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Effects of cadmium chloride on the development of rainbow trout *Oncorhynchus mykiss* early life stages

H. M. Lizardo-Daudt*† and C. Kennedy*‡

*Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6 Canada and †Universidade do Vale do Rio dos Sinos, Sao Leopoldo, RS 93 022-000, Brazil

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The sub-chronic (28–56 days) effects of exposure to low concentrations of cadmium (Cd; 0·05, 0·25, 0·50 and 2·50 μg l^{-1}) shortly following fertilization on embryos, larvae and juvenile rainbow trout *Oncorhynchus mykiss* were examined. Premature hatching occurred at lower concentrations (0·05 and 0·25 μg l^{-1} Cd), however, delayed hatching was seen in the 2·50 μg l^{-1} Cd group, with >90% of hatching occurring on the last day of the hatching period. Larval growth was negatively affected by Cd exposure in a concentration-dependent manner. Larvae exposed to 2·50 μg l^{-1} Cd were 13·9 ± 0·8% shorter in total length (L_T) and weighed 22·4 ± 3·5% (mean ± S.E.) less than controls at the end of the exposure period. Plasma sex steroid concentrations (oestradiol in juvenile females and 11-ketotestosterone in juvenile males) were elevated (four- to 10-fold over controls) in exposed fish in both males and females, following 28 days of exposure to 0·05, 0·25 and 0·50 μg l^{-1} Cd, respectively. These results suggest that environmentally realistic concentrations (in the μg l^{-1} range) of Cd can affect the development of *O. mykiss* impacting embryos, larvae and juvenile fish. © 2008 The Authors
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Key words: cadmium; development; early life stages; endocrine disruption; trout.

INTRODUCTION

Reproductive and developmental function involves complex processes that are integrated through the nervous, endocrine and reproductive systems in vertebrates (Jalabert *et al.*, 2000). The physiological mechanisms involved in these processes and their sensitivity to disruption have become an area of increasing concern since the development and reproduction of humans and wildlife are considered to be potentially at risk from environmental pollutant exposure (Arcand-Hoy & Benson, 1998; Kime, 1999).

Exposure to aquatic contaminants that interfere with endocrine function [endocrine disrupting compounds (EDCs)] may affect one or several components

‡Author to whom correspondence should be addressed. Tel.: +1 778 782 5640; fax: +1 778 782 3496; email: ckennedy@sfu.ca

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of the piscine reproductive system including the gonads, liver, sperm and eggs, reproductive behaviour and may also affect subsequent development, culminating in alterations in population dynamics (Arcand-Hoy & Benson, 1998; Kime, 1999).

Heavy metal exposure has been linked to endocrine disruption in several aquatic species (Hontela, 1998; Depledge & Billinghurst, 1999; Hewitt & Servos, 2001). Cadmium (Cd) in particular, has been shown to modulate endocrine pathways in teleosts *in vivo* and *in vitro* (Thomas & Khan, 1997; Hontela, 1998). The hypothalamic-pituitary-gonadal (HPG) axis is a prime target of Cd toxicity (Arcand-Hoy & Benson, 1998; Henson & Chedrese, 2004) and Cd acts centrally in disrupting steroidogenesis at the level of the pituitary gland (Tilton *et al.*, 2003), as well as acting directly on steroidogenic tissues including the gonads. Cadmium and other metals have been shown to both stimulate and inhibit sex steroid production depending on the metal concentration, species and sex of the animal (Sangalang & O'Halloran, 1973; Kime, 1984; Thomas & Khan, 1997).

Although Cd can act as an endocrine disruptor in gonadal steroidogenesis, the ultimate repercussions of its effects on reproduction and development are inconsistent, particularly when fishes are exposed to low environmentally realistic concentrations. Cadmium concentrations in surface waters are generally very low, ranging from $<0{\cdot}1$ to $8{\cdot}9$ $\mu g\ l^{-1}$ (Canadian Environmental Protection Act, 1994). Studies using Cd exposures in this range have reported no effects as well as reporting reproductive dysfunction in teleosts. For example, Benoit *et al.* (1976) reported negative effects on the reproductive capabilities of first- and second-generation adult male brook trout *Salvelinus fontinalis* (Mitchill) exposed to $3{\cdot}4$ $\mu g\ l^{-1}$ Cd. Foran *et al.* (2002) reported elevated plasma oestradiol concentrations in Japanese medaka *Oryzias latipes* (Temminck & Schlegel) females exposed to 1 $\mu g\ l^{-1}$ *in ovo* and subsequently re-exposed as adults. Adults whose parents were exposed to Cd (at 1–10 $\mu g\ l^{-1}$) and those adults exposed to Cd early during development, however, reproduced normally despite Cd exposure. Recently, Tilton *et al.* (2003) examined the effects of low Cd concentrations (1–10 $\mu g\ l^{-1}$) on the reproductive axis of the same species. The authors postulated that although Cd may disrupt the hypothalamic-pituitary-gonadal (HPG) axis at multiple sites in males and females, modulating plasma sex steroid concentrations and *ex vivo* gonadal steroid release, no developmental or impaired reproductive capacity was seen. Developmental effects such as altered growth after exposure to low concentrations of Cd, however, have been previously reported in the literature (Benoit *et al.*, 1976; Rombough & Garside, 1982; Hansen *et al.*, 2002*a*).

In order to establish a causal link between exposure to a putative endocrine disruptive agent and changes at the population level, the presence of a chemical in association with an effect (*e.g.* modulation of sex steroids) is not itself sufficient. A comprehensive approach, including investigations of different life stages and different end-points at each stage (*e.g.* analysis of alterations in liver, gonads and pituitary, as well as plasma sex steroids and vitellogenin concentrations) is recommended when evaluating the effects of xenobiotics on the reproductive systems of fishes (Kime, 1999). Integrated research that incorporates measurements and the evaluation of appropriate biomarker responses, chemical

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residues and population dynamics are necessary to enable the real significance of endocrine disruption in a given environment (Taylor & Harrison, 1999). This comprehensive approach has been incorporated with more frequency into recent investigations. Various researchers have examined EDCs and their effects on both development and reproduction by examining various effects in embryos, larvae, juvenile and mature fishes. Among the measurable variables of the reproductive axis, most studies have focused on alterations in hatching, growth, morphology at the tissue and whole organismal level, as well as modulation of plasma hormones concentrations (Teather *et al.*, 2001; Tilton *et al.*, 2003; Ishibashi *et al.*, 2004).

The objectives of the present study were to investigate the effects of subchronic (28–56 days) exposures to environmentally relevant concentrations of Cd on the reproductive axis of *Oncorhynchus mykiss* (Walbaum) using a comprehensive approach, with the intention of furthering the understanding of the potential interference of low concentrations on reproduction and development in teleosts.

MATERIALS AND METHODS

CHEMICALS

Cadmium chloride ($CdCl_2$) and $NaHCO_3$ were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.) Tricaine methanesulphonate (MS222), clove oil and Ovadine® were obtained from Argent Laboratories (Redmond, WA, U.S.A.), Hilltech Canada Inc. (Vankleek Hill, Ontario, Canada) and Syndel International Inc. (Vancouver, British Columbia, Canada), respectively. Nitric acid and ethanol were obtained from Anachemia Science (Vancouver, British Columbia, Canada).

TEST ORGANISM

The animal experimentation described in this manuscript was approved by the Simon Fraser University Animal Care Committee (UACC) and adhered to guidelines outlined by the Canadian Council on Animal Care (CCAC).

