellogenin; SSC: secondary sex characteristics; HIST: gonad histology; GSI: gonadosomatic index; FEC: fecundity; SPAWN: number of spawning events; FERT: fertility; HATCH: hatchability.

**Table 4**
The lowest reported LOEC across studies for individual endpoints for fish exposed to 17β-trenbolone (TB), 5α-dihydrotestosterone (DHT) or methyltestosterone (MT) under either freshwater or saline conditions. Bold indicates under which salinity the lowest $LOAEC_{LOW}$ was observed.

| Contaminant | | Condition | Endpoint LOAEC[a] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Males | | | | | Females | | | | | Other | | | |
| | | | 11KT | T | VTG | HIST | GSI | E2 | T | VTG | HIST | GSI | SSC | FEC | FERT | HATCH |
| TB | Concentration range (ng/L) | Fresh | 5–50,000 | 5–50,000 | 5–50,000 | 50–5000 | 5–50,000 | 5–50,000 | 5–50,000 | 5–50,000 | 5–50,000 | 5–50,000 | 5–50,000 | 5–50,000 | 5–50,000 | 5–50,000 |
| | | Saline | 50–500 | – | 5–5000 | 50–5000 | 50–5000 | – | – | 5–5000 | 10–5000 | 5–5000 | 10–5000 | 5–5000 | 5–5000 | 5–5000 |
| | Number of experiments | Fresh | 1 | 1 | 13 | 9 | 13 | 2 | 2 | 13 | 10 | 13 | 8 | 1 | 1 | 1 |
| | | Saline | 0 | 0 | 5 | 2 | 3 | 0 | 0 | 5 | 3 | 5 | 1 | 2 | 2 | 2 |
| | Lowest | Fresh | 50,000 | >50,000 | **500** | **50** | >5000 | 500 | 500 | 50 | **10** | **500** | **50** | **50** | **500** | **500** |
| | | Saline | – | – | >5000 | >5000 | >5000 | – | – | 50 | 1000 | 1000 | 200 | 1000 | 1000 | 5000 |
| | | Saline:Fresh | – | – | >10 | >100 | – | – | – | 1 | 100 | 2 | 4 | 20 | 2 | 10 |
| DHT | Concentration range (ng/L) | Fresh | 0.05–5 | 0.05–5 | 10–100 | 0.05–5 | 0.05–100 | 0.05–5 | 0.05–5 | 10–100 | 0.05–5 | 0.05–100 | 10–100 | 0.05–5 | – | – |
| | | Saline | 0.05–100 | 0.05–100 | 10–100 | 0.05–50 | 0.05–100 | 0.05–100 | 0.05–100 | 10–100 | 0.05–5 | 0.05–100 | – | 0.05–5 | – | – |
| | Number of experiments | Fresh | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 0 |
| | | Saline | 3 | 3 | 1 | 2 | 3 | 2 | 2 | 1 | 1 | 2 | 0 | 1 | 0 | 0 |
| | Lowest | Fresh | 5 | >5 | **32** | >5 | >5 | 0.5 | 0.5 | **10** | >5 | >5 | 10 | >5 | – | – |
| | | Saline | 5 | 10 | 100 | >5 | >5 | 0.5 | 0.5 | 100 | >5 | >5 | – | **0.05** | – | – |
| | | Saline:Fresh | 1 | – | 3 | – | – | 1 | 1 | 10 | – | – | – | <0.01 | – | – |
| MT | Concentration range (ng/L) | Fresh | – | – | 0.001–50 | 0.025–50 | 0.001–50 | – | – | 0.025–50 | 0.025–50 | 0.001–50 | 0.025–50 | 0.025–50 | 0.025–50 | 0.025–50 |
| | | Saline | 0.001–1 | 0.001–1 | 0.001–1 | – | 0.001–1 | 0.001–1 | 0.001–1 | 0.001–1 | – | 0.001–1 | – | – | – | – |
| | Number of experiments | Fresh | 0 | 0 | 3 | 2 | 3 | 0 | 0 | 2 | 2 | 4 | 2 | 2 | 2 | 1 |
| | | Saline | 2 | 2 | 2 | 0 | 2 | 2 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 0 |
| | Lowest | Fresh | – | – | 1 | 0.4 | >0.1 | – | – | 0.2 | 0.025 | **0.05** | 0.025 | 0.05 | 0.05 | 0.05 |
| | | Saline | 0.1 | 0.01 | >0.1 | – | >0.1 | 0.01 | 0.01 | **0.1** | – | >0.1 | – | – | – | – |
| | | Saline:Fresh | – | – | – | – | – | – | – | 0.5 | – | >2 | – | – | – | – |

