1 *Article*

2 Assessment of the Impacts of Anthropogenic
3 Activities on a Large River Using Longfin Eel as a
4 Bioindicator

5 **Olivier Champeau [1*], Jamie M. Ataria [1], Grant L. Northcott [2], Gen Kume [3], Andrew Barrick [1] and**
6 **Louis A. Tremblay [1*]**

7 1 Cawthron Institute, Nelson 7010, New Zealand
8 2 Northcott Research Consultants Ltd, Hamilton 3200, New Zealand
9 3 Faculty of Fisheries, Kagoshima University, Kagoshima, Japan
10 * Correspondence: olivier.champeau@cawthron.org.nz (O.C.), louis.tremblay@cawthron.org.nz (L.A.T.)

11 **Abstract:** The Matāura River is the sixth largest river system in New Zealand and has long been
12 subject to agricultural, industrial, and residential land use activities. The catchment has geographic
13 and economic value and is of great cultural importance for local Māori, who have concerns over
14 potential adverse impacts that anthropogenic stressors exert on the health of the river. There is a
15 dearth of information on the impacts of these stressors towards the health of native species such as
16 the longfin eel *Anguilla dieffenbachii*. This study assessed the environmental status of the Matāura
17 River using biological and chemical methodologies incorporating *A. dieffenbachii* as a bioindicator
18 species for exposure to combined anthroprogenic stressors. A range of biomarker endpoints were
19 measured in caged and wild-caught eels (when available) to characterize site-specific responses to
20 combined anthropogenic stressors. While there was no clear indication of cumulative impacts
21 moving from pristine headwaters to the lower reaches of the Matāura River biomarkers of
22 xenobiotic metabolization were induced in *A. dieffenbachia* and there was evidence of chemical
23 contamination in sediments and tissues.

24 **Keywords:** New Zealand; biomarkers; environmental health assessment; *Anguilla dieffenbachii*
25

26 1. Introduction

27

28 River systems span a broad range of ecosystems and can transport chemicals and particles long
29 distances before being deposited in the ocean. As headwaters progress through the landscape, they
30 can potentially accumulate contaminants, in part due to increasing anthropogenic activity and
31 pressure. This can impact multiple ecosystems with increasing severity as contaminants accumulate
32 along the river, particularly in zones of reduced energy that typically predominate in lower reaches.
33 To develop a comprehensive assessment, a 'whole catchment approach' of ecosystem health can
34 provide valuable information on the nature and severity of various aspects of river health as well as
35 identify potentially impacted sites. An assessment of ecosystem health at multiple points along a
36 river system can provide evidence of whether there are potential adverse effects on the environment
37 due to anthropogenic activity, and insight into their potential sources. In this sense the Matāura River,
38 which lies in the Murihiku (Southland) region of the South Island of New Zealand, is an ideal case
39 study. It is New Zealand's sixth longest river with a length of 240 km and encompasses the Southland
40 region's second largest catchment (5,360 km²) in both area and water flow [1,2].
41 The Matāura River originates near Eyre Peak in the Taka Ra Haka Conservation Park (Eyre
42 Mountains) located to the west of the Kingston arm of Lake Wakatipu in Northern Southland. The
43 headwaters are fed predominantly by rainfall, with a significant spring snowmelt contribution to the
44 springtime flow. The Waikaia River is the largest of the Matāura's numerous tributaries, comprising
45 just over half of the catchment area (1360 km²) at their confluence [1,2]. From Gore, the Matāura River

46   flows south through a long fertile river plain where it becomes more entrenched and its gradient
47   gradually decreases before entering the Toetoes Estuary, a small lagoon-type estuary, prior to
48   discharging into the sea at Fortrose at the bottom of the South Island [2]. The Matāura River and its
49   tributaries have long been valued worldwide by sports fisherman for the quality of the brown trout
50   fishery it maintains.
51   Agricultural land use predominates in the surrounding catchments and is likely to be a major
52   contribution of non-point source inputs into the river. The towns along the river are sites of significant
53   agriculturally based industries including meat works, dairy factories and wood processing mills, all
54   discharging wastewater into the river. These towns provide point source entry of stormwater and
55   treated municipal wastewater. The Matāura River has significant historical and spiritual relevance
56   for local Māori tribes, and there is growing public concern over the decline of water quality and the
57   health of the river.
58   In New Zealand there is a paucity of information regarding the effects of chemical contaminants
59   on native species. The Australasian component of the Society of Environmental Toxicology &
60   Chemistry (SETAC) Global Horizon Scanning Project identified the question: "What are the
61   combined effects of very low levels of multiple contaminants?" as a priority research question for the
62   region [3]. This highlights the need to investigate the health status of riverine systems under pressure
63   from multiple stressors by the application of relevant test species. Tuna, the Māori name for
64   freshwater eels in New Zealand, have been previously used in environmental studies due to their
65   facultative catadromous nature and long life cycles facilitating interactions with contaminated
66   sediment and prey for extended periods of time [4,5] In addition to these features, their naturally
67   high lipid content can potentially lead to the rapid and extensive accumulation of hydrophobic
68   organic contaminants [6] resulting in eels being recognized as useful bio-indicators of the effects of
69   localized aquatic pollution [7]. Longfin eels (*Anguilla dieffenbachii*) are the only freshwater eel endemic
70   to New Zealand and are widely distributed throughout the country's lakes and rivers, making them
71   an ideal species for assessing ecotoxicological hazards in aquatic ecosystems. *A. dieffenbachii* is a
72   treasured (taonga) and an iconic food species of current and historical importance for the wellbeing
73   of Māori, the indigenous people of New Zealand.
74   The aim of this study was to investigate the influence of anthropogenic pressures at seven
75   sites along the Matāura River system, particularly the potential effects of non-point-source and
76   diffuse inputs using *A. dieffenbachii* The study had two major components: i) analysis of sediments
77   and biological tissue for the presence of selected priority chemical contaminants and ii) an assessment
78   of sublethal effects towards *A. dieffenbachii*. To achieve this health condition indices and a series of
79   enzymatic exposure biomarkers were measured to assess the potential impact of combined
80   anthropogenic stressors within caged and resident eels in the Matāura River, New Zealand.
81

