1 # BIOINDICATOR SPECIES FOR EROD ACTIVITY MEASUREMENTS: A REVIEW WITH
2 AUSTRALIAN FISH AS A CASE STUDY

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21 Running head: Bioindicator species for EROD activity measurements

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23 **1 Abstract**

24 The conversion of ethoxyresorufin to the fluorescent product resorufin by the enzyme
25 ethoxyresorufin-*O*-deethylase (EROD) enables researchers to rapidly measure the
26 upregulation of this protein in response to exposure to a range of organic contaminants
27 e.g. PAH, PCBs. The EROD activity assay has been widely used to examine the effects of
28 such pollutants on fish taxa in both laboratory and field studies. This review is intended to
29 provide fundamental information for researchers using this EROD activity as an endpoint,
30 including methods used in the assay, the species studied to date, the background EROD
31 levels which would be expected for these species and when available, the EROD activity
32 induction potential for these species. While the focus in on Australian studies, many
33 species listed in this review have a worldwide distribution and the information presented
34 may be extend to other bioindicator fish species. Common shortfalls in the published
35 literature leads to recommendations: it is recommended to have multiple laboratory
36 control or field reference groups as basal EROD activity might vary with biotic and/or
37 abiotic factors. The use of native species over introduced species offers no advantages,
38 with EROD activity induction potential (x-fold) being similar for native or introduced fish
39 species. Similarly, in laboratory studies, EROD activity induction potential is comparable
40 for lab/hatchery-reared fish to the activity observed in field-caught animals. Because
41 EROD activity is often reported to reduce at high concentrations of toxicants, laboratory
42 studies should use a carefully considered range of contaminant concentrations rather
43 than a single exposure concentration, as a single exposure concentration may be on the
44 decreasing side of the response curve. The measurement of serum sorbitol
45 dehydrogenase (SDH) activity in conjunction with EROD activity is recommended to insure

46 liver functions are not jeopardized by high contaminant levels. In field studies, and
47 depending on the variability observed in EROD activity within a given fish species, a
48 number of 8 to 13 non-reproductively active fish, preferably of similar age and sex, per
49 site is recommended to improve the chances of detecting a significant difference in EROD
50 activity, if one does exist. Finally, an inter-continental comparison of EROD activity
51 induction potential (expressed as x-fold relative to a control or reference group) suggests
52 that highly inducible species can be found on all continents, with reported EROD activity
53 induction as high as 135-fold in exposed groups but more commonly less than 5-fold
54 relative to laboratory control or field reference groups.

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59 Keywords: EROD activity; fish; native; introduced; induction potential; bioindicator;
60 biomarker; Australia

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2 Introduction

Aquatic ecosystems are a sink for many organic chemicals such as polycyclic aromatic hydrocarbons (PCBs), organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs) and polychlrorinated dibenzo-dioxin/furan (PCDDs/PCDFs) (Perelo 2010). The impacts of such contaminants are often only identified once organism or population level effects are evident (Adams 2001). Biochemical markers (biomarkers) representing the initial, sub-organism, biological response to contaminants have been developed to aid in the detection of early indications of environmental impact (van der Oost et al. 2003). One of the most widely used biomarkers of exposure to organic pollution is the measurement of hepatic ethoxyresorufin-*O*-deethylase (EROD) activity in fishes (Whyte et al. 2000). Increased levels of EROD are indicative of elevated detoxification activity induced upon exposure to organic pollution (van der Oost et al. 2003). Induction of EROD activity above basal levels is not equivalent across fishes (Whyte et al. 2000) and, the selection of species with an appropriate potential for induced EROD activity in response to contamination is crucial to the early assessment of environmental impacts (Adams 2001).

This paper reviews the literature describing studies on EROD activities in marine, freshwater, and estuarine fish species under laboratory and field settings in an Australian context. It focuses on providing guidance for species selection in future studies through an evaluation of differences in basal EROD activity and induction potential across species from different provenances (geographic, wild vs lab/hatchery reared, native vs introduced). The review uses Australia as a case study as the fish fauna from that country could provide insights on the role that endemism and evolutionary isolation might play in explaining EROD induction potentials. The information provided may, however, be

85 extended to other bioindicator fish species occurring worldwide. Whyte *et al.* (2000)
86 extensively reviewed literature on EROD induction and described many of the factors that
87 influence the induction, conduct of the assay, and interpretation of results. Here we build
88 on that review using recent research and describe a different set of species and
89 population characteristics to specifically answer the following questions:

90 1) In laboratory studies, is there a difference in the EROD activity induction potential
91 between lab/hatchery-reared and wild-caught fish?

92 2) In field studies, is there a difference in the EROD activity induction potential
93 between Australian native and introduced species?

94 3) Is there a geographic difference in EROD activity induction potential at the
95 continental scale?

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97 2.1 Mechanism of CYP1A induction

98 The bioactivity of many xenobiotics and endogenous ligands is mediated via high affinity
99 binding to the aryl hydrocarbon receptor (AhR). While the AhR promiscuously binds many
100 xenobiotic compounds (Denison and Heath-Pagliuso 1998), high affinity ligands are
101 generally planar, aromatic and hydrophobic (Denison 1991) and include amongst others
102 tetrachlorinated dibenzo-*p*-dioxins (TCDDs), some polychlorinated biphenyls (PCBs),
103 polycyclic aromatic hydrocarbons (PAHs) and some organochlorine pesticides (OCPs)
104 (Fent 2001; Oropeza-Hernandez *et al.* 2003; Sierra-Santoyo *et al.* 2000). Limited numbers
105 of endogenous AhR ligands have been identified (Andreola *et al.* 1997; Rannug *et al.*
106 1987). The AhR/ARNT (aryl hydrocarbon nuclear translocator) dimer activates members

107 of the Ah-gene battery, resulting in a pleiotropic response including the production of
108 cytochrome P450-1A (CYP1A), immuno-toxicity, hepatic damage, carcinogenesis,
109 reproductive toxicity, and neurotoxicity (see Mandal 2005 for review).

110 The adverse effects generated by AhR ligands can occur via Phase I metabolism of the
111 ligand to toxic intermediates (Huff *et al.* 1994; Machala *et al.* 2001). The best-studied of
112 these is the CYP1A and epoxide hydrolase catalysed metabolism of benzo(a)pyrene to
113 DNA adduct forming BaP-7,8-dihydrodiol-9,10-epoxide (BPDE) (Hecht 2003; Varanasi *et*
114 *al.* 1986). AhR binding by other ligands, may result amongst other effects in the activation
115 of genes associated with the formation of tumours (oncogenes, Puga *et al.* 2000) or
116 suppression of immune responses (Camacho *et al.* 2001).

117 **2.2 CYP1A induction as a biomarker of exposure**

118 The environmental concentration of some contaminants can correlate with measured
119 body burdens in animals in intimate contact with the environmental matrix but, in cases
120 where animals rapidly metabolise rather than accumulate certain contaminants, the
121 measurement of body burden concentrations becomes irrelevant. Further, the mere
122 presence of a contaminant in the body of an organism does not necessarily translate to
123 toxicity (van der Oost *et al*., 2003) and the concentration at which this occurs is
124 dependent on a range of variables. Hence simply measuring environmental
125 concentrations and/or body burdens is insufficient to infer toxicity (van der Oost *et al.*
126 2003). Inherent spatial and temporal variability in contaminant concentrations merely
127 suggests that toxicity might be under- or over-estimated.

128 In ecotoxicological studies, such chemical analyses are used as supporting tools to the
129 measurements of biomarkers of *exposure* and *effect*. The induction of CYP1A is a
130 biomarker of *exposure* of (primarily) vertebrates to organic contaminants, allowing an
131 assessment of the contaminant-driven activation of the AhR gene battery (Whyte *et al.*
132 2000). Joined with contaminant burden in the organism or its organs, EROD activity
133 induction can suggest the onset of chemically induced metabolic alterations at the
134 subcellular level.

135 **2.3 EROD activity as a measure of CYP1A induction**

136 CYP1A and CYP1A mRNA can both be measured directly but this is time-consuming and
137 expensive where large numbers of samples are to be analysed (e.g., field populations)
138 (Miller et al. 2004). Measurement of the CYP1A-catalysed deethylation of ethoxyresorufin
139 to a fluorescent product, resorufin, (via the activity of ethoxyresorufin-*O*-deethylase;
140 EROD) has allowed more cost-effective and timely quantification of AhR ligand binding
141 and aryl hydrocarbon – (Ah-) gene activation. The correlation between EROD activity and
142 CYP1A content has been established in both laboratory and field studies (DeVito 1996;
143 Miller *et al.* 2004).

144 EROD activity varies between species and contaminants (Whyte *et al.* 2000). Ring-tests
145 have also shown that measured EROD activity varies greatly between laboratories (Banni
146 *et al.* 2008; Baršienė *et al.* 2006). In these tests the most repeatable results were achieved
147 by describing the influence of exposure on EROD activity not as the absolute enzymatic
148 activity but as the *pattern* of responses among the exposed and non-exposed fish.

149 Commonly, the EROD activity in contaminant-exposed fish is compared to the activity in
150 control or reference fish on an x-fold basis (Smith and Gagnon 2000; Webb et al 2005a).

151 In a review of the use of the EROD biomarker, Whyte *et al.* (2000) discussed many of the
152 factors which may affect the measurement of EROD activity under field conditions; (i)
153 some environmental contaminants alter the structure of AhR and/or CYP1A (e.g.,
154 organotins react with amino acids on the CYP1A protein; Boffetta *et al.* 1997); (ii) an
155 increase in EROD activity with age has been reported (Burgeot *et al.* 1996) but while
156 Collier *et al.* (1993) recommend the use of juvenile fish to minimise the effects of
157 reproduction and migration, factors such as ease of capture and adequate size of hepatic
158 tissue often lead to the analysis of larger (usually older) fish; (iii) interactions between
159 estrogens and AhR binding and activation and CYP1A production mean that
160 reproductively active female fish have consistently lower EROD activities than other
161 exposed fish of that species (Arukwe and Goksoyr 1997; Goksoyr and Forlin 1992; Navas
162 and Segner 2000; Sanchez *et al.* 2007; Waxman 1988; Webb *et al.* 2005a) – this
163 interaction has been widely studied but the mechanisms responsible remain unclear
164 (reviewed by Biró *et al.* 2002).

165 Thus, while the biomonitoring of organic contaminants using EROD activity in fish is a
166 cost-effective assessment tool allowing the prioritisation of sites for further investigation
167 using more expensive techniques such as chemical analysis of sediments, water, and flesh
168 (Clancy 2008; Whyte *et al.* 2000), care must be taken in its use and in the selection of
169 target species and individuals.

2.4 Assay methods for EROD activity measurements

While the selection of a bioindicator species with a high EROD activity induction potential is desirable, the method used for measuring EROD enzymatic activity might also influence results and interpretation. A number of the papers reviewed here refer to the original source for the measurement of CYP1A content by EROD activity (i.e., Burke and Mayer, 1974). This original method used microsomes isolated from the livers of 3-methylcholanthrene (3-MC) treated hamsters and rats. It elegantly demonstrated the temporal increase in fluorescence due to the *O*-deethylation of ethoxyresorufin to resorufin mediated by a NADPH-cytochrome P450 reductase. It described the consequence of the exposure of the animals to 3-MC in terms of changes to the apparent K_m (nM) and V_{max} standardised to the amount of protein in the sample (nmol/min/mg protein).

Where this original method is not cited, the papers discussed in this document refer (explicitly or otherwise) to adaptations to this method and to standardised methods published by government, academic or industry groups. The majority of these are based on the method developed by Pohl and Fouts (1980), who described a rapid method of EROD activity measurement in rat liver microsomes using a defined reaction time that allowed a number of assays to be run in parallel. Proteins in the reaction mixture were precipitated by methanol, stopping the reaction at the desired point. The addition of bovine serum albumin (BSA) and/or EDTA to the incubation mixture provided a homogenous reaction medium and increased fluorimetric measurements (Pohl and Fouts 1980). Both compounds increased the enzymatic activity and corrected variations in plots

192 of the resorufin produced against the concentration of microsomal protein. They also
193 showed that including Mg^{2+} (in this case as $MgSO_4$) in the incubation mixture markedly
194 increased the fluorescence signal.

195 In the 1990s, the method of Pohl and Fouts (1980) was adapted for the use in other
196 vertebrates (including fish). The most cited method for measurement of EROD activity in
197 Australian fish is the seminal technical report by Hodson *et al.* (1991). That report gave
198 recommendations for study design, sample collection and storage, data handling and
199 QA/QC, and detailed methods for measuring EROD activity in fish liver (based on the
200 method of Pohl and Fouts 1980). A strong correlation between results gained using the
201 isolated microsomes and those using the S9 (post-mitochondrial suspension) fraction was
202 reported leading to the increasing use of the S9 fraction in EROD activity measurements
203 (presumably for ease of preparation and time-saving issues).