[a] 11KT: 11-ketotestosterone; T: testosterone; E2: 17β-estradiol; VTG: vitellogenin; SSC: secondary sex characteristics; HIST: gonad histology; GSI: gonadosomatic index; FEC: fecundity; SPAWN: number of spawning events; FERT: fertility; HATCH: hatchability.

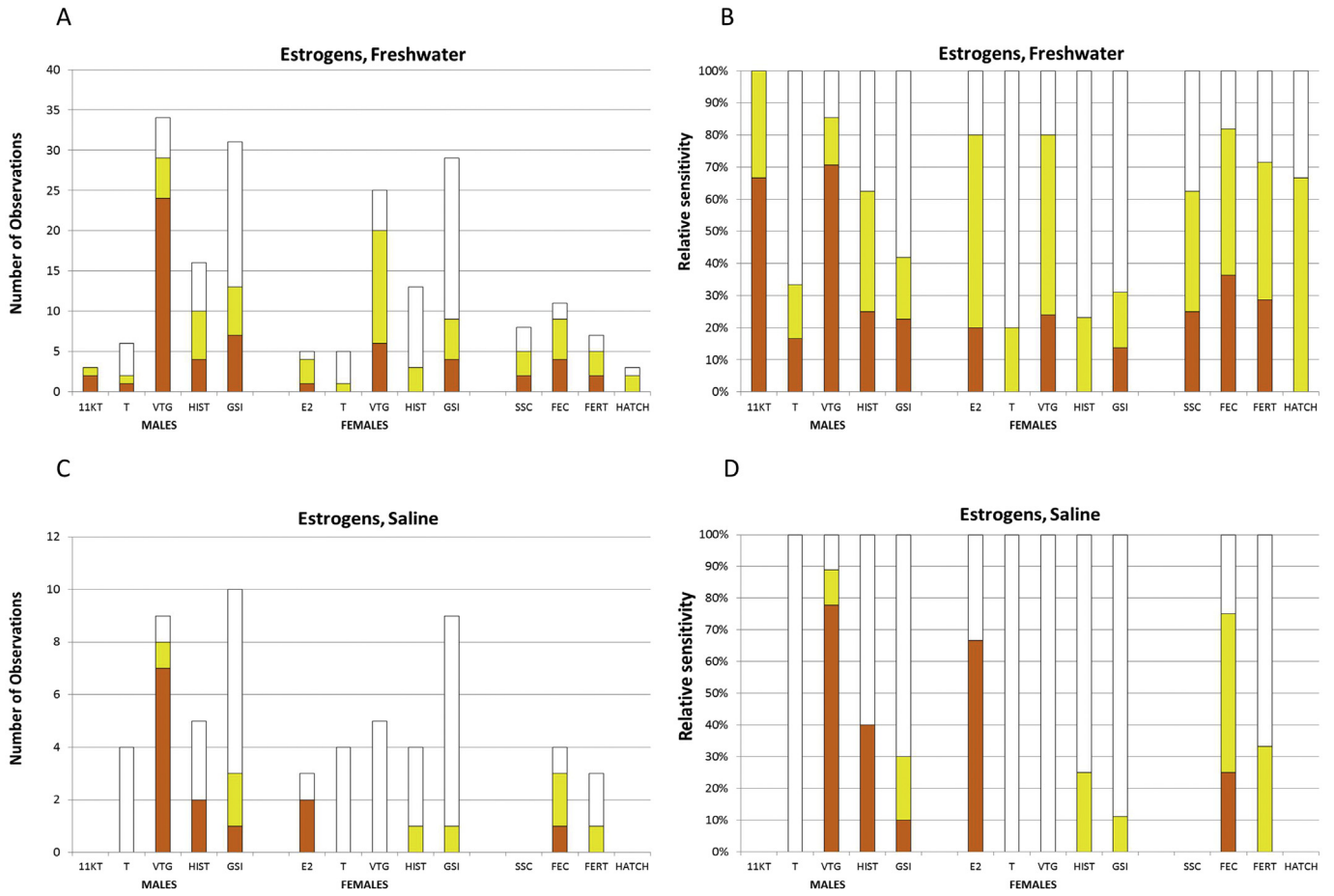


**Fig. 1.** Endpoint sensitivity for estrogens under fresh (a, c) and saline (b, d) exposure conditions. The number of experiments reported with lowest observed effect concentration (LO) is shown in orange. The number of studies in which observed effects were reported above the LOEC within the same study is shown in yellow. The number of studies in which no observed effects was reported at concentrations higher than the maximum tested concentration is shown in white. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

experiments), but not under saline conditions (effects only observed in 20% of experiments; Fig. 2).

Overall, E2 and fecundity were the only two endpoints identified as being sensitive for detecting both estrogenic and androgenic effects (Figs. 1 and 2). In >75% of experiments a significant change in fecundity was observed, regardless of salinity and estrogenic or androgenic mode of action. A significant response in E2 levels was observed in >65% of experiments.

## 4. Discussion

The outcomes of this semi-quantitative review suggest that the salinity at which standard reproductive bioassays (with small-bodied fish) are performed may be an important factor influencing effective concentration to EDCs. In general, the concentration at which $LOAEC_{LOW}$ was observed was more frequently lower when fish were exposed under freshwater conditions compared to saline conditions. This is especially true for the model estrogenic EDCs considered in our analysis (EE2 and E2), but also for model androgens (TB, DHT and MT), although the response pattern was less apparent. The influence of salinity on the observed LOAEC has been