Oncorhynchus mykiss eggs and sperm were obtained from two ripe males and two ripe females that were bred under standardized conditions at the Fraser Valley Trout Hatchery in Abbotsford, British Columbia, Canada. The procedure for artificial insemination was based on Pankhurst *et al.* (1996). Briefly, ovulating fish were anaesthetized with clove oil in ethanol (1:9), at a final concentration of *c.* 40 mg l^{-1} of clove oil, removed from the anaesthetic bath and the abdomen and urogenital area of a ripe fish were dried with an absorbent cloth. Eggs were then hand-expressed by holding the fish head-up and putting light pressure on the abdomen starting just below the head to just above the pelvic fins. Fish were given a slight shake and pressure was again applied to remove as many eggs as possible. Eggs were expressed into a dry plastic ziploc container (previously soaked in distilled water for 24 h to remove any residues) and placed on bubble wrap on ice inside a cooler until fertilized. Sperm was obtained following a similar procedure and added directly to the container containing the eggs. Sperm, eggs and ovarian fluid from two females and two males were pooled and gently mixed. Five minutes following the addition of milt to eggs, water was added and fertilized eggs allowed to water-harden for another 20 min. Subsequently, embryos were disinfected with 100 mg l^{-1} of Ovadine® for 15 min, washed gently in water and kept in cold water (*c.* 8° C) until exposure. Any dead or broken eggs were gently removed.

Juvenile *O. mykiss* (mean ± S.E. 68·40 ± 0·77 g) were obtained from Sun Valley Trout Farms (Mission, British Columbia, Canada) and acclimated for at least 4 weeks in 1200

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1 tanks supplied with dechlorinated municipal water at 8° C, range ±2° C before an experiment. Water conditions were: hardness 6·8 mg l^{-1} $CaCO_3$, dissolved oxygen >90% saturation and pH 6·8. Fish were maintained under a 12L:12D photoperiod and fed with commercial trout food *ad libitum* (3 mm pellets: Ewos Canada Ltd, Surrey, British Columbia, Canada).

CADMIUM EFFECTS ON EGG AND LARVAL STAGES

Exposure conditions for embryos and larvae were based on guidelines provided by the Organization for Economic Cooperation and Development (OECD, 1992). Viable embryos (perfectly spherical and translucent) were randomly transferred as a monolayer into eight separate egg incubators 4 h post-fertilization. Each incubator consisted of a single chamber containing 371 ± 5 (mean ± S.E.) embryos. Incubators (exposures were performed in duplicate) were connected to water reservoirs which supplied various concentrations of Cd (0, 0·05, 0·25 and 2·50 μg l^{-1} as $CdCl_2$) to incubators. The lowest concentrations used (0·05 and 0·25 μg l^{-1} Cd) are below the repeated lowest-observed-effect concentration (LOEC; 0·47 μg l^{-1}) for freshwater fishes in soft water (Canadian Environmental Protection Act, 1994). The highest concentration used (2·50 μg l^{-1}) corresponded to <10% of the 200 h concentration killing 50% of the test animals (LC_{50}) for steelhead (*O. mykiss*) alevins (>27 μg l^{-1}) reported by Chapman (1978) and the 96 h LC_{50} (37·9 μg l^{-1} Cd) for *O. mykiss* larvae [100 mg fish; Buhl & Hamilton (1991)]. Each egg incubator consisted of a modified polyethylene 3 l bottle in which water upwelled into the chamber and through a 0·2 mm nylon mesh which supported the embryos, and exited through a screened outlet on the upper section of the incubator. Water was recirculated from incubators to the reservoirs with a constant water flow of 500 ml min^{-1}, which maintained water O_2 saturation >90% at all times. Water in each reservoir was renewed (50%) every other day through the entire exposure period. Water temperature in incubators was maintained at 11·5° C, range ±1·5° C.

After a 40 days exposure, 30 hatched larvae at the swim-up stage from each incubator were removed and transferred to individual rearing aquaria (0·50 l) connected to reservoirs which supplied these tanks with the same Cd concentrations as in the incubators (exposures were in duplicate). Larvae were maintained under a 12L:12D photoperiod and fed *ad libitum* with commercial trout food (crushed 1·2 mm extruded pellets; Ewos Canada) every other day before reservoir water renewal. Excess food and organic material in tanks was removed daily. Larvae were exposed to Cd for a further 16 days following transfer to rearing aquaria.

Due to the large number of larvae in each incubator, hatching was measured in '10% steps' based on Pickova *et al.* (1997). Each tray was assessed using an overlying plexiglass template divided into 10 sections to improve the accuracy of counts. Hatching was recorded as the number of hatched larvae per batch of embryos per degree day (°D = days from fertilization × incubation temperature in ° C). Hatching was recorded from 273 °D (first day of observed hatched larvae) until 328 °D (when all embryos were hatched). Any mortality was recorded daily for the duration of the experiment. When a heartbeat could be visually observed, cessation of beating was the criterion for mortality; prior to this assessment, the opaqueness of embryos was the criterion for mortality (Brauner & Wood, 2002).

Sub-samples of three to five larvae from each rearing aquarium were removed at 35 days (pre-swim-up stage) and 56 days of exposure (post-swim-up) for mass (M) and total length (L_T) measurements. Larvae were sacrificed by immersion in buffered MS-222 (0·1 g l^{-1}).

Approximately 100 larvae at the swim-up stage were sampled from each rearing aquarium at day 40 of exposure and assessed for morphological deformities. Larvae were sacrificed by immersion in buffered MS-222 (0·1 g l^{-1}) and immediately examined without preservation using a dissecting microscope. Three possible malformation categories were evaluated: spinal, craniofacial and yolk-sac abnormalities according to von Westernhagen (1988) and Kennedy *et al.* (2000). Spinal deformities included abnormal

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curvature of the spine, such as lordosis or scoliosis. Craniofacial abnormalities included ocular abnormalities (reduced eye diameter, absence or supranumerary eyes) and jaw malformations (size reduction, abnormal structure or absence), and supranumerary heads. Yolk-sac abnormalities included incomplete yolk circulation or patches of necrotic tissue. With exception of one larva exposed to 2·50 μg l^{-1} Cd that showed prominent spinal deformity (lordosis), craniofacial abnormality (jaw malformation) and which was excluded from the study, all larvae exhibited one major type of malformation along with one or more minor deformities. Therefore, each animal was included in only one of the three malformation categories, which corresponded to the major deformity observed.

CADMIUM EFFECTS IN JUVENILE FISH

Juvenile fish were randomly divided into five groups, consisting of three replicate tanks for each group. Each flow-through tank (130 l) received a different concentration of Cd as $CdCl_2$: 0, 0·05, 0·25, 0·50 and 2·50 μg l^{-1} Cd. Concentrations of Cd in the ppb range were used to assess sublethal effects, as the acute toxicity values for Cd to various life stages and sizes of *O. mykiss* in the literature range from 0·35 to 4000 μg l^{-1} (USEPA, 2007). Mortality was not an end-point of this study, and according to the end-point agreement by the UACC, fish were removed and euthanized by over anaesthetization with MS222 when signs of toxicity such as erratic swimming and loss of equilibrium occurred. Tanks were subdivided into two compartments ($n = 3$ for each compartment). Fish were exposed to Cd continuously for 28 days. The water flow in each tank was 0·9 l min^{-1} and Cd concentrations were maintained using Mariotte bottles (Hontela *et al.*, 1996). Fish were fed *ad libitum* (3 mm pellet; Ewos Canada) every other day until 24 h before sampling. Excess food and all the organic material in tanks were removed daily with minimum disturbance to the fish.

