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**PHOTOINDUCED LETHAL AND SUBLETHAL TOXICITY OF RETENE, A
RESIN ACID DERIVED PAH, TO COREGONID LARVAE**

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1 **Abstract** — A comparative investigation on the acute phototoxicity of retene to
2 vendace (*Coregonus albula*) and whitefish (*Coregonus lavaretus*), both having
3 pelagial larvae in spring, was conducted. In order to test the concept of early warning
4 of sublethal biomarkers in relation to lethality to posthatch stages, we examined the
5 effects of ultraviolet-B (UV-B) and retene on the levels of CYP1A and HSP70 by
6 exposing the animals to elevated levels of these factors for 48 and 72 h, respectively.
7 While UV-B and retene on their own were not lethal, simultaneous retene and UV-B
8 exposure caused very high mortality to both species. **The LC50 (lethal**
9 **concentration 50%, i.e. the concentration at which half of the larvae died) of**
10 **retene as a precursor was 41 µg/L for vendace and 15-16 µg/L, depending on UV**
11 **dose, for whitefish.** Retene evoked substantial induction of CYP1A in larvae of both
12 species and UV-B induced CYP1A in whitefish. In vendace, no effect on HSP70
13 levels by any factor was observed. In whitefish, however, UV-B radiation and water
14 retene alone upregulated HSP70, but no additive response was detected. CYP1A is a
15 biomarker of exposure to retene in both species. HSP70 is an early warning signal of
16 UV-B exposure in whitefish. As a species vendace appears to be more resistant to the
17 phototoxicity of retene than whitefish, as indicated by the higher tolerance.

18

19

20 **Keywords** — Phototoxicity Fish Retene Ultraviolet-B Cytochrome
21 P4501A Heat shock protein 70

22

INTRODUCTION

UV-B radiation (280 – 315 nm) levels have increased due to stratospheric ozone depletion [1]. Enhanced UV-B radiation poses a threat to organisms due to its potential to cause harmful biological effects, directly or indirectly. Of the latter effects of UV-B, photoinduced toxicity of environmental contaminants like polynuclear aromatic hydrocarbons (PAHs) has been documented [2]. Pelagic or semipelagic stages of animals, such as planktonic larvae of coregonid fishes, may potentially be exposed to such a combination in aquatic ecosystems.

Many PAHs can absorb energy from the UV spectrum of sunlight thereby resulting in excited state molecules. The excited state is lost resulting either in the production of active oxygen (photosensitization) or modification of the PAH to new chemical species (photomodification) [2]. Active oxygen is biologically damaging and also the photomodification products are often more toxic than the parent compound.

Retene (7-isopropyl-1-methylphenanthrene) is a PAH that is formed from resin compounds during incomplete combustion of resinuous softwood and via action of anaerobic microbes [3-4]. In addition to being formed from coniferous resins, it may originate from algal and bacterial precursors [5]. High concentrations of retene (**up to 1.6 mg/g dry weight**) have been found in pulp mill sludges and sediments downstream of pulp and paper mill discharges [6-9]. As a natural product, it is also

47 present in sediments not close to pulp mills, although at negligible concentrations (< 4
48 **ng/g dry weight**) [6, 10].

49

50 Retene is not acutely lethal to fish at water-soluble concentrations [11]. On the
51 other hand, its subchronic toxicity is high (10 – 32 µg/l) as evidenced by teratogenic
52 effects, most distinctly the blue sac disease in fish embryos and larvae [12]. The acute
53 toxicity of retene to *Daphnia magna* is enhanced under UV-B radiation [13].

54

55 Autumn-spawning vendace and whitefish lay their eggs on lake bottom where
56 they may come in contact with sediment retene, shown to be bioavailable to fish [14].

57 **Newly hatched larvae may also be exposed to retene in water or particulate**
58 **material [9].** After hatching the positively phototactic larvae of both species swim
59 near the surface for the first months, thus likely to be exposed to episodes of solar
60 UV-B [15].

61

62 The objective of this study was to examine the effects of sole and
63 simultaneous exposures to retene and UV-B on larval vendace and whitefish. **The**
64 **focus was on UV-B because its levels are rising and because retene has an**
65 **absorption peak in the UV-B region of the sunlight spectrum [13]. The endpoints**
66 **were mortality and two inducible proteins, CYP1A and HSP70.** We suggested that
67 these proteins, while serving as early response biomarkers, allow comparison of the
68 two coregonid species at the larval stages.