previously described for other contaminants, such as various metals and PAHs (Hall and Anderson, 1995; Wood et al., 2004; Blanchard and Grosell, 2005; Ramachandran et al., 2006; Shukla et al., 2007). However, to our knowledge this is the first study to confirm this phenomenon using an innovative approach for semi-quantitative review, based on available response data for common steroidal EDCs. Short-term reproductive tests using small-bodied freshwater species are commonly applied by regulatory agencies such as the US EPA (EPA, 2011) and the OECD (OECD) to investigate the potential impacts of EDCs on the environment. Our results are therefore important, since they highlight the need to consider both freshwater species, and species that normally inhabit estuarine or marine environments (e.g. three-spine sticklebacks, sheepshead minnow and mummichog) to accurately predict and assess the impacts of EDCs on aquatic biota.

The mechanism underlying differences in responsiveness to EDCs at varying salinities are poorly understood. Recent work exploring uptake of EE2 by mummichog under a range of salinities (0, 16 and 32 ppt) found a significant increase in EE2 uptake at brackish (16 ppt) compared to freshwater (0 ppt) and seawater (32 ppt) conditions (Blewett et al., 2013). This difference might be due to differences in gill morphology under different salinities (Blewett et al., 2013). One obvious explanation is that differences are associated with differential species sensitivity, and that the influence of salinity on responsiveness may be more coincidence than cause. However, another study found no significant difference in EE2 uptake by the brackish mummichog exposed under freshwater conditions compared to several freshwater species (Blewett et al., 2014). This supports our findings because it suggests that differences in uptake, and potentially in responsiveness to EDCs may be more related to the salinity of the exposure medium, as