Fish were sampled from each replicate tank in each treatment group on exposure days 7, 14, 21 and 28. At each sampling time, fish from alternate compartments were quickly removed, sacrificed by over anaesthetization with buffered MS222. Fish were weighed and blood samples (1 ml) were taken by caudal puncture. Plasma was separated by centrifuging blood at 16 000 *g* for 4 min and subsequently frozen at −80° C for the determination of sex steroid concentrations. Gonads were removed and weighed (M_G) for calculation of the gonado-somatic index (I_G), $I_G = M_G M^{-1}$. Mortality was recorded daily for the duration of the experiment.

Concentrations of 11-ketotestosterone (11-KT) were determined in male plasma and oestradiol concentrations in female plasma using EIA kits (Cayman Chemical Company, Ann Arbour, MI, U.S.A.). Sub-samples (50 μl) of plasma were used for sex steroid analysis. Each 96 well steroid antibody-coated plate was run with a standard curve based on a serial dilution of a stock solution (10 ng ml^{-1} for 11-KT and 100 ng ml^{-1} for oestradiol). Intra-assay and interassay coefficients of variation (c.v.) for 11-KT were 3·1 and 1·2%, respectively ($n = 5$). Intra-assay and interassay c.v. for oestradiol were 3·8 and 3·6%, respectively ($n = 3$). All measurements were performed on a Spectra Max 420 microplate reader (Molecular Devices Corporation, Sunnyvale, CA, U.S.A.) and absorbances read at 412 nm.

CADMIUM ANALYSIS

Water samples for metal analysis were collected (in each exposure system midway through exposures, in duplicate) in polyethylene bottles, preserved by acidification with concentrated nitric acid at a pH <2·0 and stored at 4° C until analysis. Aqueous Cd concentrations were measured using inductively coupled plasma – optical emission spectroscopy with Varian Vista PRO-axial view ICP-OES (detection limit 0·2 ng ml^{-1} Cd) by Cavendish Analytical Laboratories, Vancouver, British Columbia, Canada. Since analytical confirmations were always >80% of nominal values (0: 0·0184 ± 0·0053, 0·05: 0·0363 ± 0·0042, 0·25: 0·2569 ± 0·0186, 0·50: 0·4732 ± 0·0180 and 2·50: 2·1320 ±

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0·0511 μg l^{-1} Cd; mean ± S.E.) results are reported in terms of nominal concentrations (Tilton *et al.*, 2003).

STATISTICAL ANALYSIS

Statistical analyses were performed using JMP IN 4.0.3 (SAS Institute Inc., Cary, NC, U.S.A.). The experimental data were analysed by one-way or two-way ANOVA followed by Tukey–Kramer HSD tests. Hatching was assessed by repeated measures ANOVA followed by Pillai's trace test and subsequent ANOVA to locate the days when hatching was significantly different between groups. Differences were considered significant at $P \leq 0{\cdot}05$. Some of the data required ln transformation in order to meet the assumption of homogeneity of variance, although non-transformed data are shown in the figures. Results are expressed as means ± S.E. Since no significant differences between *M* of male and female juvenile fish were observed, data from both sexes were pooled.

RESULTS

EGGS AND LARVAE

The mortality of embryos ranged from 11·94 ± 0·55 to 14·37 ± 1·03% and there were no significant differences between controls and any exposed group ($F_{3,4}$, $P > 0{\cdot}05$). Hatching occurred from 273 until 328 °D (Fig. 1). Hatching was significantly different between groups over time ($F_{3,4}$, $P < 0{\cdot}001$). Embryos exposed to 0·25 μg l^{-1} Cd began hatching earlier than all the other groups and at 294 °D showed a significantly higher per cent hatch compared to all other groups ($F_{3,4}$, $P < 0{\cdot}001$). At 316 °D, all groups, with the exception of the 2·50 μg l^{-1} Cd group, had reached their highest per cent hatch. Between 316 and 328 °D, almost all embryos from the 2·50 μg l^{-1} Cd group hatched and although delayed, hatching occurred to the same total per cent hatch as the other groups. Cumulative per cent hatch was not different between groups at

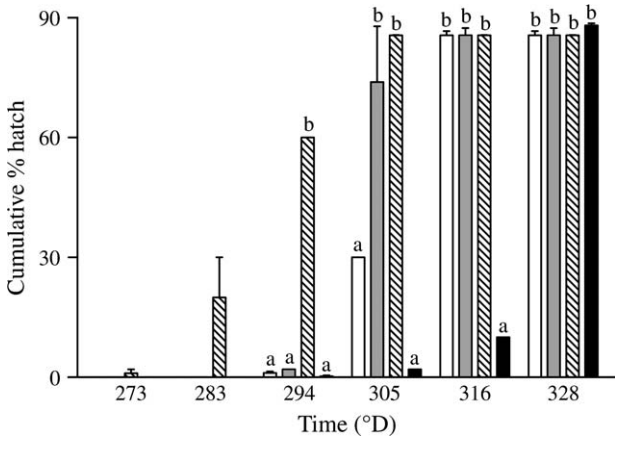

FIG. 1. Cumulative per cent hatching of rainbow trout eggs exposed to (□) 0, (▩) 0·05, (▨) 0·25 and (■) 2·50 μg l^{-1} cadmium from 273 to 328 degree days (°D). Statistical comparisons were made between concentrations at each time point. Treatments with different lower case letters were significantly different from each other ($P < 0{\cdot}05$). Values are means ± S.E.

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328 °D (85·63 ± 1·03, 86·13 ± 1·77, 85·64 ± 0·04 and 88·05 ± 0·55% for control, 0·05, 0·25 and 2·5 μg l^{-1} Cd groups, respectively) ($F_{3,4}$, $P > 0·05$).

Cumulative mortality of larvae at 56 days of Cd exposure ranged from 2·89 ± 0·77 to 5·54 ± 1·16% with no significant differences between exposed and control groups ($F_{3,4}$, $P > 0·05$). Total per cent malformations in control larvae were 22·93 ± 2·29%. Total per cent malformations in Cd-exposed groups were 13·85 ± 5·93, 20·38 ± 6·76 and 23·72 ± 6·76% for the 0·05, 0·25 and 2·50 μg l^{-1} Cd groups, respectively. No significant differences between exposed and control groups ($F_{3,4}$, $P > 0·05$) existed in the total frequency of deformities. Spinal malformations, craniofacial and yolk-sac abnormalities were observed in all groups, with spinal deformities being the most frequent. Among fish which exhibited spinal malformations, lordosis occurred in 87·33 ± 2·92% and scoliosis occurred in 12·67 ± 2·92%. Supranumerary heads was the major craniofacial abnormality observed, with only one larva showing jaw malformation (2·50 μg l^{-1} group) and another showing three eyes (0·50 μg l^{-1} group). Among yolk-sac deformities, two larvae from the control group and one larva from the 2·50 μg l^{-1} group showed patches of necrotic tissue; all the others had incomplete yolk circulation.