69

70 Cytochrome P4501A (CYP1A), a heme-containing microsomal
71 monooxygenase, catalyzes the first step in the biotransformation of many xenobiotics.

72 **It was chosen as an endpoint because it is known to be induced by many PAHs,**
 73 **including retene [12, 16]. The aim was to investigate whether the induction**
 74 **differs between species and whether UV-B has an effect on the induction caused**
 75 **by retene (e.g. if more potent CYP1A inducers are generated by UV-B).**

76

77 Heat shock protein 70 (HSP70) is a class of stress proteins with a molecular
 78 weight of 70 – 72 kDa. Cells express some proteins of this family constitutively (heat
 79 shock cognates, HSC's) and some are upregulated by a variety of environmental
 80 stressors **that generate denatured proteins**, including UV radiation and bleached
 81 kraft pulp mill effluent [17-19]. **HSP70 is believed to have a protective role against**
 82 **tissue damage as it binds to denatured proteins and attempts to restore their**
 83 **tertiary structure and function [19].**

84

85 MATERIALS AND METHODS

86

87 *Organisms and water quality*

88

89 The developing eggs of vendace (Lake Pyhäselkä stock) were obtained from
 90 the University of Joensuu and those of whitefish (Rautalampi stock) were obtained
 91 from a local hatchery (Laukaa). Once transferred to the University of Jyväskylä in
 92 late March, the embryos were further incubated and hatched in flow-through hatchery
 93 cones at 6 – 8 °C. The water was colourless (<5 mg Pt/L), **filtered groundwater with**
 94 **pH 6.6 – 7**, having a high penetrability for UV-B (65% in 15 cm). **The water**
 95 **hardness (Ca + Mg) was 1.3-1.4 mmol/L and conductivity 26.2 mS/m.** One-day

old larvae were kept in the same well-aerated water after hatching and used for the experiments.

98

Exposure system and sampling

100

The vendace and whitefish larvae (**45 and 25 per replicate, respectively**) were carefully transferred to retene (nominal concentrations 3.2; 10, 25, 32 and 100 µg/L and 10, 32 and 100 µg/L, respectively) or control treatments (water only or 0.1% dimethylsulfoxide (DMSO), used as carrier) with large-bore pipettes and pre-exposed for 24 hours in Pyrex glass bowls. The depth and volume of pre-aerated water were 5 cm and 1 liter, respectively. A portion of the water was changed daily. **There were three replicates for each treatment.** After a 24-h accumulation period, the larvae were further exposed to retene and, in addition, irradiated with either UV-B or visible light. UV-B was provided in the laboratory using a fluorescent lamp (UVB-313, Q-Panel, Cleveland, OH, USA). Ultraviolet-C (UV-C; under 280 nm), **which in the nature is blocked by the atmosphere and does not reach the surface of the earth,** was blocked with a cellulose diacetate filter (Clarifoil, Derby, UK), replaced after each 3-hour UV-B radiation. Control treatments without UV-B received visible light (TLD 36 W/950 daylight, Philips, Eindhoven, Netherlands). The photoperiod was 16h:8h (light:dark).

116

The larvae received UV-B or visible light 3 h per day (between 24 – 27 hours and 48-51 hours after the beginning) to mimic midday exposure, for two days. UV was quantified using Hamamatsu Photonic Multichannel Spectral analyser (model PMA-11), measuring the wavelength area 280 – 380 nm. The UV-B intensities were

121 measured at the water surface in the beginning and the end of each experiment **at**
 122 **various spots to ensure equal doses for all replicates in each treatment.** The daily
 123 doses were 2.8 kJ/m² for the lower UV-B intensity and 5.4 kJ/m² for the higher,
 124 calculated as CIE-weighted (**Commission Internationale de l'Eclairage;**
 125 **International Commission on Illumination**) J/m², i.e., **the action spectrum specific**
 126 **for human erythema [20].** These UV-B doses correspond with slightly subambient
 127 (7%) and 80% increase relative to the maximum daily doses in Finland (**approx. 60 –**
 128 **70 °N**) in early May. The vendace were exposed only to the higher and the whitefish
 129 to both intensities.

130

131 The larvae were sampled after 3 days (72 hours) from the start by
 132 anesthetizing the animals with MS222 (50 mg/L, 2 min) and freezing in liquid
 133 nitrogen. The samples were transferred to –80 °C and preserved there until processed
 134 further.