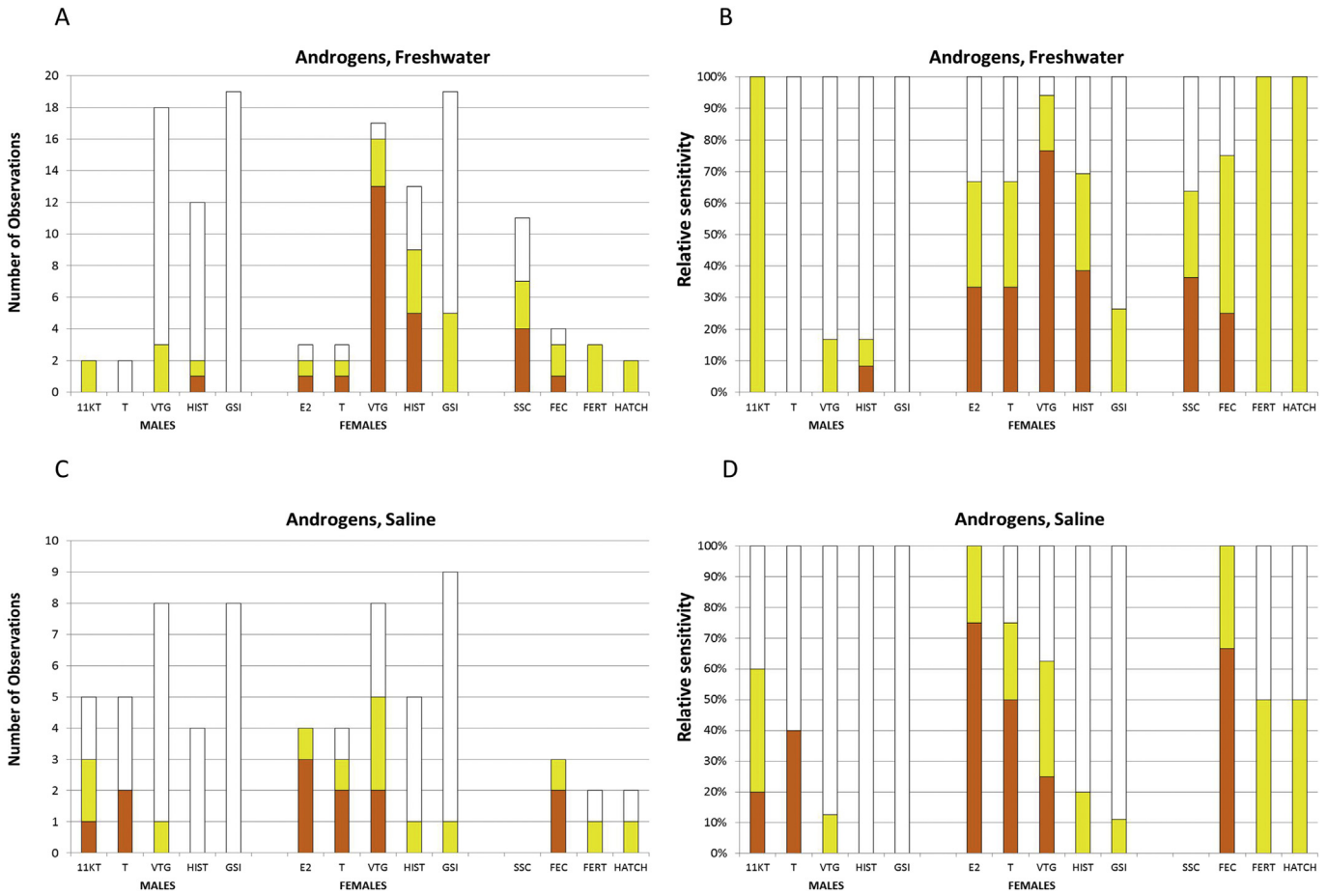


**Fig. 2.** Endpoint sensitivity for androgens under fresh (a, c) and saline (b, d) exposure conditions. The number of experiments reported with lowest observed effect concentration (LO) is shown in orange. The number of studies in which observed effects were reported above the LOEC within the same study is shown in yellow. The number of studies in which no observed effects was reported at concentrations higher than the maximum tested concentration is shown in white. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

opposed to basic differences in species sensitivity. Interestingly, tissue-specific accumulation differed across species in that study, with increased accumulation in the liver and gallbladder in mummichog, as well as Japanese medaka (*Oryzias latipes*), compared to fathead minnow, goldfish (*Carassius auratus*), zebrafish and rainbow trout (Blewett et al., 2014). As such, further research exploring differences in uptake, elimination, and bioaccumulation of EDCs are needed to better understand the influence of salinity.