The mass (M) and length (L) of larvae determined at 35 and 56 days of exposure to Cd are shown in Fig. 2. Changes in M and L varied with time and Cd concentration ($M, F_{7,77}, P < 0·001$; total length, $L_T, F_{7,77}, P < 0·001$). The L_T controls were 22·2 ± 0·1 mm at 35 days and 25·4 ± 0·1 mm at 56 days. The L_T of controls exposed to 0·05 and 0·25 μg l^{-1} Cd was not significantly different from controls at 35 days, however, larvae from the 2·50 μg l^{-1} Cd group were significantly shorter (*c.* 10%) compared to all other groups at this time point ($F_{3,19}$, $P < 0·001$). The L_T of larvae at day 56 exhibited a similar trend ($F_{3,58}$, $P < 0·001$). The M of control larvae were 91·66 ± 1·71 and 95·80 ± 1·31 mg after 35 and 56 days, respectively. The M of larvae exposed to any Cd concentration was not significantly different from controls at 35 days, however, larvae exposed to 2·50 μg l^{-1} Cd weighed significantly less than larvae exposed to 0·25 μg l^{-1} Cd ($F_{3,19}$, $P < 0·01$). Larvae exposed to 0·25 and 2·50 μg l^{-1} Cd for 56 days weighed significantly less than larvae exposed to the same Cd concentrations for 35 days. Larvae exposed to 2·50 μg l^{-1} Cd, weighed less than all other groups after 56 days of exposure ($F_{3,58}$, $P < 0·001$).

JUVENILE FISH

Mortality in juvenile *O. mykiss* occurred at the highest Cd concentration. No significant difference in mortality was seen between control and the 0·05, 0·25 and 0·50 μg l^{-1} Cd groups ($F_{3,15}$, $P > 0·05$) by 28 days of exposure. Mortality was 75·71 ± 6·49% for fish exposed to 2·50 μg l^{-1} Cd after only 7 days of exposure and therefore continued exposure of this group was discontinued.

The M of control fish at day 28 was 70·13 ± 5·79 g. No significant differences were found between control and Cd-exposed groups ($F_{3,28}$, $P > 0·05$). The I_G was 0·12 ± 0·01 for females and 0·06 ± 0·01 for males with no treatment or time effect in either group (females: $F_{3,26}$, $P > 0·05$; males: $F_{3,34}$, $P > 0·05$). Plasma sex steroid concentrations from fish exposed to varying concentrations of Cd for 7 and 28 days are shown in Fig. 3. Plasma oestradiol concentrations

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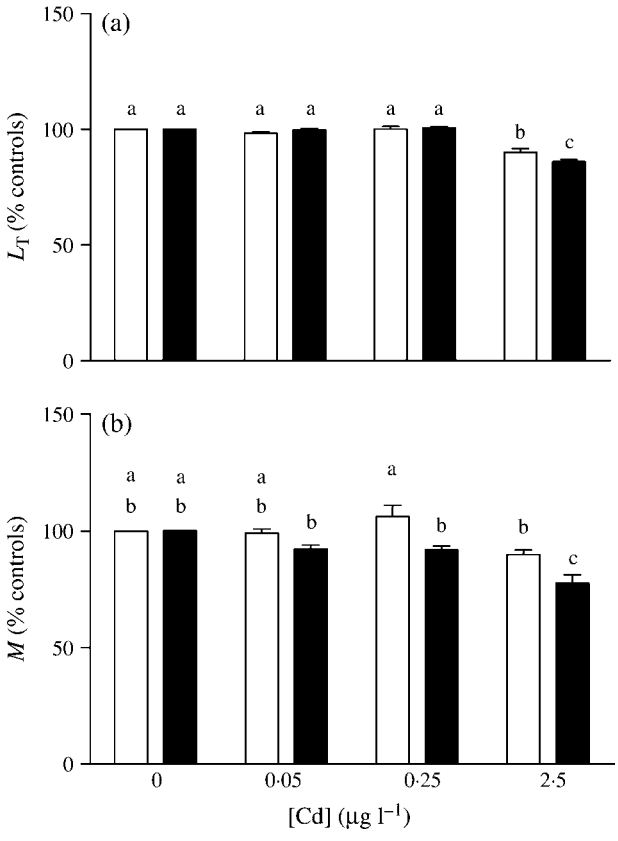

FIG. 2. Growth measurements: (a) total length (L_T) and (b) mass (M) for *Oncorhynchus mykiss* larvae exposed to 0, 0·05, 0·25 and 2·50 μg l^{-1} cadmium for 35 (■, $n = 5$–6) and 56 days (□, $n = 11$–18). Control values represent M or L at each time point. Treatments with different lower case letters were significantly different from each other ($P < 0·05$). Values are means ± S.E.

in control female fish were 3·89 ± 0·44 pg ml^{-1} through the entire exposure period, which was constant with time. Plasma oestradiol concentrations varied with time and the various Cd concentrations ($F_{15,47}$, $P < 0·001$). Plasma oestradiol concentrations were higher than control values for the 0·05 and 0·50 μg l^{-1} Cd groups after 7 days (297·16 ± 47·62 and 323·15 ± 35·67% of controls, respectively; $F_{3,8}$, $P < 0·01$). After 14 days of Cd exposure, oestradiol concentrations in exposed fish ranged from 36·12 ± 0·00 to 145·07 ± 13·08% of controls with no significant difference between controls and Cd treated groups ($F_{3,13}$, $P < 0·01$). At 21 days of exposure to Cd, no significant effect on oestradiol concentrations was evident, and values ranged from 110·84 ± 26·72 to 184·36 ± 42·21% of controls ($F_{3,15}$, $P > 0·05$). By 28 days, plasma oestradiol concentrations were significantly higher in all Cd-exposed groups (668·14 ± 64·61, 450·57 ± 77·93 and 548·68 ± 73·64% of controls in the 0·05, 0·25 and 0·50 μg l^{-1} Cd groups, respectively) ($F_{3,10}$, $P < 0·001$).

Plasma 11-KT concentrations in control fish were 13·39 ± 2·51 pg ml^{-1} for the entire exposure period and were not affected by time. Plasma 11-KT

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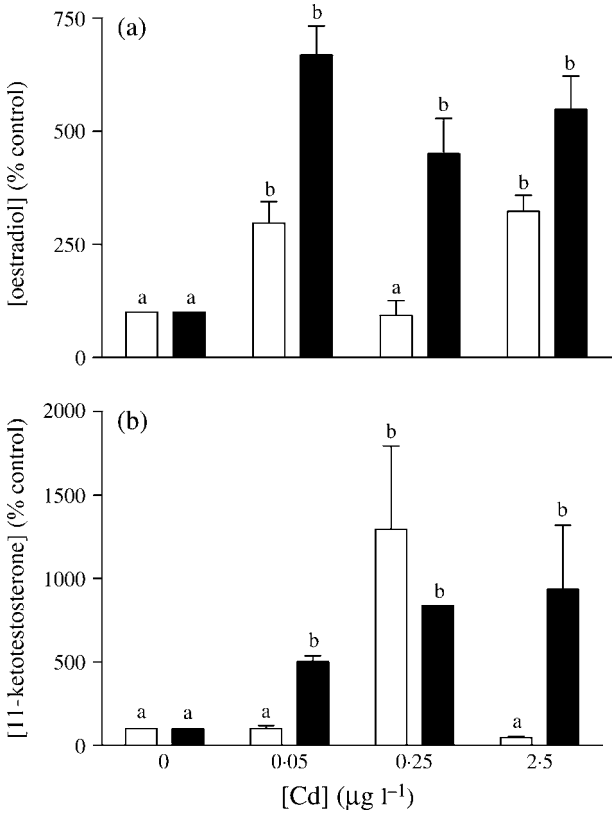