204 With the emergence of microplate technologies, methods were adapted to allow rapid
205 assessment of EROD activity using a 96 well fluorescence plate reader with reduced assay
206 volumes (Eggens and Galgani, 1992). Four publications concerned with Australian fish
207 species cite the work of Gunther *et al.* (1997) or van den Heuvel *et al.* (1999) who
208 combined previous methods using a fluorescence plate reader and the S9 fraction to
209 produce perhaps the most rapid method for the measurement of large numbers of
210 samples. An additional advantage of the microplate measurement is that it offered the
211 possibility of measuring hepatic EROD activity when very little biological tissue was
212 available.

213 No standard method is currently employed in studies on EROD activities. Rather,
214 researchers tend to optimise methods to their capacities (e.g., available laboratory
215 equipment) and in some cases, large differences exist. The excitation and emission
216 wavelengths used range from 510-574 and 584-596 nm respectively (excitation and
217 emission maxima were reported by Burke and Mayer (1974) as 560nm and 586nm
218 respectively) but the majority used the recommendation of Hodson *et al.* (1991) (Ex 530,
219 Em 585). Amongst those methods using a fixed reaction time, this time varied from 2-10
220 minutes. There was also variation in the source of the reducing agent NADPH. Most of the
221 methods called for the addition of low concentrations of NADPH to the incubation
222 mixture (while ensuring that it is not limiting in the reaction), while others, particularly
223 those based on the work of Holdway *et al.* (1994), use an NADPH regenerating system
224 (NADP, $MgCl_2$, KCl, glucose-6-phosphate, glucose-6-phosphate dehydrogenase) which
225 significantly reduces the cost of performing the assay.

226 Each of these variations in methods has the potential to affect the measured activity and
227 the results gathered in different laboratories (even on the same species at similar life-
228 stages) are likely to vary. Given the results of inter-laboratory studies elsewhere (Banni *et*
229 *al.* 2008; Baršienė *et al.* 2006), the relative x-fold induction (exposed relative to control or
230 reference fish) should remain comparable.

231 Along with the selection of an assay method, the experimental design needs to include
232 several test concentrations in order to evaluate a maximum EROD activity in any given
233 fish species. Notably, in a number of laboratory studies (e.g., Mercurio *et al.* 2004;

234 Tugiyono and Gagnon 2001; van Dam *et al.* 1999) the maximum induction of EROD
235 activity was not at the highest exposure concentration. In the original methods of Burke
236 and Mayer (1974) the increase in resorufin concentration in the presence of CYP1A was
237 only linear up to a certain point. In fact, the product of the reaction was an inhibitor of
238 the reaction. The result is that where the production is very high the reaction will be
239 slowed. A lack of linearity may also be due to incorrect methodologies where the
240 substrate, rather than the CYP1A in the sample (S9 or microsomal preparation) is in
241 excess (Mercurio *et al*. 2004). These two explanations, however, do not account for the
242 decrease in EROD induction above a certain exposure concentration. A more likely
243 explanation is that above a certain exposure concentration other chronic pathologies may
244 affect the production of CYP1A (e.g., liver damage: Webb and Gagnon 2007). This
245 demonstrates the importance of characterizing the CYP1A induction fully. A single test
246 concentration is insufficient to describe the induction potential as a researcher has no
247 way of knowing if this response is on the increasing or decreasing arm of the response
248 curve.

249 Some Australian laboratory studies (e.g., Webb *et al.* 2008) present the results of both
250 EROD activity and serum concentration of sorbitol dehydrogenase (SDH) as a biomarker
251 of hepatocellular damage. This is in response to a study by Holdway *et al.* (1994) which
252 reported that as SDH increased, EROD activity decreased. The authors suggested that
253 hepatocellular damage reduced the ability of the liver to synthesise CYP1A and that such
254 damage could cause the underestimation of exposure where EROD activity is measured
255 alone. Such assumption is supported by another study where it was demonstrated that

256 elevated serum SDH activity in fish preceded the onset of histopathology by several days
257 (Webb and Gagnon 2007). Thus coupling of EROD activity and serum SDH activity can be
258 useful to ensure the validity of the measurement of the EROD activity.

259

260 3 Review Methodology

261 In order to assist researchers in the selection of the most appropriate bioindicator species
262 for a given research question, we have conducted a review of the available literature. We
263 searched the published literature using the initial search terms "EROD" and "fish" in
264 Scopus with no time of publication constraints. The test species used in each of the 1219
265 articles retrieved from the initial search was cross-matched against occurrence records in
266 FishBase (Froese and Pauly 2016). Articles were retained for the review if there was an
267 occurrence in either Australia or New Zealand. For *Onchorynchus mykiss* and *Carassius*
268 *auratus* there were a very large numbers of studies reporting EROD activity in these
269 species. For these species articles with the highest reported fold induction were retained.

270 From retained articles, information on the provenance (hatchery or wild-caught), life-
271 stage, and sex were entered into a database. Separate searches determined the natural
272 habitat of the fish (marine, estuarine, freshwater) and whether they were native to
273 Australia. Details of test protocols (exposure route, toxicant, concentration range and
274 replication) and results (basal EROD or EROD activity in control/reference animals,
275 maximum induction (x-fold induction) were extracted from each article and added to the
276 database. Calculation of maximum x-fold induction was calculated as "maximum x-fold =

277 maximum EROD in exposed fish / basal EROD in control or reference fish”. Not all articles
278 reported EROD activity separately for sexes (e.g. some studies use juvenile fish) so the
279 calculated maximum x-fold induction was based on the group having the highest EROD
280 activity over the EROD activity in control or reference group.

281 A total of 85 entries were made to the database for (51 for laboratory based studies and
282 34 for field based studies). In some cases there were multiple entries from single studies
283 in which multiple species were tested.

284 **4 Australian fish: a special case**

285 The Australian continent has been essentially geologically isolated since the mid-
286 Cretaceous, resulting in a highly endemic fish fauna. Despite the introduction of a number
287 of exotic species over the past 220 years, endemism is highly localised with few species
288 occurring throughout the continent. Moreover, Australia has a large and ecologically
289 diverse coastline including tropical reef, warm and cool temperate waters, and estuarine
290 mangrove systems. Freshwater systems are similarly ecologically diverse with latitudinal
291 differences in climate (particularly the seasonality of rainfall) producing permanent
292 streams with large seasonal changes in flow and discharge, ephemeral streams and lakes,
293 and/or permanent inland wetlands. The extreme environment and the highly localised
294 endemism has often forced ecotoxicological investigations to use fish species with poorly
295 known biology and often with unknown EROD activity induction potential.

296 Until recently, Australian researchers relied on ecotoxicological data on non-native fish
297 species in deriving trigger values and assessing risk to native ecosystems. Since Australian

298 native fish species are adapted to a very different set of extreme environmental
299 conditions, responses to chemical and environmental stressors are likely to be different,
300 relative those fish in other continents (research question no. 3). Consequently, standard
301 testing techniques have been developed for a variety of Australian native fish species
302 leading to the derivation of more reliable and relevant guideline values for the protection
303 of the Australian environment (ANZECC and ARMCANZ 2000).

304 While the review by Whyte *et al.* (2000) (and other similar reviews) remains a valuable
305 resource for all researchers using the EROD activity bioassay, only one Australian study
306 (i.e., Holdway *et al.,* 1994) was covered. Thus to date there has been no review of the
307 EROD activity measured in Australian fish species, and how the potential for EROD activity
308 in these species compares to the potential induction in fish found on other continents.

309 **5 Laboratory Studies**

310 Laboratory studies often use extreme exposure concentrations or doses in order to
311 trigger a response. Nevertheless, laboratory trials can inform on the relative EROD activity
312 induction potential of various fish species exposed to single toxicants or to mixtures of
313 contaminants.

314 **5.1 Study Species**

315 EROD measurements on fish exposed under laboratory conditions to contaminants with
316 known or unknown CYP1A induction potential have been performed on 25 species that
317 occur within Australia. Of these 16 are considered native and 9 are introduced species
318 (Table 1). Given the well-documented inter-species difference in EROD induction, the

319 ratio of native to introduced Australian species studied under laboratory conditions is
320 very low. While many introduced species present convenient model organisms as their
321 biology is well understood, efforts are required to investigate native species, particularly
322 with a view of establishing baselines against which future impacts can be assessed.

323 The fishes studied in the published literature included in this review were a combination
324 of native and introduced marine (7), estuarine (7) and freshwater (11) species. Hoese *et*
325 *al.* (2006) describe over 4000 species of marine fishes in Australia compared to 443
326 estuarine and 258 freshwater fish species. Given this relatively high marine fish diversity
327 in Australia, the study of EROD induction in marine fishes is grossly under-represented.
328 Whyte *et al.* (2000) reviewed the estimated basal EROD activity in 98 species of which
329 58% were marine and 35% freshwater species (with the remainder catadromous,
330 anadromous or estuarine). The relatively high proportion of freshwater species in which
331 EROD activity is reported most likely reflects the greater impacts of human settlements
332 around freshwater environments.

333 This bias toward freshwater and estuarine species is likely a reflection of social and
334 scientific concerns regarding contamination by organic compounds in freshwater and
335 estuarine environments which are often immediately subject to contamination by
336 adjacent industrial and urban developments. However, with increasing coastal
337 development and resource extraction in Australia, it is important that future efforts
338 include a range of marine fish species from a wide variety of taxonomic groups. Particular

339 attention is required on marine species with wide geographic distributions yet small
340 home ranges.

341 While selecting a bioindicator species for laboratory studies, one might consider using
342 field-caught animals or laboratory/hatchery-reared fish stocks. Examination of the EROD
343 activity potential of field-caught fish and lab/hatchery-reared fish under laboratory
344 conditions presents no obvious advantage regarding EROD induction potential, as similar
345 EROD activity induction potential can be achieved with animals sourced from the field or
346 farmed (Webb and Gagnon 2013) (Figure 1, data from Table 1, T-test, $t_{48} = 1.443$, $p =$
347 0.155). The use of lab/hatchery-reared fish can however present experimental
348 advantages such as a uniform genetic background, an homogenous fish size and disease-
349 free animals that feed on the food provided. The use of native versus introduces species
350 is discussed later in this review.

351 **5.2 Single Toxicant Studies**

352 The model contaminants most often used in laboratory studies were PCB isomers (PCB
353 126), PCB mixtures (Aroclor 1254), PAHs, and known EROD inducers (β-naphthoflavone,
354 DTPA). Across most laboratory studies, intraperitoneal (i.p.) injection with β-
355 naphthoflavone (βNF) consistently resulted in the highest EROD fold induction (often >
356 than 20-fold relative to control fish) and is therefore a reliable positive control for EROD
357 activity investigations. However, i.p. injection of yellowtail trumpeter (*Amniataba*
358 *caudavittata*) with neither B(a)P (5 µg/kg) or βNF (10 µg/kg) resulted in the significant
359 induction of EROD activity (Webb *et al.* 2008; Table 1). Similarly, some laboratory trials

360 suggested that the common blowfish *Torquigener pleurogramma* is not responsive to
361 injections by PCB126 or β -naphtoflavone (Gagnon, unpublished data). Similarly, the Perth
362 hearing is too sensitive to the stress of capture and handling to be collected from the wild
363 and used in laboratory experiments where handling of the fish is required (Webb and
364 Gagnon 2002b). The measurement of EROD induction by specific compounds on
365 individual species under laboratory conditions can confirm (or otherwise) their suitability
366 for the detection of AhR ligands in pre-release or caging studies. It is clear from the range
367 of fold-induction in different species exposed to the same compound that the value of
368 this bioindicator to detect exposure to, and uptake of, specific classes of contaminants
369 under field conditions is difficult to predict. Hence, EROD induction is a biomarker of
370 exposure to be used in conjunction with other measures of exposure and effects.

371 Only two studies cited EROD activity in Australian native fish exposed to individual PAHs.
372 EROD activity was not significantly induced in crimson spotted rainbowfish by immersion
373 in 400 μg/L naphthalene (Pollino *et al.* 2009) but was significantly induced (8.9 fold) in
374 aereolated grouper exposed to B(a)P (12.5 μg/kg/day) in diet (Au *et al.* 2004). PAH
375 mediated EROD activity induction is stronger in high molecular weight (4 – 5 ring) PAHs
376 (e.g., chrysene, B(a)P) than in lower molecular weight PAHs (e.g., naphthalene,
377 phenanthrene) (reviewed by Whyte *et al.* 2000). Future work needs to focus on these
378 higher molecular weight compounds including benzo(b)fluoranthene,
379 benzo(k)fluoranthene, benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene – those high
380 molecular weight PAHs defined as European Commission priority substances (European
381 Union 2008).

382 **5.3 Mixtures**

383 A number of laboratory studies have expanded on single toxicant exposures and
384 demonstrated EROD activity induction in Australian species exposed to complex mixtures
385 (e.g. van Dam et al. 1999; Moose et al. 1996; Gagnon et al. 1998). Common industrial
386 effluents from kraft mills, wastewater treatment plants and oil refineries have all
387 demonstrated increased EROD activity induction in fish species found in Australian
388 waters, however there is an overall lack of such studies on native species. Only one
389 laboratory study (Woodworth *et al.* 1998) has demonstrated significant EROD activity
390 induction in Australian native species (*Parablennius tasmanianus* and *Galaxius maculatus*)
391 exposed to mixtures of contaminants. There is a need to continue efforts to demonstrate
392 the utility of this endpoint to indicate exposure to AhR ligands in complex mixtures in
393 order to guide field-based studies in the selection of suitable bioindicator species relevant
394 to the study location.