A previous semi-quantitative review applied a similar approach to compare endpoint sensitivity in fathead minnow, zebrafish and Japanese medaka exposed to EDCs (Dang et al., 2011a). The present study expanded this evaluation to include a total of 12 different species to facilitate comparison of responsiveness in studies performed under freshwater versus saline conditions. The number of chemicals was also reduced for the present analysis, to include only those estrogenic and androgenic EDCs that have been studied under both freshwater and saline conditions with small-bodied reproductive fish bioassays. By focusing the analysis in this manner, our study identified several differences in comparative endpoint sensitivity between exposure under saline and freshwater conditions. Most notably, changes in VTG levels in female fish were identified as a sensitive endpoint to assess estrogenic EDCs under freshwater conditions, but this was not the case for studies carried out under saline conditions. Similarly, 11KT levels in males was found to be highly sensitive under freshwater conditions, but much less so under saline conditions. As discussed, a limited number of studies have explored the influence of salinity on responsiveness of fish to EDCs, but several studies have explored the influence of salinity on sexual maturation, including vitellogenesis and steroidogenesis. For example, female striped mullet (*Mugil cephalus*) exhibited greater vitellogenesis in saline compared to freshwater conditions (Tamaru et al., 1994), and plasma steroid levels were unaffected by salinity in female black bream (*Acanthopagrus butcheri*) whereas males of this species exhibited increased 11KT in saline conditions (Haddy and Pankhurst, 2000). These examples support our hypothesis that salinity is an important factor that can influence sensitivity of fish to EDCs, and also corroborates the differences in responsiveness of VTG and 11KT identified between sexes.

Our results suggest that the most sensitive endpoints in fish exposed to both estrogenic and androgenic EDCs are E2 levels and altered fecundity in females. This is consistent with a previous review on short-term reproductive tests that similarly identified E2 as a highly sensitive endpoint. Importantly, that study also found E2 to exhibit the best correlation with changes in fecundity (Bosker et al., 2010b), highlighting the importance in assessing both of these endpoints when evaluating the effects of EDCs on fish reproduction. However, our results differ somewhat from the study by Dang et al. (2011a) who reported fecundity, VTG and gonad histology to be the most sensitive endpoints. Specifically, our results indicate that female VTG is not a sensitive endpoint for

assessing estrogenic EDCs under saline conditions, and that male VTG levels are not sensitive to androgenic compounds under freshwater or saline conditions. Finally, histological assessment of the gonads showed only a moderate chance of finding significant effects for androgens under freshwater conditions, but not for any other scenario. The difference in outcomes may reflect the difference in approach. Specifically, the present study included a greater number of species but focussed on fewer chemicals compared to the study performed by Dang et al. (2011a).

To conclude, this is the first study to our knowledge to systematically assess the potential influence of salinity on reproductive effects in fish exposed to common environmental EDCs. We found that fish generally respond to lower levels of both estrogenic and androgenic contaminants when exposed under freshwater conditions. In addition, our analysis revealed minor differences in endpoint sensitivity, which represents useful information for ensuring that the most sensitive endpoints are targeted for reproductive bioassays with small-bodied fish. The most sensitive endpoints in the literature, regardless of estrogenic or androgenic mode of action, or differences in salinity, were identified as E2 levels in female fish and fecundity. Overall, these findings support the hypothesis that salinity may be an important factor that can influence the effects of EDCs on fish reproduction, stressing the importance of taking this variable into account to achieve comprehensive environmental risk assessment. Considering the potential importance for influencing study outcomes, future experimental research is warranted to explicitly explore differential sensitivity in common model small-bodied fish species exposed under different salinities.

## Competing interests

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.05.063.

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