FIG. 3. Sex steroids concentrations as per cent of control values in juvenile *Oncorhynchus mykiss* (a) females and (b) males exposed to 0 (control), 0·05, 0·25 and 0·50 μg l^{-1} cadmium for 7 (■) and 28 (□) days, $n = 3$–5. Treatments with different lower case letters were significantly different from each other ($P < 0·05$). Values are means ± S.E.

concentrations varied with time and Cd exposure ($F_{5,42}$, $P < 0·001$). After 7 days of Cd exposure, male fish showed significantly higher 11-KT concentrations in the 0·25 μg l^{-1} Cd group compared to controls (1296·34 ± 136·29% of controls; $F_{3,11}$, $P < 0·001$). After 14 and 21 days of Cd exposure, levels of 11-KT in exposed fish ranged from 63·75 ± 24·93 to 179·17 ± 77·15% of controls with no significant difference between controls and any treatment group (14 days: $F_{3,12}$, $P > 0·05$; 21 days: $F_{3,11}$, $P > 0·05$). Plasma 11-KT concentrations were significantly higher in exposed *v.* control fish for all concentrations of Cd after 28 days of exposure, and values ranged from 501·80 ± 36·15 to 935·85 ± 385·02% of controls ($F_{3,8}$, $P < 0·01$).

DISCUSSION

In this sub-chronic study, early life stages (embryos and larvae) and juvenile fish were exposed to low environmentally realistic concentrations of Cd with the intention of assessing the potential for adverse effects on the reproductive

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and developmental axes of *O. mykiss* using a comprehensive approach. As far as is known, this is the first study to demonstrate that low μg l^{-1} Cd concentrations affect *O. mykiss* reproductive and developmental variables.

Early life stage and juvenile fish responded very differently to the Cd exposure concentrations used in this study. It was not the intention of this study to examine lethality as an end-point; however, some mortality did occur and yielded important information regarding Cd toxicity to various teleost life stages. Cadmium was not acutely toxic to either embryos or larvae at the concentrations used, however, at the highest concentration tested (2·50 μg l^{-1} Cd), *c.* 80% of test juvenile fish died after 7 days of exposure. LC_{50} values described in the literature for *O. mykiss* vary widely (from 0·35 to 4000 μg l^{-1}) depending on various factors including the time of exposure, hardness of water and size and developmental stage of the fish (Chapman, 1978; Hansen *et al.*, 2002*b*; Lacroix & Hontela, 2004). The present results demonstrate clearly that juvenile *O. mykiss* are more sensitive to Cd than earlier life stages. A shift in the primary site of Cd uptake from the more robust integument in newly hatched larvae, to the fragile branchial epithelium in older fish may account for the increase in sensitivity in the later developmental stage (Rombough & Garside, 1982). It is also conceivable that an increase in the number of uptake sites, from primarily the integument in newly hatched larvae, to both the integumentary and branchial epithelium in yolk-sac larvae, to the integument, branchial and gastrointestinal routes in late larvae and juveniles could influence sensitivity. It has been demonstrated that the majority of the Cd present in carp *Cyprinus carpio* L. eggs exposed to polluted river water was confined to the outer membranes with only a small amount moving into the embryo (van Anholt *et al.*, 2002). After hatching, however, the accumulation of Cd increased rapidly in larvae following yolk-sac resorption and the onset of food uptake (Cd-contaminated organic matter and macroinvertebrates). Further investigations are necessary to address the relative importance of the various uptake routes to overall Cd toxicity and their importance to the susceptibility of different life stages of fishes.

Hatching started at 294 °D in controls, with mass hatching at 316 °D, slightly earlier than previously reported values for rainbow trout under typical conditions (*c.* 330 °D) (Wright *et al.*, 2003). Cadmium affected the time embryos took to hatch; however, the effects did not occur in a strict concentration-dependent manner. Hatching began earlier in embryos exposed to the two lower concentrations of Cd compared to controls. Delayed hatching, however, was observed in embryos exposed to the highest concentration of Cd. Interestingly, the final per cent hatch was the same in all the groups by 328 °D. Changes in time to hatch are common in fish embryos exposed to sublethal concentrations of pollutants, and Cd has been shown to induce premature hatching in carp and herring *Clupea harengus* L. and delayed hatching in salmonids (von Westernhagen, 1988). Delayed hatching also occurred in *C. carpio* eggs exposed to 10–50 μg l^{-1} Cd although no effects on eggs exposed to concentrations <5 μg l^{-1} were observed (Witeska *et al.*, 1995; Calta, 2001). Despite differences in the sensitivity between those species (*C. carpio* and *O. mykiss*), it seems that higher Cd concentrations delay hatching in fishes. No clear explanation for this phenomenon exists, but it is possible that Cd may affect the activity of chorionase, the

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enzyme responsible for the dissolution of the egg envelope (Calta, 2001). The present results also demonstrated early hatching associated with the lowest Cd concentrations. Divalent cations such as Ca^{2+} and Mg^{2+} have been shown to stimulate and inhibit chorionase activity: high concentrations can inhibit activity and low concentrations can be slightly stimulating (Yamagami, 1973). Therefore, it is possible that Cd^{2+} may both inhibit and stimulate chorionase activity, thus inducing early or delayed hatch depending on its concentration.

Body mass and length have been previously used as toxicity end-points in studies on the effects of Cd in fishes (Calta, 2001; Nguyen & Janssen, 2002; Tilton *et al.*, 2003). Larval M and L_T were negatively affected at the highest concentration of Cd and, to a lesser extent, at 0·25 μg l^{-1} Cd, suggesting a concentration-dependent response. In addition, a time-dependent effect on growth could also be identified: fish exposed to 0·25 and 2·50 μg l^{-1} Cd showed reduced mass gain with time compared to controls. Specific growth rates of 1–2% per day have been described for salmonids during early life stages and under normal conditions (Uysal & Alpbaz, 2002), a value slightly higher than the 0·6% observed in the present study. Feeding regimes in toxicological studies using fishes will vary depending on the contaminant under investigation (Dave, 1985; Manning *et al.*, 1999; Foran *et al.*, 2002). Since Cd may readily complex with organic material present in water and potentially decrease toxicity (USEPA, 2001; Van Ginneken *et al.*, 2001), the feeding regimes in the early life stage experiments in the present study were modified to be at the lower end of standard feeding protocols in order to minimize the presence of extra organic material in rearing aquaria which could interfere with water quality (Manning *et al.*, 1999). Independent of feeding regime, reduced larval growth was also observed in Atlantic salmon *Salmo salar* L. exposed to 0·47 μg l^{-1} Cd (Rombough & Garside, 1982), *S. fontinalis* exposed to 3·4 μg l^{-1} (Benoit *et al.*, 1976) and *C. carpio* exposed to concentrations as low as 10 μg l^{-1} Cd (Calta, 2001). Cadmium as Cd^{2+} may reduce the uptake of Ca^{2+}, due to their similar ionic size, and consequently compete for ionic binding sites in fishes. Reduced Ca^{2+} uptake may lead to slowed growth in teleosts (Chang *et al.*, 1998; Meinelt *et al.*, 2001). In addition, delays in growth hormone expression during *O. mykiss* development have been shown in Cd-contaminated water at a concentration of 100 μg l^{-1} Cd (Jones *et al.*, 2001). These studies indicate that several mechanisms may be involved in Cd's effects on teleost growth.