395 **5.4 Laboratory Study Outcomes**

396 Variation in the basal EROD activity induction measured for the same species in different
397 studies was quite high in some cases. The crimson spotted rainbowfish (*Melanotaenia*
398 *fluviatilis*) was used as a test species in three separate published studies (Pollino *et al.*
399 2009; Pollino and Holdway 2003; van Dam *et al.* 1999) and the basal EROD activity
400 reading varied between 8.3 and 264 pmol/min/mg protein for male fish and 12.3 and 185
401 pmol/min/mg protein for female fish. In two laboratory studies (Codi *et al.* 2004;
402 Mercurio *et al.* 2004) using immature barramundi (*Lates calcarifer*) basal EROD activity
403 levels varied between 3.4 and 54 pmol/min/mg protein. EROD activity in adult fish

404 (particularly female fish) varies annually with the reproductive cycle and other
405 environmental parameters such as temperature (Whyte *et al.*, 2000). This can be
406 overcome by the use of male fish and only comparing across fish of similar age and with a
407 known life history. There are also methodological sources of variation. Since measured
408 EROD activity is based on reaction rate, slight differences in reagent concentration,
409 reaction temperature, incubation times, and S9 preparation methods may result in
410 differences in measured activity. With the aim of producing comparable results for
411 widespread freshwater and marine species it is important that common methods be
412 defined and followed. While the expression of EROD induction as a fold induction is
413 preferable the definition and reporting of basal EROD activity is required and should form
414 part of reporting of EROD activity.

415 **6 Field Studies**

416 Field studies typically exhibit higher variability and lower levels of EROD activity
417 compared to laboratory studies however, the measurement of EROD activity in field-
418 caught specimens provides insights into the bioavailability of contaminants under real-life
419 conditions. In field studies, the EROD induction potential of the selected bioindicator
420 species and the confounding factors (such as reproductive activity, Wunderlich et al.
421 2015) are additional aspects that need to be carefully considered in the study design.

422 **6.1 Choice of species**

423 Northern hemisphere studies using EROD activity as a biomarker have focused on
424 widespread local species (e.g., rainbow trout, Atlantic salmon, fathead minnow, white
425 sucker) and the induction of EROD activity in these fish has been well characterized. It is

426 clear from the studies cited here that none of the Australian species examined for EROD
427 activity are as well studied as their northern counterparts. Twenty-four separate field-
428 based studies in Australian and New Zealand were found citing EROD activity in native
429 and introduced fish species (Table 2). Here again, there was a tendency toward studying
430 freshwater species with the studies covering 20 individual species in total, of which 7
431 were marine species, 3 were estuarine species and the remaining were either freshwater
432 (8) or anadromous (2) species. Four studies used fish native to Australian waters that are
433 also native to Chilean (Fossi *et al.* 1995; Gavilán *et al.* 2001) and Portuguese estuaries
434 (Ferreira *et al.* 2004), and Taiwanese waters (Chen *et al.* 1998). Each of these studies
435 focused on the potential impacts of a range of stressors (e.g., known organic
436 contamination, oil production activities, human recreational activities, or agricultural
437 runoff).

438 Of the 10 native Australian species studied in field studies only 3 (*Platycephalus bassensis*,
439 *Acanthopagrus butcheri* and *Lates calcarifer*) had been previously or concurrently studied
440 in the laboratory to assess basal EROD activity and the induction potential of the species.
441 Without this assessment it is difficult to truly estimate the EROD activity induction
442 potential of a given fish species following exposure of the animal to environmental
443 xenobiotics.

444 In most field studies there is a community of fish species from which (a) study species can
445 be selected and it is considered important to indicate the reasons for this choice. In the
446 literature reviewed here the reasons given for choice include ease of capture (Rawson *et*

447 *al.* 2009) to a species of high commercial and recreational importance (Codi King *et al.*
448 2005; Klumpp *et al.* 2007; van den Heuvel *et al.* 2006); relevant justification where
449 protection of these species is also of primary importance. However where overall
450 protection of fish communities is desired, it may be more appropriate to consider other
451 factors such as whether the species is sedentary and/or has a small home range (Holdway
452 *et al.* 1994; Shaw *et al.* 2004). A limited number of field studies have investigated multiple
453 species collected from field sites in an attempt to identify the most appropriate species as
454 a suitable bioindicator (Chaplin *et al.* 1997; Codi King *et al.* 2005). When selecting more
455 than one bioindicator species from the field, the use of native bioindicator species over
456 introduced ones provides no clear advantage to field studies as native and introduce
457 species have comparable EROD activity induction potential, with inter-species variability
458 within natives or within introduced also being similar (Figure 2, data extracted from Table
459 2). In field studies conducted in Australia, there is no difference between the maximum
460 EROD activity induction potential of native fish relative to the one form introduced
461 species (Figure 2, T-test, $t_{20} = 0.751$, $p = 0.941$).

462 From the limited data available, EROD activity induction in barramundi (a tropical
463 euryhaline species) (Codi *et al.* 2004; Humphrey *et al.* 2007) and sand flathead (a
464 temperate marine to estuarine species) (Gagnon and Holdway 2002; Holdway *et al.* 1994)
465 has been repeatedly demonstrated to reflect expected contamination gradients in field
466 studies. This is not to say that these are the most sensitive Australian species available or
467 that they will be appropriate under all conditions. While it would be instructive to
468 comment on the relative sensitivity of species under field conditions (i.e., the ability of a

469 researcher to correctly assume bioavailability of environmental contaminants using the
470 EROD activity), the sheer lack of repeated use of a range of species makes this impossible.
471 To date, the demonstration of a correlation between EROD activity and known
472 contamination remains to be confirmed in an Australian native freshwater fish. However,
473 a number of freshwater fish species non-native to Australia have been shown in other
474 countries (and in the case of *Gambusia holbrooki* – [Rawson *et al.* 2009]) to be suitable in
475 such studies. These include common carp (Solé *et al.* 2003), redfin perch (Kantoniemi *et*
476 *al.* 1996), rainbow trout (Orrego *et al.* 2006) and tilapia (*Oreochromis niloticus*, native to
477 Africa but now has a worldwide distribution, Chen *et al.* 2001). Amongst the continents,
478 the most commonly used species in studies using EROD activity as biomarker include
479 tilapia, silver croaker (*Plagioscion squamosissimus*) in South America, Atlantic salmon
480 (*Salmo salar*), rainbow trout (*Onchorhynchus mykiss*) and white sucker (*Catostomus*
481 *commersoni*) in North America, while in Europe the sea bass (*Dicentrachus labrax*), the
482 mullet (*Mugil cephalus*) and the flounder (*Platichthys flesus*) have shown to be highly
483 inducible species. Table 3 lists recent studies where high EROD activity induction has been
484 reported in the above fish species.

485 It is noteworthy that while many studies have reported low or non-significant EROD
486 induction in some fish species, highly inducible species are found worldwide, and no
487 continent has more or less highly inducible species than another. While less studies using
488 EROD activity as a biomarker of exposure have to date been conducted in developing
489 countries, the potential for using EROD induction as a biomarker of exposure to AhR
490 ligand inducer is ubiquitous.

491 The use of these exotic species in Australian field studies may be highly appropriate as a
492 screening tool. The question of how well the measured metabolic exposure of these
493 exotics matches the metabolic exposure in co-occurring native fish is an open question
494 that requires investigation. There are instances where field collected fish have not
495 showed any induction of EROD above estimated basal levels (e.g., coral trout: Klumpp *et*
496 *al.* 2007). Future field studies intending to collect these species from the field should first
497 attempt to induce EROD activity above basal level by the contaminant(s) of interest under
498 laboratory conditions.

499 **6.2 Degree of EROD activity induction in field conditions**

500 Expectedly, the fold-induction in field studies conducted in Australia was generally lower
501 than that reported in laboratory studies where exposure can be guaranteed by an i.p.
502 injection of known concentrations (generally at higher than environmental
503 concentrations). The maximum fold induction (22-fold) in a fish native to Australia in a
504 field study was in the mullet (*Mugil cephalus*) which were exposed to PAHs (Bozcaarmutlu
505 *et al.* 2015). The second highest induction reported was a 20- fold in the same species
506 *Mugil cephalus* exposed to untreated domestic sewage and industrial effluent (Ferreira *et*
507 *al*. 2000), while a 10.8-fold induction was measured in the estuarine barramundi in
508 Queensland where exposure to agricultural herbicide runoff was suspected (Humphrey *et*
509 *al.* 2007). It was common, however, for the majority of field studies to report significant
510 induction of EROD activity above basal levels (where these are estimated) in the range of
511 1.5 – 4.5 fold induction.

512 In many cases, a basal EROD activity was not estimated from a “non-impacted” set of
513 animals negating the chance to calculate any fold-induction (Table 3). Instead the
514 researchers focused on establishing the presence of, or lack of, a gradient of response, or
515 identifying temporal and/or spatial variations (Holdway *et al.* 1994; Klumpp *et al.* 2007;
516 Webb and Gagnon 2002a; Webb *et al.* 2005a; Webb *et al.* 2005b). It is common that no
517 pristine reference sites where the targeted species is present can be identified; in which
518 case the use of laboratory depurated fish can represent a surrogate to reference fish
519 (Webb and Gagnon 2013).

520 **6.3 Confounding Factors**

521 Given that the presence of endogenous 17β-estradiol can impact the measurement of
522 EROD activity by inhibiting CYP1A activity (Burkina et al, 2015; Biró *et al.* 2002) most of
523 the studies on Australian fish have attempted to account for fish sex and developmental
524 stage. Most have collected and measured EROD activity in both male and female fish and
525 either included these in a 2-factor analysis to look for significant interaction (sex x site)
526 terms (Holdway *et al.* 1994) or tested for differences between the sexes and, where these
527 do not exist, pooled the data prior to analysis between groups (Webb *et al.* 2005a; Webb
528 *et al.* 2005b). Other studies have excluded all female fish from the analysis (Rawson *et al.*
529 2009) or targeted juvenile fish (and included male and female fish) with the assumption
530 that 17β-estradiol should be equal between males and females at this life-stage (van den
531 Heuvel *et al.* 2006). Where sample sizes are limited, ethical and statistical considerations
532 dictate that the former of these approaches is more appropriate. But where sample size is
533 no problem, and particularly where invasive species are used, removing all females or

534 adults from EROD activity analyses may reduce variation and enhance accuracy in the
535 interpretation of the data. Based on field data where black bream (*Acanthopagrus*
536 *butcheri*) was collected, Gagnon and Hodson (2012) have suggested that in field studies a
537 minimum of 8 non-reproductive adult fish per species per site (or 8 fish per sex if
538 reproductively active) have to be collected in order to have 80% chance to identify an
539 inter-site difference of 3-fold EROD activity induction at $\alpha = 0.05$ (although the number of
540 fish per sex per species increases to 12 for a statistical power of 0.95 at $\alpha = 0.05$, Gagnon
541 and Hodson, 2012). In a similar exercise, Flammarion and Garric (1999) estimated, using
542 EROD activity data from field-collected chub (*Leuciscus caphalus*) and gudgeon (*Gobio*
543 *gobio*), that a 2-fold induction required a minimum of 13 fish at $\alpha = 0.05$ and a power of
544 0.80 (80% chances of detecting a difference). According to the inter-individual variability
545 observed in a given species, it appears that a minimum fish number between 8 and 13
546 need to be collected in order to detect an inter-site difference of 2- to 3-fold EROD
547 activity induction.

548 In field studies it is (by definition) more difficult to control for variation in biological and
549 physico-chemical parameters which may influence the measurement of the endpoint
550 (Whyte *et al.* 2000). Most of the field studies on Australian fish to date have focussed on
551 a single sampling period (Codi *et al.* 2004; Klumpp *et al.* 2007; Rawson *et al.* 2009).
552 Others, however, have used multiple collection times and describe variation between
553 seasons and across years (Webb *et al.* 2005a; Webb *et al.* 2005b). By sampling fish in
554 more than one season the impact of environmental factors (e.g. water temperature)
555 thought to impact EROD activity (Whyte *et al.* 2000) can by teased out, though it is

556 difficult to suggest whether this may be due to variation in these factors or the
557 contamination source causing the increase in the activity.