135

136 *Positive controls for Western blotting*

137

138 The positive control used for Western blotting of CYP1A was obtained by
 139 injecting four one-year-old juvenile whitefish with 30 µg/g β-naphthoflavone in 95%
 140 ethanol : olive oil (1:1). The fish were further kept at 12 °C in a 40-liter aquarium for
 141 4 days, anesthetized with MS-222 (50 mg/L) and sacrificed. The organs were
 142 removed and frozen immediately in liquid nitrogen. Once thawed on ice the livers
 143 were homogenized with potassium gluconate buffer (70 mmol/L, pH 7.8) and
 144 centrifuged for five minutes (3000 x g). The supernatant was used for the blotting.

145

Heat-shocked vendace and whitefish larvae were used as positive controls for the responsiveness and Western blotting of HSP70. Larvae acclimated at 6 °C were held in 16 °C for 1 hour, transferred back to 6 °C and sampled after 3 hours. The animals were frozen in liquid nitrogen and preserved in –80 °C.

150

151 *Western immunoblotting*

152

Larvae frozen in liquid nitrogen (30 – 40 mg, corresponding to 10 larvae of vendace or 5 larvae of whitefish) were homogenized in a glass homogenizer with potassium gluconate buffer (5 mmol/L MgSO₄, 5 mmol/L NaH₂PO₄, 40 mmol/L HEPES, 70 mmol/L potassium gluconate, 150 mmol/L sorbitol, pH 7.8) [21]. The homogenates were centrifuged at 1000 g for 5min.

158

Total protein concentration of the supernatant was determined by modified Lowry method (BioRad DC, Bio Rad Laboratories, Hercules, CA, USA) adapted for 96-well plates [22]. Five µl of each diluted sample was pipetted into a microtiter plate, in triplicate. Similarly, 5 µl aliquots of protein standards (bovine serum albumin, 0 – 1.5 mg/ml) were pipetted into the 96-well plate. An aliquot of 25 µl of Bio-Rad reagent A was added and 200 µl reagent B pipetted into each well. The plate was put into the microplate reader for mixing, incubated for 15 min and absorbance read at 690 nm.

167

The CYP1A and HSP70 determination was made by Western blot after sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) separation (100 and 20 µg protein / lane, respectively), using a Mini-Protean II apparatus [21,

23-24]. The positive controls were the liver of β -naphthoflavone injected juvenile whitefish (3.15 μ g protein / lane) and heat-shocked vendace and whitefish larvae (20 μ g protein / lane) for CYP1A and HSP70, respectively. Samples were diluted in a buffer solution (0.125 mol/L Tris-HCl, 2% SDS, 20% glycerol, 0.02% bromophenol blue, 5% 2-mercaptoethanol) and loaded onto gels after heating (4 min, 95 °C). Proteins were run on a SDS-PAGE gel for 1 h ($V = 100$ V for 5 min, 200 V for 55 min), after which gels were soaked in blotting buffer (25 mmol/L Tris, 192 mmol/L glycine, 20% methanol, pH 8.3). Proteins were transferred to a nitrocellulose membrane (Trans-Blot Transfer Medium, Bio-Rad) using Bio Rad Mini-Protean II apparatus at 100 V for 90 min. The membrane was stained with 0.2 % Ponceau S in 3 % trichloroacetic acid for 1 min to confirm protein transfer, rinsed twice with Tris-buffered saline-Tween (TBST; 0.9% NaCl, 10 mmol/L Tris, 0.1% Tween-20, pH 7.4) and incubated in blocking buffer (9% non-fat dry milk in TBST) for 14 h in 4 °C.

The blot was probed with 3 μ g/ml anti-CYP1A (Mab 1-12-3, kindly provided by Dr. John Stegeman) or 1:5000 anti-HSP70 (MA3-006, Affinity BioReagents, Golden, CO, USA) in blocking buffer for 2 h. These antibodies recognize CYP1A or HSP70, respectively, of many species including fish, amphibians, and mammals (Murphy SP, Fox S, Myers MP, Morimoto RI. Unpublished data. Affinity Bioreagents) [25]. After washing (TBST, 1x15 min, 2x5 min), the blot was probed with secondary antibody, 1:3000 peroxidase labeled anti-mouse IgG (A9044, Sigma-Aldrich Chemie, Steinheim, Germany), in TBST for 1 hour. After washing (TBST, 1x15 min, 2x5 min) the immunodetection was performed via enhanced chemiluminescence using Star-Glo chemiluminescent substrate (ICN Biomedicals,

Irvine, CA, USA). Hyperfilm ECL[®] high performance chemiluminescence film (Amersham Pharmacia, Uppsala, Sweden) was used for visualization.