Larval malformations were observed in both control and exposed fish with an average frequency of 20·2 ± 2·6%, however, no significant differences between treatment groups were found. This frequency falls within the normal range reported for salmonids (0–21%) (Ciereszko *et al.*, 1999; Kennedy *et al.*, 2000; Aegerter & Jalabert, 2004). In fact, this level of incidence of larval abnormalities under normal conditions is relatively common in cultured fishes (Aubin *et al.*, 2005). Regardless of the observed frequency of malformed larvae in controls, the present results indicate that Cd does not act as a teratogen at the concentrations used. As far as is known, teratogenic effects in embryos exposed to Cd concentrations <500 μg l^{-1} have never been cited in the literature, even though previous studies using higher Cd concentrations (mg l^{-1} range) have demonstrated concentration-dependent teratogenic effects in fishes (Pragatheeswaran *et al.*, 1989; Nguyen & Janssen, 2002; Chan & Cheng, 2003).

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Kime (1999) reviewed strategies for assessing the effects of pollutants on fish reproduction and suggested several variables to measure which would indicate gonadal dysfunction, including both I_G and plasma sex steroid concentrations. The I_G was not affected by the concentrations of Cd used in the present study. Similar results were observed in winter flounder *Pseudopleuronectes americanus* (Walbaum) exposed to 25 and 50 μg l^{-1} Cd (Pereira *et al.*, 1993). On the other hand, in the present study, plasma sex steroid concentrations were significantly increased in juvenile fish following Cd exposure for 28 days with values substantially higher in both males and females exposed to all Cd concentrations. Plasma 11-KT (males) and oestradiol (females) concentrations in mature salmonids are *c.* 100 ng ml^{-1} and 1 to 60 ng ml^{-1} for 11-KT and oestradiol, respectively. In immature fishes, values <1 ng ml^{-1} for both hormones have been reported (van Bohemen & Lambert, 1981; Pottinger *et al.*, 1996; McQuillan *et al.*, 2003). Even at low concentrations, sex steroids are important for sex differentiation and the onset of puberty in juvenile fishes (Patinõ, 1997; Consten *et al.*, 2002). Previous studies with female Atlantic croaker *Micropogonias undulatus* (L.) exposed to 1 mg l^{-1} Cd for 40 days (Thomas, 1989) and male *S. fontinalis* exposed to 1 μg l^{-1} Cd for 93 days (Sangalang & Freeman, 1974) resulted in elevated plasma sex steroid concentrations. Singh (1989), however, reported reduced plasma testosterone and oestradiol concentrations in swamp eel *Monopterus albus* (Zuiew) exposed to 3 mg l^{-1} Cd. Moreover, plasma oestradiol concentration was increased in female *O. latipes* exposed to 5 μg l^{-1} Cd for 49 days, but decreased in fish exposed to 10 μg l^{-1} Cd for the same period of time (Tilton *et al.*, 2003). *In vitro* studies show similarly inconsistent results: increases and decreases in sex steroid production have been seen following Cd exposure (Sangalang & O'Halloran, 1973; Kime, 1984; Thomas & Khan, 1997). It is clear that Cd can modulate plasma sex steroid concentrations, however, the mechanisms of action of Cd in disrupting steroidogenesis in gonads has not been extensively examined in teleosts. In mammals, it has been suggested that Cd may act at multiple sites in the intracellular signalling pathway of testosterone synthesis, encompassing both stimulatory and inhibitory sites (Laskey & Phelps, 1991). Therefore, estimates of inhibition or stimulation of this hormone is probably a summation of both actions, a phenomenon that may also occur in teleosts. Another possible site of Cd action is at the pituitary and hypothalamic level. Studies in mammals have shown the modulation of pituitary hormones *in vivo* (gonadotropin, prolactin, ACTH, growth hormone and thyroid-stimulating hormone) following exposure to Cd (Lafuente & Esquifino, 1999; Lafuente *et al.*, 2003, 2004). Cadmium has been shown to affect pituitary morphology (degenerative lesions) (Pundir & Saxena, 1992) and to increase and decrease pituitary hormones (gonadotropins and prolactin) *in vitro* and *in vivo* in fishes (Thomas, 1989; Fu & Lock, 1990; Mukherjee *et al.*, 1994). In the field, fishes from polluted sites (heavy metals, including Cd) have shown altered pituitary morphology (Mousa & Mousa, 1999).

Seasonal variations in plasma concentrations of sex steroids have been linked to different phases of the reproductive cycle in *O. mykiss*: 11-KT peaks during the period of spermiation (Scott *et al.*, 1980) and oestradiol peaks during vitellogenesis, immediately before the ovulation period (Scott & Sumpter, 1983). Modulation of plasma sex steroid concentrations by xenobiotics could affect the reproductive cycle by producing mature gametes outside the normal spawning

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season, and thus compromising the viability of offspring (Thomas, 1989). Although plasma steroid modulation was assessed only in juvenile fish in the present study, the high levels of sex steroids observed for both sexes after 28 days of Cd exposures indicate that timing of reproduction could be affected in more mature fish.

The results of this study demonstrate that Cd can disrupt several components of the reproductive and developmental axes in *O. mykiss* following subchronic exposure to extremely low Cd concentrations. In fact, Cd acute and chronic toxicity tests indicate that salmonids are the most sensitive fish group as a whole are supported by the present data (Canadian Environmental Protection Act, 1994; Hansen *et al.*, 2002*a*). Moreover, Cd impaired the three life stages studied very differently, with juveniles being affected at the lowest concentrations (although different end-points were used at each stage). Since environmentally realistic concentrations of Cd were used, it is evident that additional field studies are needed to evaluate the potential impact of this metal on the reproductive success of fishes and wildlife.

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References

Aegerter, S. & Jalbert, B. (2004). Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **231,** 59–71.

van Anholt, R. D., Spanings, F. A. T., Knol, A. H., van der Velden, J. A. & Wenderlaar Bonga, S. E. (2002). Effect of iron sulphur dosage on the water flea (*Daphnia magna* Straus) an early development of carp (*Cyprinus carpio* L.). *Archives of Environmental Contamination and Toxicology* **42,** 182–192. doi: 10.1007/s00244-001-0001-x

Arcand-Hoy, L. D. & Benson, W. (1998). Fish reproduction: an ecologically relevant indicator of endocrine disruption. *Environmental Toxicology and Chemistry* **17,** 49–57.

Aubin, J., Gatesoupe, F. J., Labbé, L. & Lebrun, L. (2005). Trial of probotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture Research* **36,** 758–767. doi: 10.1111/j.1365-2109.2005.01280.x

Benoit, D. A., Leonard, E. N., Christensen, G. M. & Fiandt, J. T. (1976). Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). *Transactions of the American Fisheries Society* **105,** 550–560.

van Bohemen, C. G. & Lambert, J. G. D. (1981). Estrogen synthesis in relation to estrone, estradiol, and vitellogenin plasma levels during the reproductive cycle of female rainbow trout, *Salmo gairdneri*. *General and Comparative Endocrinology* **45,** 105–114.