558 **6.4 Outcomes of field studies**

559 Measurement of the EROD biomarker in Australian studies has, in most cases provided
560 useful information as the authors of these studies interpret wider data within individual
561 studies. In Australian field studies the biomarker has been used in two different scenarios;
562 where a specific contamination source (or sources) is suspected (Codi King *et al.* 2005)
563 and where a comparison of field sites is desired to guide management priorities (Gagnon
564 and Holdway 2002; Holdway *et al.* 1994; Webb and Gagnon 2002a). In the first of these
565 cases the results of some studies reported that EROD activity has confirmed the exposure
566 of fish to the contamination source as well as confirming the bioavailability of the
567 contaminants (Codi *et al.* 2004; Humphrey *et al.* 2007) but other studies failed to find
568 EROD activity in fish which were expected to be exposed to CYP1A inducers in the
569 environment (Codi King *et al.* 2005). Even where patterns of EROD activity differ from
570 those excepted *a priori* this can indicate that contamination sources are not readily
571 bioavailable for uptake by organisms, or that the contaminant mixture has less potency
572 than expected to induce EROD activity in fish. Almost all the studies which focus on inter-
573 site comparisons are able to explain the apparent spatial trends in EROD activity using
574 site-specific factors such as industrial site usage (e.g., presence of a passenger jetty:
575 Webb and Gagnon 2002) or remediation history (Rawson *et al.* 2009). The primary value
576 in studies describing inter-site differences without prior knowledge of point source

577 contamination is in providing an indication of where management work on further
578 characterising contamination and the associated environmental risks should focus.

579 **7 Field vs. Laboratory Data**

580 Laboratory studies have often been conducted with the intent of determining the
581 suitability of a species for use in pollution monitoring. Whereas field studies usually aim
582 at detecting exposure to a pollutant, and inform managers of potential impacts on
583 aquatic ecosystems.

584 EROD activity induction responses measured in laboratory studies are often difficult to
585 relate to effects that may occur in the environment (Clancy 2008). Laboratory results can
586 either overestimate or underestimate induction in field populations as, in most cases,
587 laboratory fish are exposed to a single chemical at concentrations intended to induce a
588 response. These concentrations may not relate to levels actually found in the 'real world'
589 and laboratory observations validated with field research will be considered the most
590 reliable (Clancy 2008).

591 Only three Australian native species (all estuarine) could be found for which both
592 laboratory and local field data was available. In each of these cases EROD activity
593 induction was demonstrated in the laboratory by i.p. injection with either PCB126 or
594 β-naphthoflavone. For barramundi (50 mg/kg β-NF) and sand flathead (0.1 mg/kg
595 PCB126) a 20-fold induction of EROD above basal levels was achieved in the laboratory
596 with the maximum field based induction of about 10-fold for barramundi and about 2-
597 fold for sand flathead. In each of these cases the laboratory and field estimated basal

598 EROD activities were very similar (Table 1 and Table 2). For black bream injection with
599 0.01 mg/kg PCB126 caused a 9-fold induction of EROD activity and the fish with the
600 highest EROD activities where about 5-fold higher than those with the lowest (results
601 across 3 studies). These comparisons indicate that laboratory based estimates of basal
602 EROD activity are likely to be appropriate for field studies. This will be of use where a
603 suitable reference site cannot be found although it would be advisable to use the data
604 gathered by this method as comparative within an individual species rather than
605 attempting to compare fold-induction between studies. Further, for these species, where
606 EROD activity induction was measured in the laboratory researchers have subsequently
607 been able to measure EROD activity induction in field situations where contamination is
608 expected, demonstrating the value of such laboratory testing prior to the use of a
609 particular species for field studies.

610 Only one Australian publication (Codi *et al.* 2004) could be found which reported both
611 laboratory and field data on EROD activity induction in the same species of fish. By
612 presenting both sets of data together these authors were able to indicate approximate
613 toxic equivalencies. While other research groups have published such data separately,
614 concurrent publication allows direct comparison and evaluation of the degree of
615 exposure as opposed to presence or absence of exposure.

616 The various methods of EROD analysis generate comparable results especially when the
617 enzymatic activities are expressed as a relative induction over a laboratory control group

618 or field reference site (Whyte *et al.* 2000) and it is therefore relevant to seek a species
619 known to demonstrate a highly inducible EROD activity.

620 EROD activity induction potential does vary between species, and the detection of
621 exposure to inducing compounds is facilitated by the use of a bioindicator species which
622 is highly inducible but has low basal EROD activity. Considered on a x-fold induction
623 potential, native Australian species appear to have a comparable EROD activity induction
624 potential when tested under laboratory conditions to that of introduced species (Figure
625 1), potentially offering the researcher a wider range of bioindicator species to choose
626 from. The selection of a highly inducible species can provide a better discrimination
627 between treatment/sites groups as well as reduce the number of organisms required to
628 identify a difference between treatments/sites. It might be strategic to use an Australian
629 native fish species over an introduced one, when available, to conduct laboratory studies
630 when EROD activity induction is an endpoint as EROD activity can later be compared
631 between laboratory and field results.

632

633 In summary, the examination of published EROD activity data reveals that:

634 1) in laboratory studies, there is no significant difference in the EROD activity
635 induction potential between lab/hatchery-reared and wild-caught fish (Figure 1);
636 2) in field studies, is there no difference in the EROD activity induction potential
637 between Australian native and introduced species (Figure 2); and

638 3) there is no difference in EROD activity induction potential between Australian
639 native fish and fish from other continents (Table 3).

640

641 **8 Recommendations**

642 1) It is advisable that every field study using the EROD activity preferably utilizes multiple
643 reference sites (as close as possible to zero impact) and that these sites be used to
644 provide an estimate for the basal EROD activity level and its variability. In
645 environments where at least one reference site is not available a baseline/reference
646 condition can be determined by utilising fish captured from the same locality and
647 depurated under clean laboratory conditions (Antunes *et al.* 2007; Ferreira *et al.*
648 2004). Since background EROD activity levels appear to vary within species as well as
649 with site and method (see paragraphs 5.4 and 6.1) it is usually insufficient to rely on
650 previously published basal levels (although these will provide a valuable guide as to
651 the expected value). Without an estimate of basal EROD activity level no inference
652 can be made as to potential exposure to EROD activity inducers.

653 2) As with all ecotoxicological studies, it is important to use ecologically relevant species
654 in laboratory studies. This review shows that a number of Australian species across
655 the breadth of the continent have now been demonstrated to have increased EROD
656 activity induction potential in response to organic pollution. The maximum EROD
657 activity induction potential appears to be similar for native species as for introduced
658 species. The use of native fish captured from the wild or sourced from lab/hatchery
659 stocks has no influence on the EROD activity induction potential tested under

660 laboratory conditions (see section Choice of species). When possible, it is therefore
661 preferable to select a native species for laboratory studies using EROD activity
662 endpoints.

663 3) Given the consistently reported lack of monotonic increase in EROD activity with
664 contaminant concentration, laboratory studies should use a carefully considered
665 range of contaminant concentrations. A single concentration test may not give an
666 accurate EROD activity value if the concentration is on the decreasing side of the
667 response curve (see section Assay methods).

668 4) Measurement of serum SDH activity in conjunction with EROD activity is
669 recommended (see section Assay methods). This ensures that the liver has not
670 sustained damage which may reduce the ability to produce CYP1A and therefore
671 underestimate the exposure to organic contamination made by measuring EROD
672 activity alone.

673 5) Consistency in selection of sex, age and size class will improve the confidence in the
674 validity of measured EROD activities. In field studies, and depending on the variability
675 observed in EROD activity within a given fish species, a number of 8 to 13 non-
676 reproductively active fish per species per treatment or site is recommended to
677 improve the chances of detecting a significant difference in EROD activity, if one does
678 exist (see section on confounding factors).

679

680

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684

9 REFERENCES

Adams S. M. (2001) Biomarker/bioindicator response profiles of organisms can help differentiate between sources of anthropogenic stressors in aquatic ecossystems. Bioindicators. *Biomarkers* **6**, 33-44.

Andreola F., Fernandez-Salguero P. M., Chiantore M. V., Petkovich M. P., Gonzalez F. J. & De Luca L. M. (1997) Aryl hydrocarbon receptor knockout mice ($AHR^{-/-}$) exhibit liver retinoid accumulation and reduced retinoic acid metabolism. *Cancer Res.* **57**, 2835-8.

Antunes P., Gil O., Ferreira M., Vale C. & Reis-Henriques M. A. (2007) Depuration of PCBs and DDTs in mullet under captivity clean conditions. *Chemosphere* **67**, S58-S64.

ANZECC & ARMCANZ. (2000) *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. Australian and New Zealand Environmental and Conservation Council and Agricultural and Resource Management Council of Australia and New Zealand.

Arantes Felício A., Martins Parente T. E., Regina Maschio L., Nogueira L., Rodrigues Venancio L. P., de Freitas Rebelo M., Schlenk D. & de Almeida E. A. (2015) Biochemical responses, morphometric changes, genotoxic effects and CYP1A expression in the armored catfish *Pterygoplichthys anisitsi* after 15 days of exposure to mineral diesel and biodiesel. *Ecotoxicol. Environ. Saf.* **115**, 26-32.

Arukwe A. & Goksoyr A. (1997) Changes in three hepatic cytochrome P450 subfamilies during a reproductive cycle in turbot (*Scophthalmus maximus* L.). *J. Exp. Zool.* **277**, 313-25.

705 Au D. W. T., Chen P. & Pollino C. A. (2004) Cytological changes in association with
706 ethoxyresorufin O-deethylase induction in fish upon dietary exposure to benzo[a]pyrene.
707 *Environ. Toxicol. Chem.* **23**, 1043-50.

708 Bakthyar S. & Gagnon M.M. (2011) Comparison of biomarker responses following one
709 dose of benzo(a)pyrene administered to three native Australian fish species. *J. Roy. Soc.*
710 *West. Austral*. 465-72.

711 Banni M., Bouraoui Z., Ghedira J., Clerandeau C., Guerbej H., Narbonne J. F. & Boussetta
712 H. (2008) Acute effects of benzo[a]pyrene on liver phase I and II enzymes, and DNA
713 damage on sea bream *Sparus aurata*. *Fish Physiol. Biochem.* **35**, 293-9.

714 Baršienė J., Dedonytė V., Rybakovas A., Andreikėnaitė L. & Andersen O. K. (2006)
715 Investigation of micronuclei and other nuclear abnormalities in peripheral blood and
716 kidney of marine fish treated with crude oil. *Aquat. Toxicol.* **78, Supplement**, S99-S104.

717 Beg M. U., Al-Bahloul M., Jacob P. G., Beg K. R., Al-Matrouk K. & Abdel-Elah K. (2001)
718 Biomarker response in sheim (*Acanthopagrus latus*) exposed to polycyclic aromatic
719 hydrocarbons. *Bull. Environ. Contam. Toxicol.* **67**, 210-6.

720 Biró A., Pállinger É., Major J., Jakab M. G., Klupp T., Falus A. & Tompa A. (2002)
721 Lymphocyte phenotype analysis and chromosome aberration frequency of workers
722 occupationally exposed to styrene, benzene, polycyclic aromatic hydrocarbons or mixed
723 solvents. *Immunol. Lett.* **81**, 133-40.

724 Boffetta P., Jourenkova N. & Gustavsson P. (1997) Cancer risk from occupational and
725 environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Causes Control* **8**,
726 444-72.

727 Bozcaarmutlu A, Sapmaz C., Kaleli G., Turna S. & Yenisoy-Karakaş S. (2015) Combined
728 use of PAH levels and EROD activities in the determination of PAH pollution in flathead
729 mullet (*Mugil cephalus*) caught from the West Black Sea coast of Turkey. *Environ. Sci.*
730 *Pollut. Bull.* ***22,*** 2515-25.

731 Brumley C. M., Haritos V. S., Ahokas J. T. & Holdway D. A. (1995) Validation of biomarkers
732 of marine pollution exposure in sand flathead using Aroclor 1254. *Aquat. Toxicol.* **31**, 249-
733 62.

734 Burgeot T., Bocquene G., Porte C., Dimeet J., Santella R. M., Garcia De La Parra L. M.,
735 Pihol-Leszkowicz A., Raoux C. & Galgani F. (1996) Bioindicators of pollutant exposure in
736 the northwestern Mediterranean sea. *Mar. Ecol. Prog. Ser.* **131**, 125-41.

737 Burke M. D. & Mayer R. T. (1974) Ethoxyresorufin: direct fluorometric assay of a
738 microsomal *O*-dealkylation which is preferentially inducible by 3-methylcholanthrene.
739 *Drug Metab. Dispos.* **2**, 583-8.

740 Burkina V., Zlabek V. & Zamaratskaia G (2015) Effetcs of pharmaceuticals present in
741 aquatic environment on Phase I metabolism in fish. *Environ. Toxicol. Chem*. **40**, 430-44.

742 Camacho I. A., Hassuneh M. R., Nagarkatti M. & Nagarkatti P. S. (2001) Enhanced
743 activation-induced cell death as a mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin
744 (TCDD)-induced immunotoxicity in peripheral T cells. *Toxicology* **165**, 51-63.

745 Cavanagh J. E., Burns K. A., Brunskill G. J., Ryan D. A. J. & Ahokas J. T. (2000) Induction of
746 Hepatic Cytochrome P-450 1A in Pikey Bream (*Acanthopagrus berda*) Collected from
747 Agricultural and Urban Catchments in Far North Queensland. *Mar. Pollut. Bull.* **41**, 377-84.

748 Celander M., Leaver M. J., George S. G. & Förlin L. (1993) Induction of cytochrome P450
749 1A1 and conjugating enzymes in rainbow trout *(Oncorhynchus mykiss*) liver: A time course
750 study. *Comp. Biochem. Physiol. Part C Toxicol. Pharmcol.* **106**, 343-9.