The exposed films were scanned and, for densitometric analysis, the pictures were analyzed using Scion Image 4.0.2. Different blots were made comparable to each other by calibrating them with positive controls, fixing each positive control at a value of 1.

Statistical analysis

Differences in mortality between control and treatment groups were tested using two-way analysis of variance (ANOVA) and Tukey's test. LC50 values were calculated using Probit analysis. Values for CYP1A and HSP70 induction were log-transformed and the differences between control and treatment groups tested using two-way ANOVA and Tukey's test. The level of significance of $p < 0.05$ was considered as statistically significant.

RESULTS

Lethality and behavioural effects

UV-B or retene alone caused no acute mortality. However, UV-B radiation increased the toxicity of retene significantly. On day three, after two daily 3-h periods of UV-B, most vendace larvae in 100 µg/L retene treatments were dead (Fig. 1). All whitefish larvae in 100 µg/L and nearly all in 32 µg/L retene treatments died by day

three, at both UV-B intensities. **As retene in itself did not cause lethality, we call the type of toxicity as retene-based lethality potential, but the concentrations are expressed as those of the precursor, retene. The LC50 values (72 h) calculated from nominal concentrations of the precursor, retene, were 41 µg/L (95% confidence limits 30 and 58 µg/L) for vendace, and 16 µg/L (13, 20 µg/L) and 15 µg/L (13, 17 µg/L) for whitefish exposed to the lower and higher dose of UV-B, respectively. Vendace is statistically more tolerant, but the whitefish exposed to lower and higher dose of UV-B do not differ statistically from each other.**

The vendace larvae exposed to 100 µg/L retene and receiving UV-B radiation showed coughing and decreased motility after the first and second UV exposure. The whitefish larvae showed similar symptoms but even more frequently. Symptoms typically lasted for 3 – 4 hours.

CYP1A induction

Retene caused induction of CYP1A both in the presence and absence of UV-B radiation in both species. In vendace the CYP1A induction was approximately proportional to the concentration of retene, being greatest with the highest concentration (100 µg/L). In vendace, UV-B radiation alone caused no induction of CYP1A (Fig. 2).

In whitefish the induction was greatest with 32 µg/L retene, being slightly submaximal ($p > 0.05$, Tukey) with the highest retene concentration applied (100 µg/L). The lower UV-B intensity caused induction in non-retene-exposed whitefish

larvae. In retene-exposed whitefish (10 µg/L **only, because the higher concentrations of retene as precursor were lethal**) the lower UV-B level also seemed to have an additive effect, but this finding was not statistically significant ($p > 0.05$, Tukey) (Fig. 3).

When comparing the maximal responses of CYP1A, the inducibility of vendace was slightly higher than that of whitefish ($p > 0.05$, Tukey). The positive control gave a strong dark band in the Western blots even with the low protein amount used (3.15 µg / lane).

HSP70 induction

In vendace, neither UV-B nor retene had an effect on HSP70 induction that was statistically significant. Also the HSP70 level of the positive control, heat shocked vendace, was similar to control, showing no induction (Fig. 4).

In whitefish, the lower intensity of UV-B radiation caused an induction of HSP70 in retene non-exposed animals. Also the higher intensity seemed to induce HSP70, but this finding was not statistically significant ($p > 0.05$, Tukey). Retene upregulated HSP70 slightly in larvae not exposed to UV-B, but only the induction by the highest concentration was statistically significant because of high variability among individuals. Even though simultaneous exposure to retene and UV-B seemed to elevate HSP70 levels, this finding was not statistically significant (Fig. 5). The HSP70 level of the positive control, heat-shocked whitefish, was 2.5 times that of the control fish.

DISCUSSION

In the clearest oligotrophic lakes in Finland, UV-B can penetrate deeper than 1 metre and, as larval whitefish and vendace are positively phototactic and swim near the surface for the first weeks, in some years they are likely exposed to episodes of exceptional solar UV-B [15, 26]. Additionally, related to climatic change, up to 90% increase in CIE-weighted UV doses have been predicted in northern latitudes [27]. The UV daily doses used in this study, 2.8 kJ/m² and 5.4 kJ/m², were realistic and correspond with a slightly (7%) subambient or an 80% increase relative to the maximum daily doses in Finland in early May.