Brauner, C. J. & Wood, C. M. (2002). Effects of long-term silver exposure on survival and ionoregulatory development in rainbow trout (*Oncorhynchus mykiss*) embryos and larvae, in the presence and absence of added dissolved organic matter. *Comparative Biochemistry and Physiology C* **133,** 161–173.

Buhl, K. J. & Hamilton, S. J. (1991). Relative sensibility of early stages of arctic grayling coho salmon, and rainbow trout to nine inorganics. *Ecotoxicology and Environmental Safety* **22,** 184–197.

© 2008 The Authors

Calta, M. (2001). Effects of aqueous cadmium on embryos and larvae of mirror carp. *Indian Journal of Animal Sciences* **71,** 885–888.
Canadian Environmental Protection Act (1994). Cadmium and its compounds. *Environment Canada Health Canada Priority Substances List Assessment Report*
Chan, P. K. & Cheng, S. H. (2003). Cadmium-induced ectopic apoptosis in zebra fish embryos. *Archives of Toxicology* **77,** 69–79. doi: 10.1007/s00204-002-0411-1
Chang, M. H., Lin, H. C. & Hwang, P. P. (1998). Ca^{2+} uptake and Cd^{2+} accumulation in larval tilapia (*Oreochromis mossambicus*) acclimated to waterborne Cd2+. *American Journal of Physiology* **274,** R1570–R1577.
Chapman, G. A. (1978). Toxicities of cadmium, copper, and zinc to four juvenile stages of Chinook salmon and steelhead. *Transactions of the American Fisheries Society* **107,** 841–847.
Ciereszko, A., Dabrowski, K., Lin, F. & Liu, L. (1999). Protective role of ascorbic acid against damage to male germ cells in rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* **56,** 178–183.
Consten, D., Keuning, E. D., Bogerd, J., Zandbergen, M. A., Lambert, J. G. D., Komen, J. & Goos, H. J. Th. (2002). Sex steroids and their involvement in the cortisol-induced inhibition of pubertal development in male common carp, *Cyprinus carpio* L. *Biology of Reproduction* **67,** 465–472.
Dave, G. (1985). The influence of pH on the toxicity of aluminium, cadmium, and iron to eggs and larvae of the zebra fish, *Brachydanio rerio*. *Ecotoxicology and Environmental Safety* **10,** 253–267.
Depledge, M. H. & Billinghurst, Z. (1999). Ecological significance of endocrine disruption in marine invertebrates. *Marine Pollution Bulletin* **39,** 32–38.
Foran, C. M., Peterson, B. N. & Benson, W. H. (2002). Influence of parenteral and developmental cadmium exposure on endocrine and reproductive function in Japanese medaka (*Oryzias latipes*). *Comparative Biochemistry and Physiology C* **133,** 345–354.
Fu, H. & Lock, R. A. C. (1990). Pituitary response to cadmium during the early development of tilapia (*Oreochromis mossambicus*). *Aquatic Toxicology* **16,** 9–18.
Hansen, J. A., Welsh, P. G., Lipton, J. & Suedkamp, M. J. (2002*a*). The effects of long-term cadmium exposure on the growth and survival of juvenile bull trout (*Salvelinus confluentus*). *Aquatic Toxicology* **58,** 165–174.
Hansen, J. A., Welsh, P. G., Lipton, J., Cacela, D. & Darley, A. D. (2002*b*). Relative sensitivity of bull trout (*Salvelinus confluentus*) and rainbow trout (Oncorhynchus mykiss) to acute exposures of cadmium and zinc. *Environmental Toxicology and Chemistry* **21,** 67–75.
Henson, M. C. & Chedrese, P. J. (2004). Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. *Experimental Biology and Medicine* **229,** 383–392.
Hewitt, M. & Servos, M. (2001). An overview of substances present in the Canadian aquatic environment associated with endocrine disruption. *Water Quality Research Journal of Canada* **36,** 191–213.
Hontela, A. (1998). Interrenal dysfunction in fish from contaminated sites: in vivo and in vitro assessment. *Environmental Toxicology and Chemistry* **17,** 44–48.
Hontela, A., Daniel, C. & Ricard, A. C. (1996). Effects of acute and sub acute exposures to cadmium on the interrenal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology* **35,** 171–182.
Ishibashi, H., Matsumura, N., Hirano, M., Matsuoka, M., Shiratsuchi, H., Ishibashi, Y., Takao, Y. & Arizono, K. (2004). Effects of triclosan on early life stages and reproduction of medaka *Oryzias latipes* and induction of hepatic vitellogenin. *Aquatic Toxicology* **67,** 167–179.
Jalabert, B., Baroiller, J. F., Breton, B., Fostier, A., Le Gac, F., Guiguen, Y. & Monod, G. (2000). Main neuro-endocrine, endocrine and paracrine regulations of fish reproduction, and vulnerability to xenobiotics. *Ecotoxicology* **9,** 25–40.
Jones, I., Kille, P. & Sweeney, G. (2001). Cadmium delays growth hormone expression during rainbow trout development. *Journal of Fish Biology* **59,** 1015–1022. doi: 10.1006/jfbi.2001.1718

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Journal compilation © 2008 The Fisheries Society of the British Isles,

Kennedy, C. J., McDonald, L. E., Loveridge, R. & Strosher, M. M. (2000). The effect of bioaccumulated selenium on mortalities and deformities in eggs, larvae, and fry of a wild population of cutthroat trout (*Oncorhynchus clarki lewisi*). *Archives of Environmental Contamination and Toxicology* **39,** 46–52. doi: 10.1007/s002440010078

Kime, D. E. (1984). The effect of cadmium on steroidogenesis by testes of the rainbow trout, *Salmo gairdneri*. *Toxicology Letters* **22,** 83–88.

Kime, D. E. (1999). A strategy for assessing the effects of xenobiotics on fish reproduction. *Science of the Total Environment* **225,** 3–11.

Lacroix, A. & Hontela, A. (2004). A comparative assessment of the adrenotoxic effects of cadmium in two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, *Perca flavescens*. *Aquatic Toxicology* **67,** 13–21.

Lafuente, A. & Esquifino, A. I. (1999). Cadmium effects on hypothalamic activity and pituitary hormone secretion in the male. *Toxicology Letters* **110,** 209–218.

Lafuente, A., Cano, P. & Esquifino, A. I. (2003). Are cadmium effects on plasma gonadotropin, prolactin, ACTH, GH and TSH levels, dose-dependent? *BioMetals* **16,** 243–250.

Lafuente, A., Gonzalez-Carracedo, A., Romero, A., Cano, P. & Esquifino, A. I. (2004). Cadmium exposure differentially modifies the circadian patterns of norepinephrine at the median eminence and plasma LH, FSH and testosterone levels. *Toxicology Letters* **146,** 175–182.

Laskey, J. W. & Phelps, P. V. (1991). Effect of cadmium and other metal cations on in vivo Leydig cell testosterone production. *Toxicology and Applied Pharmacology* **108,** 296–306.

Manning, C. S., Lytle, T. F., Walker, W. W. & Lytle, J. S. (1999). Life-cycle toxicity of bis (tributyltin) oxide to the sheepshead minnow (*Cyprinidon variegatus*). *Archives of Environmental Contamination and Toxicology* **37,** 258–266.

McQuillan, H. J., Lokman, P. M. & Young, G. (2003). Effects of sex steroids, sex, and sexual maturity on cortisol production: an in vitro comparison of Chinook salmon and rainbow trout interrenals. *General and Comparative Endocrinology* **133,** 154–163.