751 Chaplin J. A., Baudains G. A., Gill H. S., Mcculloch R. & Potter I. C. (1997) Are assemblages
752 of black bream (*Acanthopagrus butcheri*) in different estuaries genetically distinct?
753 *International Journal of Salt Lake Research* **6**, 303-21.

754 Chen C.-M., Chou Liu M., Liang Shih M., Yu S.-C., Yeh C.-C., Ting Lee S., Yang T.-Y. & Hung
755 S.-J. (2001) Microsomal monooxygenase activity in tilapia (*Oreochromis mossambicus*)
756 exposed to a bleached kraft mill effluent using different exposure systems. *Chemosphere*
757 **45**, 581-8.

758 Chen C. M., Ueng T. H., Wang H. W., Lee S. Z. & Wang J. S. (1998) Microsomal
759 monooxygenase activity in milkfish (*Chanos chanos*) from aquaculture ponds near metal
760 reclamation facilities. *Bull. Environ. Contam. Toxicol.* **61**, 378-83.

761 Clancy S. (2008) DNA damage and repair: mechanisms for maintaining DNA integrity.
762 *Nature Education* **1**.

763 Codi King S., Johnson J. E., Haasch M. L., Ryan D. A. J., Ahokas J. T. & Burns K. A. (2005)
764 Summary results from a pilot study conducted around an oil production platform on the
765 Northwest Shelf of Australia. *Mar. Pollut. Bull.* **50**, 1163-72.

766 Codi S., Humphrey C., Klumpp D. & Delean S. (2004) Barramundi as an indicator species
767 for environmental monitoring in north Queensland, Australia: Laboratory versus field
768 studies. *Environ. Toxicol. Chem.* **23**, 2737-44.

769 Cohen C., Gagnon M. M. & Nugegoda D. (2006) Oil spill remediation techniques can have
770 different impacts on mixed function oxygenase enzyme activities in fish. *Bull. Environ.*
771 *Contam. Toxicol.* **76**, 855-62.

772 Collier T. K., Anulacion B. F., Stein J. E., Goksoyr A. & Varanasi U. (1993) A field evaluation
773 of cytochrome P4501A as a biomarker of contaminant exposure in three species of fish.
774 *Environ. Toxicol. Chem.* **14**, 143-52.

775 Dabrowska H., Kopko O., Góra A., Waszak I. & Walkusz-Miotk J. (2014) DNA damage,
776 EROD activity, condition indices, and their linkages with contaminants in female flounder
777 (Platichthys flesus) from the southern Baltic Sea. *Sci. Total Environ.* **496**, 488-98.

778 Della Torre C., Mariottini M., Vannuccini M. L., Trisciani A., Marchi D. & Corsi I. (2014)
779 Induction of CYP1A and ABC transporters in European sea bass (Dicentrarchus labrax)
780 upon 2,3,7,8-TCDD waterborne exposure. *Mar. Environ. Res.* **99**, 218-22.

781 Denison M. S. (1991) The molecular mechanism of action of 2,3,7,8-tetrachlorodibenzo-*p*-
782 dioxion and related halogenated aromatic hydrocarbons. *Chemosphere* **23**, 1825-30.

783 Denison M. S. & Heath-Pagliuso S. (1998) The Ah receptor: a regulator of the biochemical
784 and toxicological actions of structurally diverse chemicals. *Bull. Environ. Contam. Toxicol.*
785 **61**, 557-68.

786 DeVito M. J. (1996) Relationship between CYP1A enzyme activities and protein levels in
787 rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Toxicol. Environ. Health Part A* **47**,
788 379-94.

789 European Union. (2008) Directive 2008/105/EC. (ed E. Parliament).

790 Fent K. (2001) Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity,
791 cytochrome P450A induction potential and estrogenic activity of chemicals and
792 environmental samples. *Toxicol. In Vitro* **15**, 477-88.

793 Ferreira M., Antunes P., Gil O., Vale C. & Reis-Henriques M. A. (2004) Organochlorine
794 contaminants in flounder (*Platichthys flesus*) and mullet (*Mugil cephalus*) from Douro
795 estuary, and their use as sentinel species for environmental monitoring. *Aquat. Toxicol.*
796 **69**, 347-57.

797 Flammarion P. & Garric J. (1996) A statistical approach for classifying the extent of EROD
798 induction of fish sampled in clean and contaminated waters. *Wat. Res*. **33**: 2683-2689.

799 Flammarion P., Migeon B. & Garric J. (1996) Joint effects of copper sulphate and
800 methidathion on rainbow trout (*Oncorhynchus mykiss*) EROD and AChE activities. *Bull.*
801 *Environ. Contam. Toxicol.* **56**, 440-5.

802 Fossi M. C., Focardi S., Leonzio C., Gavilán J. F., Barra R. & Parra O. (1995) Use of
803 biomarkers to evaluate effects of xenobiotic compounds in the Biobio basin (Central
804 Chile). *Bull. Environ. Contam. Toxicol.* **55**, 36-42.

805 Frasco M. F. & Guilhermino L. (2002) Effects of dimethoate and beta-naphthoflavone on
806 selected biomarkers of *Poecilia reticulata*. *Fish Physiol. Biochem.* **26**, 149-56.

807 Froese R. & Pauly D. (2016) *www.FishBase.org (version 01/2016)*.

808 Gagnon M. M., Dodson J. J., Hodson P. V., Kraak G. V. D. & Carey J. H. (1994) Seasonal
809 effects of bleached kraft mill effluent on reproductive parameters of white sucker
810 (*Catostomus commersoni*) populations of the St. Maurice River, Quebec, Canada. *Can. J.*
811 *Fish*. Aquat. Sci. **51,** 337-47.

812 Gagnon M.M. & Hodson P.V. (2012) Field studies using fish – how many fish are enough?
813 *Mar. Pollut. Bull.*, **64**, 2871-2876.

814 Gagnon M. M. & Holdway D. A. (1998) MFO induction in Atlantic salmon (*Salmo salar*)
815 during and after exposure to Bass Strait crude oil. *Australas. J. Ecotoxicol.* **4**, 29-35.

816 Gagnon M. M. & Holdway D. A. (2000) EROD induction and biliary metabolite excretion
817 following exposure to the water accomodated fraction of crude oil and to chemically
818 dispersed oil. *Arch. Environ. Contam. Toxicol.* **38**, 70-7.

819 Gagnon M. M. & Holdway D. A. (2002) EROD activity, serum SDH and PAH biliary
820 metabolites in sand flathead (*Platycephalus bassensis*) collected in Port Phillip Bay,
821 Australia. *Mar. Pollut. Bull.* **44**, 230-7.

822 Gavilán J. F., Barra R., Fossi M. C., Casini S., Salinas G., Parra O. & Focardi S. (2001)
823 Biochemical biomarkers in fish from different river systems reflect exposure to a variety
824 of anthropogenic stressors. *Bull. Environ. Contam. Toxicol.* **66**, 476-83.

825 Goksoyr A. & Forlin L. (1992) The cytochrome *P*-450 system in fish, aquatic toxicology and
826 environmental monitoring. *Aquat. Toxicol.* **22**, 287-312.

827 He X., Nie X., Yang Y., Liu X., Pan D., Cheng Z. & Liang X. (2012) Multi-biomarker responses
828 in fishes from two typical marine aquaculture regions of South China. *Mar. Pollut. Bull.* **64**,
829 2317-24.

830 Hecht S. S. (2003) Tobacco carcinogens, their biomarkers and tobacco-induced cancer.
831 *Nat. Rev. Cancer* **3**, 733-44.

832 Hodson P. V., Kloepper-Sams P. J., Munkittrick K. R., Lockhart W. L., Metner D. A., Luxon
833 L., Smith I. R., Gagnon M. M., Servos M. & Payne J. F. (1991) Protocols for measuring

834 mixed function oxygenases of fish liver. In: *Can. Tech. Rep. Fish. Aquat. Sci.* p. 49. Maurice
835 Lamontagne Istitute, Mont-Joli.

836 Hoeger B., van den Heuvel M. R., Hitzfeld B. C. & Dietrich D. R. (2004) Effects of treated
837 sewage effluent on immune function in rainbow trout (*Oncorhynchus mykiss*). *Aquat.*
838 *Toxicol.* **70**, 345-55.

839 Hoese D. F., Bray D. J., Paxton J. R. & Allen G. R. (2006) Fishes. In: *Zoological Catalogue of*
840 *Australia* (eds P. L. Beesley and A. Wells) pp. 1-670. Australian Biological Resources Study
841 & CSIRO Publishing, Collingwood.

842 Holdway D. A., Brennan S. E. & Ahokas J. T. (1994) Use of hepatic MFO and blood enzyme
843 biomarkers in sand flathead *(Platycephalus bassensis*) as indicators of pollution in Port
844 Phillip Bay, Australia. *Mar. Pollut. Bull.* **28**, 683-95.

845 Huff J., Jucier G. & Tritscher A. (1994) Carcinogenicity of TCDD: experimental, mechanistic
846 and epidemiologic evidence. *Annu. Rev. Pharmacol. Toxicol.* **34**, 343-72.

847 Humphrey C. A., Codi King S. & Klumpp D. W. (2007) A multi-biomarker approach in
848 barramundi (*Lates calcarifer*) to measure exposure to contaminants in estuaries of
849 tropical North Queensland. *Mar. Pollut. Bull.* **54**, 1569-81.

850 James M. O., Heard C. S. & Hawkins W. E. (1988) Effect of 3-methylcholanthrene on
851 monooxygenase, epoxide hydrolase, and glutathione S-transferase activities in small
852 estuarine and freshwater fish. *Aquat. Toxicol.* **12**, 1-15.

853 Kammann U., Brinkmann M., Freese M., Pohlmann J.-D., Stoffels S., Hollert H. & Hanel R.
854 (2013) PAH metabolites, GST and EROD in European eel (*Anguilla anguilla*) as possible

855 indicators for eel habitat quality in German rivers. *Environmental Science and Pollution*
856 *Research* **21**, 2519-30.

857 Kantoniemi A., Vähäkangas K. & Oikari A. (1996) The capacity of liver microsomes to form
858 benzo[a]pyrene-diolepoxide-DNA adducts and induction of cytochrome P450 1A in feral
859 fish exposed to pulp mill effluents. *Ecotoxicol. Environ. Saf.* **35**, 136-41.

860 Klumpp D. W., Humphrey C. A. & Codi King S. (2007) Biomarker responses in coral trout
861 (*Plectropomus leopardus*) as an indicator of exposure to contaminants in a coral reef
862 environment. *Australas. J. Ecotoxicol.* **13**, 9-17.

863 Lu G., Wang C. & Zhu Z. (2009) The dose–response relationships for EROD and GST
864 induced by polyaromatic hydrocarbons in *Carassius auratus*. *Bull. Environ. Contam.*
865 *Toxicol.* **82**, 194-9.

866 Lyons M. C., Wong D. K. H., Mulder I., Lee K. & Burridge L. E. (2011) The influence of water
867 temperature on induced liver EROD activity in Atlantic cod (*Gadus morhua*) exposed to
868 crude oil and oil dispersants. *Ecotoxicol. Environ. Saf.* **74**, 904-10.

869 Machala M., Vondracek J., Blaha L., Ciganek M. & Neca J. (2001) Aryl hydrocarbon
870 receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined
871 using *in vitro* reporter gene assay. *Mutat. Res.* **497**, 49-62.

872 Mandal P. K. (2005) Dioxin: a review of its environmental effects and its aryl hydrocarbon
873 receptor biology. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **175**, 221-30.

874 Mercurio P., Burns K. A. & Cavanagh J. (2004) Testing the ecotoxicology of vegetable
875 versus mineral based lubricating oils: 2. Induction of mixed function oxidase enzymes in
876 barramundi, Lates calcarifer, a tropical fish species. *Environ. Pollut.* **129**, 175-82.

877 Miller K. A., Addison R. F. & Bandiera S. M. (2004) Hepatic CYP1A levels and EROD activity
878 in English sole: biomonitoring of marine contaminants in Vancouver Harbour. *Mar.*
879 *Environ. Res.* **57**, 37-54.

880 Möller A.-M., Hermsen C., Floehr T., Lamoree M. H. & Segner H. (2014) Tissue-specific
881 metabolism of benzo[a]pyrene in rainbow trout (*Oncorhynchus mykiss*): a comparison
882 between the liver and immune organs. *Drug Metab. Dispos.* **42**, 111-8.

883 Mondon J. A., Duda S. & Nowak B. F. (2001) Histological, growth and 7-ethoxyresorufin O-
884 deethylase (EROD) activity responses of greenback flounder *Rhombosolea tapirina* to
885 contaminated marine sediment and diet. *Aquat. Toxicol.* **54**, 231-47.

886 Mosse P. R. L., Brumley C. M., Ahokas J. T. & Holdway D. A. (1996) A preliminary
887 investigation into the use of biomarkers for the monitoring of an ocean outfall. *Environ.*
888 *Toxicol.* **11**, 113-9.