UV-B radiation has been shown to be detrimental to fishes, especially at embryonic and larval stages [28]. In our study, however, UV-B on its own had no effect on the behaviour or mortality of the larvae of vendace and whitefish. This is in accordance with former results showing that vendace and whitefish are relatively resistant to UV-B [29]. As species, therefore, they appear to be well adaptable in respect to scenarios of the future UV-climate. Importantly, however, UV-photoinduced ecotoxicity of xenobiotic chemicals may provide additional risks to adaptable species, as we hypothesized.

Retene is found at mg/L concentrations in pulp mill sludges and sediments downstream of wastewater discharges [6-9]. Preliminary elutriation trials show that sediment retene is dissolved from this matrix (up to 13 µg/L) and is bioavailable to

294 fish [14, 30]. Despite these aspects of its environmental fate, retene is not acutely
295 lethal to fish in water-soluble concentrations [11], as also verified in this study.

296

297 Certain PAHs (e.g. fluoranthene, anthracene, pyrene) have been found to be
298 acutely phototoxic to a number of aquatic organisms including microbes, plants,
299 invertebrates and vertebrates, in the presence of UV radiation [2, 31-34]. It was
300 recently discovered that retene is acutely phototoxic to *Daphnia magna* and to
301 whitefish (*Coregonus lavaretus*) in the presence of UV-B [13, 35] and this study
302 extends those observations with more realistic daily doses of UV-B and with tests of
303 another fish species, vendace. While both coregonids were sensitized due to
304 simultaneous exposure to UV-B and retene, vendace was more tolerant to this **retene-**
305 **based lethality potential** . Importantly, the lower dose of UV-B also evoked similar
306 lethality in whitefish as the higher dose, a UV-B intensity predicted for the coming
307 decades.

308

309 **The retene concentrations we used were below (10 µg/L) and over (32 and**
310 **100 µg/L) the concentrations that are likely to occur in the nature. This was**
311 **needed for a more mechanistic approach of effects. It must be noted that only**
312 **two days of simultaneous exposure to UV-B and 10 µg/L retene (a concentration**
313 **likely to occur in the nature) caused significant mortality (20 – 30%). In addition**
314 **to water as a source, retene can be accumulated by eggs from the sediment, or by**
315 **larvae from particulate material [9, 11]. Furthermore, the applied safety factors**
316 **in risk assessment with the given set of endpoints often range from 10 to 100.**

317

318 **The LC50 values were calculated with nominal concentrations of the**
319 **precursor retene, as the concentrations were measured only in the beginning of**
320 **the experiment, the measured concentrations being similar to nominal**
321 **concentrations (data not shown). In a similar but different experiment the actual**
322 **concentrations were measured also after 24 h, before the first UV-B radiation:**
323 **The concentrations had diminished so that they were about 80% of nominal**
324 **concentrations (Häkkinen, unpublished results). So the LC50 values are perhaps**
325 **optimistic compared to the real-life situation.**

326

327 As behavioural endpoints, retene caused signs of hypoxia in both species
328 during simultaneous UV-B exposure. Increased ventilation rate and coughing were
329 noted in juvenile sunfish (*Lepomis macrochirus*) and whitefish (*Coregonus lavaretus*)
330 exposed to PAH and UV in combination [31, 35]. The actions behind these symptoms
331 in whitefish larvae seem to be the functional disruption of mucous cells eventually
332 expressed as respiratory stress [35].

333

334 UV-B induces CYP1A1 gene expression in mammalian cells [36]. Irradiation
335 of amino acid tryptophan with UV causes the formation of formylated
336 indolocarbazoles, which are very potent Ah-receptor agonists [37]. Although no
337 CYP1A induction by UV-B could be detected in vendace, a slight induction (10% of
338 the maximum induction detected) was observed in whitefish exposed to the lower
339 UV-B dose (2.8 kJ/m²). In whitefish larvae exposed to the higher dose (5.4 kJ/m²),
340 however, no induction by UV-B was detected. Why? We presume that the higher UV-
341 B level may have caused damage to the skin where the CYP1A induction would have
342 taken place. This is in accordance with our earlier observation on UV-B induced skin

343 pathology in larval whitefish exposed to the same high UV-B dose as in the present
344 study [35].