Meinelt, T., Playle, R. C., Pietrock, M., Burnison, B. K., Wienke, A. & Steiberg, C. E. W. (2001). Interactions of cadmium toxicity in embryos and larvae of zebrafish (*Danio rerio*) with calcium and humic substances. *Aquatic Toxicology* **54,** 205–215.

Mousa, S. A. & Mousa, M. A. (1999). Immunocytochemical and histological studies at the hypophyseal-gonadal system in the freshwater Nile tilapia, *Oreochromis niloticus* (L.), during sexual maturation and spawning in different habitats. *Journal of Experimental Zoology* **284,** 343–354.

Mukherjee, D., Kumar, V. & Chakraborti, P. (1994). Effect of mercuric chloride and cadmium chloride on gonadal function and its regulation in sexually mature common carp *Cyprinus carpio*. *Biomedical and Environmental Sciences* **7,** 13–24.

Nguyen, L. T. H. & Janssen, C. R. (2002). Embryo-larval toxicity tests with the African catfish (*Clarias gariepinus*): comparative sensitivity of endpoints. *Archives of Environmental Contamination and Toxicology* **42,** 256–262. doi: 10.1007/s00244-001-0007-4

OECD (1992). *Guideline for Testing of Chemicals. Guideline 210: Fish, Early-Life Stage Toxicity Test*. Paris: Organisation for Economic Cooperation and Development.

Pankhurst, N. W., Purser, G. J., Van der Kraak, G., Thomas, P. M. & Forteath, G. N. R. (1996). Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*. *Aquaculture* **146,** 277–290.

Patinõ, R. (1997). Manipulations of the reproductive system of fishes by means of exogenous chemicals. *The Progressive Fish-Culturist* **59,** 118–128.

Pereira, J. J., Mercaldo-Allen, R., Kuropat, C., Luedke, D. & Sennefelder, G. (1993). Effect of cadmium accumulation on serum vitellogenin levels and hepatosomatic and gonadosomatic indices of winter flounder (*Pleuronectes americanus*). *Archives of Environmental Contamination and Toxicology* **24,** 427–431.

© 2008 The Authors

Pickova, J., Dutta, P. C., Larsson, P. O. & Kiessling, A. (1997). Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (*Gadus morhua*) stocks. *Canadian Journal of Fisheries and Aquatic Sciences* **54,** 2410–2416.

Pottinger, T. G., Carrick, T. R., Hughes, S. E. & Balm, P. H. M. (1996). Testosterone, 11-ketotestosterone, and estradiol-17 ß modify baseline and stress-induced interrenal and corticotropic activity in trout. *General and Comparative Endocrinology* **104,** 284–295.

Pragatheeswaran, V., Loganathan, B., Natarajan, R. & Venugopalan, V. K. (1989). Cadmium induced malformation in eyes of *Ambassis commersoni* Cuvier. *Bulletin of Environmental Contamination and Toxicology* **43,** 755–760.

Pundir, R. & Saxena, A. B. (1992). Chronic toxic exposure of cadmium on the pituitary gland of fish *Puntius ticto* and pattern of recoupment. *Journal of Environmental Biology* **13,** 69–74.

Rombough, P. J. & Garside, E. T. (1982). Cadmium toxicity and accumulation in eggs and alevins of Atlantic salmon *Salmo salar*. *Canadian Journal of Zoology* **60,** 2006–2014.

Sangalang, G. B. & Freeman, H. C. (1974). Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production in vivo in brook trout. *Biology of Reproduction* **11,** 429–435.

Sangalang, G. B. & O'Halloran, M. J. (1973). Adverse effects of cadmium on brook trout testis and on in vitro testicular androgen synthesis. *Biology of Reproduction* **9,** 394–403.

Scott, A. P. & Sumpter, J. P. (1983). A comparison of the female reproductive cycles of autumn-spawning and winter-spawning strains of rainbow trout (*Salmo gairdneri* Richardson). *General Comparative Endocrinology* **52,** 79–85.

Scott, A. P., Bye, V. J., Baynes, S. M. & Springate, J. R. C. (1980). Seasonal variation in plasma concentrations of 11-ketotestosterone and testosterone in male rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **17,** 495–505.

Singh, H. (1989). Interactions of xenobiotics with reproductive endocrine functions in a protogynous teleost, *Monopterus albus*. *Marine Environmental Research* **28,** 285–289.

Taylor, M. R. & Harrison, P. T. C. (1999). Ecological effects of endocrine disruption: current evidence and research priorities. *Chemosphere* **39,** 1237–1248.

Teather, K., Harris, M., Boswell, J. & Gray, M. (2001). Effects of Acrobat MZ® and Tattoo C® on Japanese medaka (*Oryzias latipes*) development and adult male behavior. *Aquatic Toxicology* **51,** 419–430.

Thomas, P. (1989). Effects of Aroclor 1254 and cadmium on reproductive endocrine function and ovarian growth in Atlantic croaker. *Marine Environmental Research* **28,** 499–503.

Thomas, P. & Khan, I. A. (1997). Mechanisms of chemical interference with reproductive endocrine function in sciaenid fishes. In *Chemically Induced Alterations in Functional Development and Reproduction of Fishes – Proceedings from a Session at the 1995 Wigspread Conference* (Rolland, R. M., Gilbertson, M. & Peterson, R., eds) pp. 29–51. Pensacola, FL: SETAC Press.

Tilton, S. C., Foran, C. M. & Benson, W. H. (2003). Effects of cadmium on the reproductive axis of Japanese medaka (*Oryzias latipes*). *Comparative Biochemistry and Physiology C* **136,** 265–276.

USEPA, (2001). *2001 Update of Ambient Water Quality Criteria for Cadmium*. Washington, DC: United States Environmental protection Agency, Office of Water and Office of Science and Technology.

Uysal, I.& Alpbaz, A. (2002). Comparison of the growth performance and mortality in abant trout (*Salmo trutta abanticus* Tortonese, 1954) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) under farming conditions. *Turkish Journal of Zoology* **26,** 399–403.

Van Ginneken, L., Bervoets, L. & Blust, R. (2001). Bioavailability of Cd to the common carp, *Cyprinus carpio*, in the presence of humic acid. *Aquatic Toxicology* **52,** 13–27.

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Journal compilation © 2008 The Fisheries Society of the British Isles,

von Westernhagen, H. (1988). Sublethal effects of pollutants on fish eggs and larvae. In *Fish Physiology*, Vol. XI (Hoar, W. S. & Randall, D. J., eds), pp. 253–346. London: Academic Press.
Witeska, M., Jesierska, B. & Chaber, J. (1995). The influence of cadmium on common carp embryos and larvae. *Aquaculture* **129,** 129–132.
Wright, P. J., Noltie, D. B. & Yillitt, D. E. (2003). Comparison of prehatch c-start responses in rainbow trout and lake trout embryos by means of a tactile stimulus test. *Transactions of the American Fisheries Society* **132,** 988–996.
Yamagami, K. (1973). Some enzymological properties of a hatching enzyme (chorionase) isolated from the freshwater teleost, *Oryzias latipes*. *Comparative Biochemistry and Physiology B* **46,** 603–616.

Electronic Reference

USEPA (2007). *Ecotox Database*. Available at http://www.cfpub.epa.gov/ecotox/quick_query.htm

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