889 Navas J. M. & Segner H. (2000) Modulation of trout 7-ethoxyresorufin-O-deethylase
890 (EROD) activity by estradiol and octylphenol. *Mar. Environ. Res.* **50**, 157-62.

891 Oropeza-Hernandez L. F., Lopez-Romero R. & Albores A. (2003) Hepatic CYP1A, 2B, 2C, 2E
892 and 3A regulation by methoxychlor in male and female rats. *Toxicol. Lett.* **144**, 93-103.

893 Orrego R., Burgos A., Moraga-Cid G., Inzunza B., Gonzalez M., Valenzuela A., Barra R. &
894 Gavilán J. F. (2006) Effects of pulp and paper mill discharges on caged rainbow trout
895 (*Oncorhynchus mykiss*): Biomarker responses along a pollution gradient in the Biobio
896 River, Chile. *Environ. Toxicol. Chem.* **25**, 2280-7.

897 Padrós J., Pelletier É., Reader S. & Denizeau F. (2000) Mutual *in vivo* interactions between
898 benzo[*a*]pyrene and tributyltin in brook trout (*Salvelinus fontinalis*). *Environ. Toxicol.*
899 *Chem.* **19**, 1019-27.

900 Perelo L. W. (2010) Review: In situ and bioremediation of organic pollutants in aquatic
901 sediments. *J. Hazard. Mater.* **177**, 81-9.

902 Pohl R. J. & Fouts J. R. (1980) A rapid method for assaying the metabolism of 7-
903 ethoxyresorufin by microsomal subcellular fractions. *Anal. Biochem.* **107**, 150-5.

904 Pollino C. A., Georgiades E. & Holdway D. A. (2009) Physiological changes in
905 reproductively active rainbowfish (*Melanotaenia fluviatilis*) following exposure to
906 naphthalene. *Ecotoxicol. Environ. Saf.* **72**, 1265-70.

907 Pollino C. A. & Holdway D. A. (2003) Hydrocarbon-induced changes to metabolic and
908 detoxification enzymes of the Australian crimson-spotted rainbowfish (*Melanotaenia*
909 *fluviatilis*). *Environ. Toxicol.* **18**, 21-8.

910 Puga A., Barnes S. J., Chang C.-Y., Zhu H., Nephew K. P., Kahn S. A. & Shertzer H. G. (2000)
911 Activation of transcription factors activator protein-1 and nuclear factor-κB by 2,3,7,8-
912 tetrachlorodibenzo-*p*-dioxin. *Biochem. Pharmacol.* **59**, 997-1005.

913 Rannug A., Rannug U., Rosenkranz H. S., Winqvist L., Westerholm R., Agurell E. &
914 Grafstrom A. (1987) Certain photooxidised derivatives of tryptophan bind with very high
915 affinity to the Ah receptor and are likely to be endogenous signal substances. *J. Biol.*
916 *Chem.* **15**, 15422-7.

917 Ratia H. M., Vehniäinen E.-R., Rusanen A. T. & Oikari A. O. J. (2014) Recovery of
918 historically contaminated watercourse polluted by the chemical wood industry: EROD
919 activity in fish as biomarker. *Soil Sediment Contam.* **23**, 211-25.

920 Rawson C. A., Tremblay L. A., Warne M. S. J., Kookana R. S., Ying G.-G., Chapman J. C. &
921 Lim R. P. (2009) Bioactivity of POPs and their effects in mosquitofish in Sydney Olympic
922 Park, Australia. *Sci. Total Environ.* **407**, 3721-30.

923 Rodrigues A. C. F., Oliveira Moneró T., Frighetto R. T. S. & Almeida E. A. (2014) E2
924 potentializes benzo(a)pyrene-induced hepatic cytochrome P450 enzyme activities in Nile
925 tilapia at high concentrations. *Environmental Science and Pollution Research* **22**, 17367-
926 74.

927 Rodrigo O., Burgos A., Moraga-Cid G., Inzunza B., Gonzalez M., Valenzuela A., Barra R. &
928 Gavilan J. F. (2006) Effects of pulp and paper mill discharges on caged rainbow trout
929 (*Oncorhynchus mykiss*): Biomarker responses along a pollution gradient in the Biobio
930 River, Chile. *Environ. Toxicol. Chem*. ***25***, 2280-7.

931 Sanchez W., Aït-Aïssa S., Palluel O., Ditche J.-M. & Porcher J.-M. (2007) Preliminary
932 investigation of multi-biomarker responses in three-spined stickleback (*Gasterosteus*
933 *aculeatus* L.) sampled in contaminated streams. *Ecotoxicology* **16**, 279-87.

934 Servizi J. A., Gordon R. W., Martens D., Lockhart W. L., Metner D. A., Rogers I. H., McBride
935 J. R. & Norstrom R. J. (1993) Effects of biotreated bleached kraft mill effluent on fingerling
936 chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* **50**, 846-57.

937 Shailaja M. S. & D'Silva C. (2003) Evaluation of impact of PAH on a tropical fish,
938 *Oreochromis mossambicus* using multiple biomarkers. *Chemosphere* **53**, 835-41.

939 Shaw M., Tibbetts I. R. & Müller J. F. (2004) Monitoring PAHs in the Brisbane River and
940 Moreton Bay, Australia, using semipermeable membrane devices and EROD activity in
941 yellowfin bream, Acanthopagrus australis. *Chemosphere* **56**, 237-46.

942 Sierra-Santoyo A., Hernandez M., Albores A. & Cebrian M. E. (2000) Sex-dependent
943 regulation of hepatic cytochrome P-450 by DDT. *Toxicol. Sci.* **54**, 81-7.

944 Smith B. J. & Gagnon M. M. (2000) MFO Induction of three Australian fish species.
945 *Environ. Toxicol.* **15**, 1-7.

946 Solé M., Raldua D., Piferrer F., Barceló D. & Porte C. (2003) Feminization of wild carp,
947 *Cyprinus carpio*, in a polluted environment: plasma steroid hormones, gonadal
948 morphology and xenobiotic metabolizing system. *Comp. Biochem. Physiol. Part C Toxicol.*
949 *Pharmcol.* **136**, 145-56.

950 Taysse L., Chambras C., Marionnet D., Bosgiraud C. & Deschaux P. (1998) Basal level and
951 induction of cytochrome P450, EROD, UDPGT, and GST activities in carp (*Cyprinus carpio*)
952 immune organs (spleen and head kidney). *Bull. Environ. Contam. Toxicol.* **60**, 300-5.

953 Tugiyono & Gagnon M. M. (2001) Pink snapper (*Pagrus auratus*) as a bioindicator of
954 aquatic environmental health in Western Australia. *Environ. Toxicol.* **16**, 449-54.

955 van Dam R. A., Ahokas J. T. & Holdway D. A. (1999) Chronic toxicity of diethylenetriamine
956 pentaacetic acid to crimson-spotted rainbowfish (*Melanotaenia fluviatilis*): effects on
957 reproduction, condition, and ethoxyresorufin-O-deethylase activity. *Ecotoxicol. Environ.*
958 *Saf.* **43**, 74-82.

959 van den Heuvel M. R., Landman M. J. & Tremblay L. A. (2006) Responses of shortfin eel
960 (*Anguilla australis)* exposed in situ to pulp and paper effluent. *J. Toxicol. Environ. Health*
961 *Part A* **69**, 1763 - 79.

962 Varanasi U., Nishimoto M., Reichert W. L. & Le Eberhart B.-T. (1986) Comparative
963 metabolism of benzo(a)pyrene and covalent binding to hepatic DNA in English sole, starry
964 flounder and rat. *Cancer Res.* **46**, 3817-24.

965 Waxman D. J. (1988) Interactions of hepatic cytochromes *P*-450 with steroid hormones:
966 regioselectivity and stereospecificity of steroid metabolism and hormonal regulation of
967 rat *P*-450 enzyme expression. *Biochem. Pharmacol.* **37**, 71-84.

968 Webb D. & Gagnon M. M. (2002a) Biomarkers of exposure in fish inhabiting the Swan–
969 Canning Estuary, Western Australia – a preliminary study. *J. Aquat. Ecosyst. Stress*
970 *Recovery* **9**, 259-69.

971 Webb D. & Gagnon M. M. (2002b) MFO induction potential of fish species native to the
972 Swan-Canning Estuary, Western Australia. *Environ. Toxicol.* **17**, 87-92.

973 Webb D., Gagnon M. M. & Rose T. (2005a) Interseasonal variability in biomarkers of
974 exposure in fish inhabiting a southwestern Australian estuary. *Environ. Toxicol.* **20**, 522-
975 32.

976 Webb D., Gagnon M. M. & Rose T. H. (2005b) Interannual variability in fish biomarkers in
977 a contaminated temperate urban estuary. *Ecotoxicol. Environ. Saf.* **62**, 53-65.

978 Webb D., Gagnon M. M. & Rose T. H. (2008) Hepatic metabolism of contaminants in the
979 Terapontid fish, yellowtail trumpeter (*Amniataba caudavittata* Richardson). *Environ.*
980 *Toxicol.* **23**, 68-76.

981 Webb D. & Gagnon M.M. (2013) Depurated fish as an alternative reference for field-based
982 biomarker monitoring. *Environ. Monit. Assess*. **185**:1331-1340.

983 Webb D., & Gagnon M.M. (2007) Serum sorbitol dehydrogenase activity as an indicator of
984 chemically induced liver damage in black bream (*Acanthopagrus butcheri*). Environ.
985 Bioindicat. 2, 172-182.

986 Whyte J. J., Jung R. E., Schmitt C. J. & Tillitt D. E. (2000) Ethoxyresorufin-*O*-deethylase
987 (EROD) activity in fish as a biomarker of chemical exposure. *Crit. Rev. Toxicol.* **30**, 347-571.

988 Wilson J. Y., Addison R. F., Martens D., Gordon R. & Glickman B. (2000) Cytochrome P450
989 1A and related measurements in juvenile chinook salmon (*Oncorhynchus tshawytscha*)
990 from the Fraser River. *Can. J. Fish. Aquat. Sci.* **57**, 405-13.

991 Wilson J. Y., Kruzynski G. M. & Addison R. F. (2001) Experimental exposure of juvenile
992 chinook (*Oncorhynchus tshawytscha*) to bleached kraft mill effluent: hepatic CYP1A
993 induction is correlated with DNA adducts but not with organochlorine residues. *Aquat.*
994 *Toxicol.* **53**, 49-63.

995 Woodworth J. G., Munday B. L. & Campin D. (1998) Evaluation of biomarkers for exposure
996 of fish to eucalypt-based pulp mill effluent and for determination of routes of exposure.
997 *Environ. Toxicol. Water Qual.* **13**, 285-96.

998 Wunderlich A. C., Silva R. J., Zica É. O. P., Rebelo M. F., Parente T. E. M. & Vidal-Martínez
999 V. M. (2015) The influence of seasonality, fish size and reproductive status on EROD
1000 activity in *Plagioscion squamosissimus*: Implications for biomonitoring of
1001 tropical/subtropical reservoirs. *Ecol. Indic.* **58**, 267-76.

1002

1003 Table 1. Summary of Australian laboratory studies using an EROD activity endpoint.

Family	Species	Native *	Habitat **	Sex ^	Life-Stage ^^	Source #	Contaminant	Exp. Route †	Tmnts	Fish per Tmnt	Max Conc	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold induction††	Reference
Bleniidae	*Parablennius tasmanianus*	N	M	A	U	W	KME	Imm/ Diet	4	10	1.00%	Holdway *et al.* 1993	0.32	1.67	5.22	Woodworth *et al.*, 1998
Centropomidae	*Lates calcarifer*	N	E	M	J	H	β-NP	I.P.	3	9	50 mg/kg	Holdway *et al.* 1994	54	1256 @10mg/kg	23.6	Mercurio *et al.*, 2004
		N	E	M	J	H	β-NP	I.P.	4	40	50 mg/ kg	Kruner & von Westerhagen 1999	3.4	145	43	Codi *et al.*, 2004
		N	E	M	J	H	B(a)P	I.P.	1	8	1.0 µg/kg	Hodson et al 1991	9	15	1.7	Bakhtyar and Gagnon 2011
Cichlidae	*Oreochromis mossambicus*	I	F	A	A	W	β-NP	I.P.	1	5	50 µg/g	Prince 1993	5.51	26.0	4.7	Chen *et al.*, 2001
		I	F	A	J	W	KME	Imm	1	5	100%	Prince 1993	5.33	19.64	19.6	Chen *et al.*, 2001
		I	F	U	J	H	Phenanthrene	I.P.	2	6	6-32 µg/g	Gunther *et al.* 1997	720	17310	24.0	Shailaja *et al.*, 2003
		I	F	U	J	H	Oil refinery effluent	Imm	1	6	25%	Gunther *et al.* 1997	1000	5100	5.1	Shailaja *et al.*, 2003
Clupeidae	*Nematalosa*	N	E	U	J	W	PCB 126	I.P.	1	5	10 µg/kg	Hodson *et al.* 1991	100%	100% Mort	-	Webb *et al.*, 2002b