345

346 Subchronic exposure of rainbow trout larvae to retene causes CYP1A
347 induction [12]. In the present study, a three-day retene exposure induced CYP1A in
348 both vendace and whitefish. Induction in vendace was highest with the highest retene
349 concentration (100 µg/L) but this concentration seemed to cause a distinctly
350 submaximal induction in whitefish. Additionally, when comparing the maximal
351 responses of CYP1A in the same larval phase of the two closely related coregonid
352 species, the inducibility of vendace is slightly higher than that of whitefish. Overall,
353 the response of CYP1A in animals exposed to combined UV-B + retene equalled the
354 response to exposure to retene alone, particularly in vendace.

355

356 HSPs are upregulated by a variety of environmental stressors, including
357 elevated temperatures, UV radiation and many xenobiotics [19]. Our results show that
358 heat shock (used as a positive control), retene and UV-B were all inefficient in
359 upregulating HSP70 levels in vendace. One may assume that the treatments we
360 applied were too mild or too short to upregulate HSP70 in larval vendace. The levels
361 of HSP70 in control vendace were slightly lower compared to the levels in control
362 whitefish (data not shown), so it is not likely that the vendace larvae were
363 unresponsive because of previous and ongoing HSP70 induction.

364

365 In contrast to vendace, **the lower dose of UV-B** induced HSP70 in whitefish
366 larvae. The LD50 (14 d) for larval whitefish is 5.1 kJ/m² (CIE-weighted) of daily
367 exposure, which corresponds to the total dose of 71 kJ/m² (O. Ylönen, personal

communication). Supposing the model of cumulative dose for an effect endpoint of UV-B, the total dose in two days ($2 \times 2.8 \text{ kJ/m}^2$) causing significant induction in HSP70 of whitefish larvae reveals an early signal being 12 times more sensitive in relation to lethality.

There was an increasing trend in HSP70 levels in whitefish exposed to retene, but the induction varied a lot among individuals and only the highest concentration of retene caused an upregulation that was statistically significant.

HSP70 induction is often considered as a protective response and the inability to upregulate HSP70 usually correlates with tissue injury [19]. This does not directly correlate with our findings where the more tolerant species, vendace, was unresponsive and the more sensible species, whitefish, was able to upregulate HSP70. Presumably vendace has other protective mechanisms making it more tolerant than whitefish.

In conclusion, larval vendace and whitefish exposed simultaneously to retene and UV-B revealed different mortality and differ also in the induction of CYP1A and HSP70. Vendace appears to be more resistant to the **retene-based lethality potential** than whitefish, as indicated by the higher tolerance. CYP1A, being elevated even at the lowest sublethal concentration used, was a biomarker of retene exposure in both species. In whitefish, HSP70 acts as an early warning signal of effect due to UV-B exposure. These sublethal responses, however, cannot be used as specific biomarkers of combined retene and UV-B exposures.

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398

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Figure 1. **Retene-based lethality potential of newly hatched vendace and whitefish. Vendace and whitefish larvae were pre-exposed to retene, followed by two 3 h daily irradiations with UV-B (2.8 or 5.4 kJ/m²) or visible light.** Bar denotes standard deviation. Groups denoted by the same letter do not differ significantly from each other ($p \geq 0.05$, Tukey). **UV-B = ultraviolet-B.**

Figure 2. CYP1A induction in larval vendace by retene and UV-B, alone or in combination. **Positive control values were fixed at a value of 1. Bar denotes standard deviation.** Number of determinations 6, each sample pooled of 10 animals. Groups denoted by the same letter do not differ significantly from each other ($p \geq 0.05$, Tukey). **UV-B = ultraviolet-B.**

Figure 3. CYP1A induction in larval whitefish by retene and UV-B, alone or in combination. No measurements of CYP1A in animals exposed to 32 µg/L and 100 µg/L retene + UV-B were done, as these treatments were lethal. **Positive control values were fixed at a value of 1. Bar denotes standard deviation.** Number of determinations 6, each sample pooled of 5 animals. Groups denoted by the same letter do not differ significantly from each other ($p \geq 0.05$, Tukey). **UV-B = ultraviolet-B.**

Fig. 4. HSP70 induction in larval vendace by retene and UV-B, alone or in combination. **Bar denotes standard deviation.** For the details, see legend to Fig. 2. **UV-B = ultraviolet-B.**

Fig. 5. HSP70 induction in larval whitefish by retene and ultraviolet-B (UV-B), alone or in combination. **Bar denotes standard deviation.** For the details, see legend to

Fig. 3. **UV-B = ultraviolet-B.**