Family	Species	Native *	Habitat **	Sex ^	Life-Stage ^^	Source #	Contaminant	Exp. Route †	Tmnts	Fish per Tmnt	Max Conc	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold induction††	Reference
	vlaminghi												Mort			
Cyprinidae	*Carassius auratus*	I	F	U	U	H	Fluorene	I.P.	5	U	10 mg/ kg	Chen *et al.* 1999	6000	16000	2.67	Lu *et al.*, 2009
		I	F	U	U	H	Fluoranthene	I.P.	5	U	10 mg/ kg	Chen *et al.* 1999	5500	12000	2.2	Lu *et al.*, 2009
		I	F	U	U	H	Benzo(b) fluoranthene	I.P.	5	U	8 mg/ kg	Chen *et al.* 1999	4800	14000	2.9	Lu *et al.*, 2009
		I	F	U	U	H	Benzo(g,h,i) perylene	I.P.	5	U	10 mg/ kg	Chen *et al.* 1999	4800	15000	3.1	Lu *et al.*, 2009
		I	F	U	U	H	Ideno(1,2,3-cd) pyrene	I.P.	5	U	10 mg/ kg	Chen *et al.* 1999	5000	26000 @5mg/kg	5.2	Lu *et al.*, 2009
	Cyprinius carpio	I	F	U	J	H	3-MC	I.P.	1	U	40 mg/ kg	Burke & Mayer, 1974	7.1	306.3	43.1	Taysse *et al.*, 1998
Galaxiidae	*Galaxias maculatus*	N	F	A	J	W	KME	Imm/ Diet	4	10	0.50%	Holdway *et al.*, 1993	0.5	2.21	4.42	Woodworth *et al.*, 1998
		N	F	A	A	W	KME	Imm/ Diet	4	10	0.50%	Holdway *et al.* 1993	2.63	13.79	5.2	Woodworth *et al.*, 1998

Family	Species	Native *	Habitat **	Sex ^	Life-Stage ^^	Source #	Contaminant	Exp. Route †	Tmnts	Fish per Tmnt	Max Conc	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold induction††	Reference
Labridae	*Notolabrus tetricus*	N	M	A	U	W	PCB 126	I.P.	3	9	100 μg/kg	Hodson *et al.* 1991	256	284	NSI	Smith *et al.*, 2000
Melanitaenidae	*Melanotaenia fluviatilis*	N	F	A	U	L	Crude oil	Imm	4	5	30% WAF/ 0.3% DCWAF	Burke & Mayer 1974	50-100	360	4.6	Pollino & Holdway., 2003
		N	F	M	A	L	DPTA	Imm	4	25	100 mg/ L	Reid 1997	8.35	11.91 @10mg/L	NSI	van Dam *et al.*, 1999
		N	F	F	A	L	DPTA	Imm	4	25	100 mg/ L	Reid 1997	12.29	12.90 @10mg/L	NSI	van Dam *et al.*, 1999
		N	F	M	A	L	Naphthalene	Imm	4	20	400 μg/L	Pollino & Holdway 2003	264	434	NSI	Pollino *et al.*, 2009
		N	F	F	A	L	Naphthalene	Imm	4	20	400 μg/L	Pollino & Holdway 2003	185	226	NSI	Pollino *et al.*, 2009
Monacanthidae	*Meuschenia freycineti*	N	E	F	U	W	PCB 126	I.P.	3	4	100 μg/kg	Hodson *et al.* 1991	118	315	2.7	Smith and Gagnon, 2000
Mugilidae	*Mugil cephalus*	N	M	U	J	W	PCB 126	I.P.	1	5	10 μg/kg	Hodson *et al.* 1991	9.2	31.7	3.5	Webb and Gagnon 2002b

Family	Species	Native *	Habitat **	Sex ^	Life-Stage ^^	Source #	Contaminant	Exp. Route †	Tmnts	Fish per Tmnt	Max Conc	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold induction††	Reference
Percichthyidae	*Macquaria novemaculeata*	N	F	U	J	H	Crude oil	Imm	4	30	49.8%	Holdway *et al.* 1994	1.95	42.3	21.7	Cohen *et al.*, 2006
Platycephalidae	*Platycephalus bassensis*	N	E	A	U	W	PCB 126	I.P.	3	13	100 µg/kg	Hodson *et al.* 1991	34.4	693	20.2	Smith and Gagnon, 2000
		N	E	U	U	W	Aroclor 1254	I.P.	5	10	400 mg/kg	Holdway *et al.* 1993	18	68	3.8	Brumley *et al.*, 1995
		N	E	A	U	W	WWTPE	Imm	3	10	2.5% (v/v)	U	15	23	NSI	Mosse *et al.*, 1996
Pleuronectidae	*Rhombosolea tapirina*	N	M	U	J	H	Cont Sed/ Contam Diet	Imm/ Diet	5	20	NA	Holdway *et al.* 1994	9	170	18.9	Mondon *et al.*, 2001
Poecillidae	*Poecilia Reticulata*	I	F	M	A	H	β-NP	Imm	5	9	1.7 mg/L	Hodson *et al.* 1991	3.5	31	8.9	Frasco *et al.*, 2002
		I	F	U	U	L	3-MC	I.P.	1	41	9 mg/kg	Pohl & Fouts (1980)	90	U	8	James *et al.*, 1988
Salmonidae	*Oncorhynchus mykiss*	I	F	U	J	H	β-NP	I.P.	3	U	0.5 mg/kg	Pohl & Fouts 1980	3.5	80.5	23	Flammarion *et al.*, 1996
		I	F	U	J	H	B(a)P	I.P.	1	3	15 mg/kg	Nakayama *et al.* 2008	8.5	1150	135	Moller *et al.* 2014

Family	Species	Native *	Habitat **	Sex ^	Life-Stage ^^	Source #	Contaminant	Exp. Route †	Tmnts	Fish per Tmnt	Max Conc	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold induction††	Reference
		I	F	U	J	H	WWTPE	Imm	4	10	70%	van den Heuvel 1999	4	11.5 @30%	2.9	Hoeger *et al.*, 2004
		I	F	U	J	L	β-NP	I.P.	1	8	10 ml/kg	Celender & Forlin 1991	800	6000	7.5	Celander *et al.*, 1993
		I	F	U	J	L	isosafrole	I.P.	1	8	10 ml/kg	Celender & Forlin 1991	200	3600	18	Celander *et al.*, 1993
	Oncorhynchus tshawytscha	I	F	U	J	H	KME	Imm	4	20	16%	Hodson *et al.* 1991	70	200	2.9	Wilson *et al.*, 2001
		I	F	U	J	H	KME	Imm	1	10	1.5%	Pohl & Fouts 1980	0.004	0.027	6.8	Servizi *et al.*, 1993
	Salmo salar	I	F	F	J	H	Crude oil	Imm	2	16	250 ppm	Hodson *et al.* 1991	25	125	5	Gagnon *et al.*, 1998
	Salvenius fontinalis	I	F	M	A	L	B(a)P	I.P.	1	6	10 mg/kg	Burke & Mayer, 1974	4.5	12	2.7	Padrós *et al.*, 2000
		I	F	M	A	L	Tributyltin	I.P.	1	6	10 mg/kg	Burke & Mayer, 1974	4.5	4.5	NSI	Padrós *et al.*, 2000
Scinaenid	*Argyosomus hololepidotus*	I	M	J	J	H	B(a)P	I.P.	1	8	1.0 µg/kg	Hodson et al 1991	21	32	1.5	Bakhtyar and Gagnon 2011
Serranidae	*Epinephelus areolatus*	N	M	J	J	H	B(a)P	Diet	4	6	12.5 µg BaP/g fish/day	Hodson *et al.* 1996	45	400	8.9	Au *et al.*, 2004

Family	Species	Native *	Habitat **	Sex ^	Life-Stage ^^	Source #	Contaminant	Exp. Route †	Tmnts	Fish per Tmnt	Max Conc	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold induction††	Reference
Sparidae	*Acanthopagrus butcheri*	N	E	U	J	W	PCB 126	I.P.	1	5	10 µg/kg	Hodson *et al.* 1991	8.1	72.2	8.9	Webb and Gagnon 2002b
	Acathopagrus latus	N	E	U	A	L	B(a)P	Diet	9	U	25 mg/kg	Beyer & Goksoyer, 1993	18.25	1080.3	59.2	Beg *et al.*, 2001
	Pagrus auratus	N	M	U	J	H	PCB 126	I.P.	5	16	1000 µg/kg	Hodson *et al.* 1991	45	170 @10µg/kg	3.8	Tugiyono and Gagnon 2001
	Pagrus auratus	N	M	J	J	H	B(a)P	I.P.	1	8	1.0 µg/kg	Hodson *et al.* 1991	17	56	3.3	Bakthyar and Gagnon 2011
Terapontidae	*Amniataba caudavittata*	N	E	U	J	W	PCB 126	I.P.	1	5	10 µg/kg	Hodson *et al.* 1991	7.0	15.9	2.3	Webb and Gagnon 2002b
		N	E	U	A	W	β-NP	I.P.	1	6	40 µM/kg	Hodson *et al.*, 1991	12.4	22.3	NSI	Webb *et al.*, 2008
		N	E	U	A	W	B(a)P	I.P.	1	6	40 µM/kg	Hodson *et al.* 1991	16.1	27.5	NSI	Webb *et al.*, 2008

1004 *N= native; I= introduced; **F= freshwater, M= marine, E= estuarine , Eu = euryhaline; ^M=male, F=female, U=unspecified; ^^ A=adult, J=juvenile, U=unspecified;

1005 #W=wild-caught; H=hatchery; L= laboratory bred; †Imm= immersion, I.P.=intra-peritoneal; ††U=unknown; NSI=non-significant; NI= not induced Contaminants are

1006 abbreviated as in text.

1007

1008 Table 2. Summary of findings in Australian and overseas field studies on fish species native to Australia.

Family	Species	Native*	Habitat**	Age^	Sex^^	Country	Location	Sites	Contaminant	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold Induction††	Reference
Anguillidae	*Anguilla australis*	N	F	U	U	NZ	Nth Is.	3	KME	U	0.13	0.33	2.6	van den Heuvel *et al.*, 2006
Carangidae	*Carangoides fulvoguttatus*	N	M	1-6 yr	A	Aust	CW	3	PFW	Holdway *et al.* 1994	20.5	34.3	1.68	Codi King *et al.*, 2005
Centropomidae	*Lates calcarifer*	N	A	U	M	Aust	QLD	2	U	Kruner & von Westerhagen, 1999	4.3	11.8	2.26	Codi King *et al.*, 2004
		N	A	U	A	Aust	QLD	5	Agric.	Kruner & von Westerhagen, 1999	3.2	34.6	10.8	Humphrey *et al.*, 2007
Chanidae	*Chanos chanos*	N	A	J	U	Taiwan	Er-Jen R.	4	Indust. WWTPE	Pohl & Fouts, 1980	46.8	196	4.2	Chen *et al.*, 1998
Cichlidae	*Oreochromis mossambicus*	I	F	J	A	Taiwan	Shin-Yin	1	BKME	Prince, 1993	20	125	6.3	Chen *et al.*, 2001
Cyprinidae	*Cyprinius carpio*	I	F	A	A	Spain	Anoia R.	1	WWTPE	Burke & Mayer, 1974	U	156	U	Solé *et al.*, 2003

Family	Species	Native*	Habitat**	Age^	Sex^^	Country	Location	Sites	Contaminant	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold Induction††	Reference
	Rutilis rutilis	I	F	A	U	Finland	Lake Vatianjarvi	3	KME	Vehniainen *et al.* 2012	1.6	1.9	1.2	Ratia *et al.* 2014
		I	F	A	U	Finland	L. Saimaa	4	KME	Burke & Mayer 1974	2.3	4.4	NSI	Kantoniemi *et al.*, 1996
Lutjanidae	*Lutjanus argentimaculatus*	N	M	U	U	China	Pearl River Delta	2	PAHs, OCs, metals	Pohl & Fouts, 1980	2.5	5	2	He *et al.*2012
	Lutjanus erythropterus	I	M	U	U	China	Pearl River Delta	2	PAHs, OCs, metals	Pohl & Fouts, 1980	4.4	7.8	1.8	He *et al.* 2012
Mugilidae	*Mugil cephalus*	I	M	J	M	Chile		3		U	7	17	2.4	Gavilán *et al.*, 2001
		I	M	U	A	Portugal	Duoro Est,	1	WWTPE	Pacheco & Sanchez, 1998	75	1500	20	Ferreira *et al.*, 2004
		N	M	U	M	Turkey	West Black Sea	5	PAHs	Burke & Mayer 1997 Arnic & Sen 1994	220	4750	22	Bozcaarmutlu *et al.* 2015

Family	Species	Native*	Habitat**	Age^	Sex^^	Country	Location	Sites	Contaminant	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold Induction††	Reference
Percidae	*Perca fluviatilis*	I	F	U	U	Finland	L. Saimma	4	KME	Burke & Mayer 1974	98	116	NSI	Kantoniemi *et al.*, 1996
		I	F	A	U	Finland	Lake Vatianjarvi	3	KME	Vehniainen *et al.* 2012	10	1.5	-	Ratia *et al.* 2014
Platycephalidae	*Platycephalus bassensis*	N	M	U	M	Aust	Vic	6	Urban. Indust. Ship.	Hodson *et al.*, 1991	21	44	2.1	Gagnon and Holdway 2002
		N	M	U	F	Aust	Vic	7	Hg, PAH, OCP, PCB	Holdway *et al.*, 1993	U	80.8	U	Holdway *et al.*, 1994
		N	M	U	M	Aust	Vic	7	Hg, PAH, OCP, PCB	Holdway *et al.*, 1993	U	198	U	Holdway *et al.*, 1994

Family	Species	Native*	Habitat**	Age^	Sex^^	Country	Location	Sites	Contaminant	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold Induction††	Reference
		N	M	U	F	Aust	Vic	6	Urban. Indust. Ship.	Hodson *et al.*, 1991	19.2	30.1	1.57	Gagnon and Holdway 2002
Poecillidae	*Gambusia holbrooki*	I	F	U	M	Aust	NSW	15	PAH, PCB, TCDD, DDT	Eggens & Galgani, 1992	1339	6002	4.5	Rawson *et al.*, 2009
Salmonidae	*Oncorhynchus mykiss*	I	F	J	U	Chile	Biobio R.	3	KME	U	0.3	2.5	9.8	Rodrigo *et al.*, 2006
	Oncorhynchus tshawytscha	I	F	J		Canada	Fraser R.	6	Indust. WWTPE	Hodson *et al.*, 1991	21	69	3.3	Wilson *et al.*, 2000
Serranidae	*Plectropomus leopardus*	N	M	U	A	Aust	QLD	6	Rec. Boat.	Kruner & von Westerhagen, 1999	U	10.5	U	Klumpp *et al.*, 2007
	Plectropomus maculatus	N	M	2-9 yr	A	Aust	CW	2	PFW	Holdway *et al.* 1994	25.4	20.4	U	King *et al.*, 2005

Family	Species	Native*	Habitat**	Age^	Sex^^	Country	Location	Sites	Contaminant	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold Induction††	Reference
Sparidae	*Acanthopagrus australis*	N	E	U	A	Aust	QLD	5	Urban	Burke & Mayer 1974	16	22	1.4	Shaw *et al.*, 2004
		N	E	U	F	Aust	QLD	5	Urban	Burke & Mayer 1974	12	30	2.5	Shaw *et al.*, 2004
	Acanthopagrus berda	N	E	U	M	Aust	QLD	7	rec. urban. Agric.	Burke & Mayer 1974	67	564	8.4	Cavanagh *et al.*, 2000
	Acanthopagrus butcheri	N	E	U	M	Aust	WA	8	rec. urban	Hodson *et al.* 1991	U	21.8	U	Gagnon and Holdway 2000
		N	E	U	F	Aust	WA	8	rec. urban	Hodson *et al.* 1991	U	12.5	U	Gagnon and Holdway 2000
		N	E	U	M	Aust	WA	7	rec. urban	Hodson *et al.* 1991	U	21.8	U	Webb and Gagnon 2002b
		N	E	U	F	Aust	WA	7	rec. urban	Hodson *et al.* 1991	U	8.0	U	Webb and Gagnon 2002b

Family	Species	Native*	Habitat**	Age^	Sex^^	Country	Location	Sites	Contaminant	EROD Method	Basal EROD (pmol/min/mg pr)	MAX EROD (pmol/min/mg pr)	Fold Induction††	Reference
		N	E	U	F	Aust	WA	5	rec. urban	Hodson *et al.*, 1991	U	17.9	U	Webb *et al.*, 2005
		N	E	U	M	Aust	WA	5	rec. urban	Hodson *et al.*, 1991	U	37.4	U	Webb *et al.*, 2005

1009 *N= native; I= introduced; **F= freshwater, M= marine, E= estuarine , Eu = euryhaline; ^^M=male, F=female, U=unspecified; ^ A=adult, J=juvenile, U=unspecified;

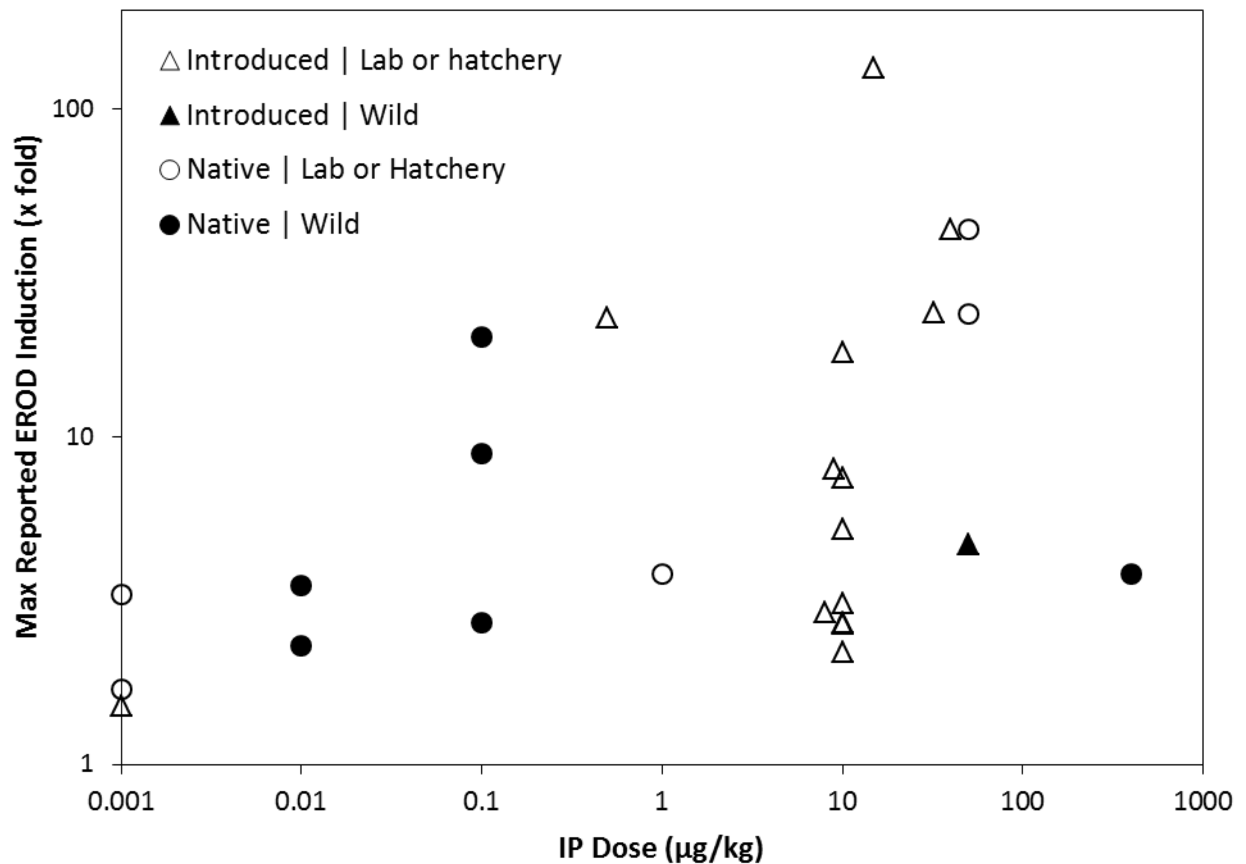
1010 ††U=unknown; NSI=non-significant; NI= not induced Contaminants are abbreviated as in text.

1011

1012 Table 3. Induction potential of a few highly inducible fish species reported around the world.

Family	Species	Common name	Continent where reported	Induction relative to ctl/ref*	Reference
Cyprinidae	*Cyprinus carpio*	Carp	Australia	43-fold	Taysse *et al.* 1998
Centropomidae	*Lates calcarifer*	Barramundi	Australia	24-fold	Mercurio *et al.* 2004
Cichlidae	*Oreochromis mossambicus*	Mozambique tilapia	Australia	24-fold	Shailaja and D'Silva 2003
Platycephalidae	*Platycephalus bassensis*	Sand flathead	Australia	20-fold	Smith and Gagnon 2000
Moronidae	*Dicentrarchus labrax*	European seabass	Europe	26-fold	Della Torre *et al.* 2014
Mugilidae	*Mugil cephalus*	Mullet	Europe	20-fold	Ferreira *et al.* 2004
Pleuronectidae	*Platichthys flesus*	European flounder	Europe	17-fold	Dabrowska *et al.* 2014
Anguillidae	*Anguilla anguilla*	European eel	Europe	5-fold	Kammann *et al.* 2013
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	N. America	135-fold	Möller *et al.* 2014
Salmonidae	*Salmo salar*	Atlantic salmon	N. America	53-fold	Gagnon and Holdway 1998
Gadidae	*Gadus morhua*	Atlantic cod	N. America	25-fold	Lyons *et al.* 2011
Catostomidae	*Catostomus commersoni*	White sucker	N. America	15-fold	Gagnon *et al.* 1994
Cichlidae	*Oreochromis niloticus*	Nile tilapia	S. America	24-fold	Rodrigues *et al.* 2014
Sciaenidae	*Plagioscion squamosissimus*	Silver croaker	S. America	7-fold	Wunderlich *et al.* 2015
Loricariidae	*Pterygoplichthys anisitsi*	Armored catfish	S. America	6-fold	Arantes Felício *et al.* 2015

1013 *Ctl/ref: control or reference groups



1014

1015 Figure 1. Maximum EROD activity induction (x-fold, pmol/min/mg pr) reported for

1016 intraperitoneal (I.P.) injected native (circles) and introduced (triangles) Australian species

1017 from lab/hatchery-reared (open symbols) and wild-caught (closed symbols) sources used in

1018 laboratory studies. The data extracted from Table 1 compares the maximum x-fold EROD

1019 activity induction potential of various fish species following exposure to a variety of EROD

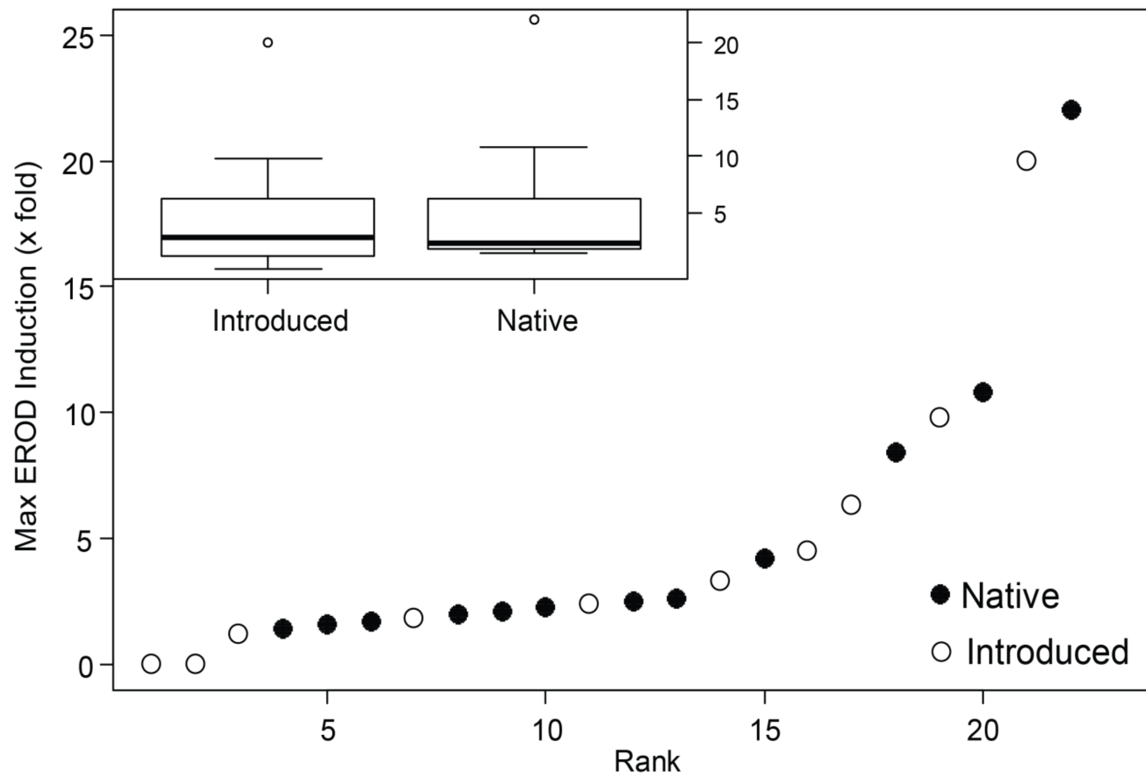
1020 activity inducers. The maximum EROD activity induction from fish sourced from lab/hatchery

1021 facilities is statistically similar ($p = 0.155$) to the maximum EROD activity induction of fish

1022 sourced from the wild.

1023

1024



1025

1026 Figure 2. Maximum EROD activity induction (x-fold, pmol/min/mg pr) observed in field

1027 studies conducted in Australia. Inter-species variability is comparable (p = 0.941) for native

1028 species and for introduced species. Data points are presented as a rank for ease of display

1029 not analysis. Data extracted from relevant references listed in Table 1.

1030

1031

1032