2. Materials and Methods

2.1. Study sites

85   Seven sites along the length of the Matāura River catchment were selected for investigation
86   following consultation with local authorities and environmental regulators (Figure 1). Key factors
87   considered in the selection of these sites included their cultural relevance to Maori, recreational value
88   (hikers, swimmers and trout fisherman, aquatic and terrestrial biodiversity, commercial value for eel
89   and trout fishing, and urban and industrial discharges.

91   The first site located in Piano Flat on the Waikaia River was close to the river's headwaters,
92   surrounded by native bush, and was the most upstream site in this study. Apart from some historical
93   gold prospection little anthropogenic activity has occurred in these headwaters. Five other sites were
94   located downstream along the length of the Matāura River. The second site was located within the
95   town boundaries of Gore and downstream from the sewage treatment plant and stormwater
96   discharges to address concerns raised by the public about unregulated overflow discharges. The

97 third site near the township of Matāura was located directly downstream of the discharge of treated
98 effluent from a meat works industry that processes lamb, sheep and cattle. It is significant that the
99 stretch of the Matāura River intersecting the township of Matāura was the first inland river site in
100 New Zealand to gain the status of mātaitai reserve (managed by indigenous locals) [8]. The fourth
101 site at Tuturau, was located downstream from the discharge of the Matāura sewage treatment plant.
102 Wyndham, the fifth site, is the location where Southland District Council discharges stormwater and
103 septic tank effluents into the Matāura River. Gorge Road, the sixth site was located in the lower
104 reaches of the Matāura River where it is surrounded by agricultural land use but is also a deposition
105 zone for sediment, making it an ideal to assess cumulative impacts from activities upstream. It is also
106 the furthest point downstream in the Matāura River outside of tidal influence. The last site was
107 located at Niagara in the adjacent Waikawa River away from the Matāura River catchment. The site
108 has cultural significance and a history of being a location of indigenous temporary settlements. It is
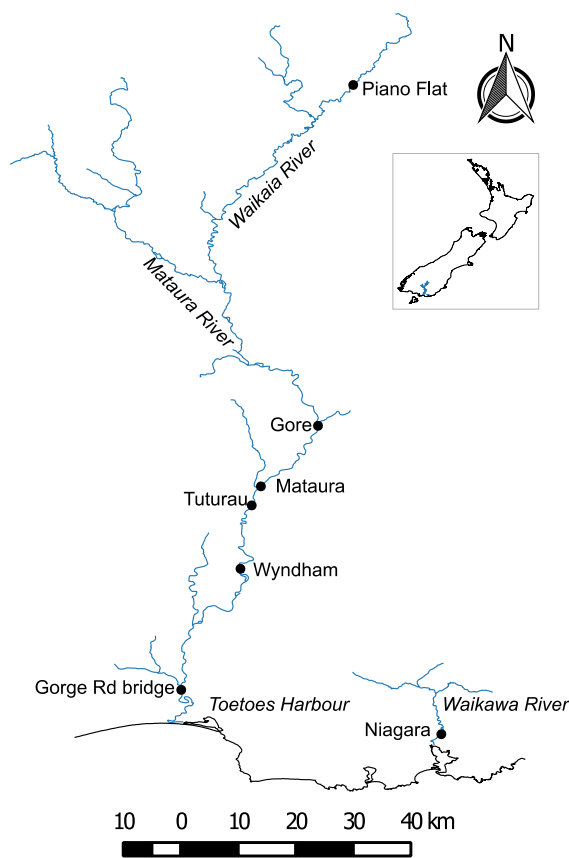109 surrounded by farmland and native bush.
110

112   **Figure 1.** Study sites of the caged and wild-caught eels on the Mataura and Waikawa Rivers.

113 *2.2. Field Setting*

114 2.2.1. Eels Caging

115   A total of 80 non-migratory longfin eels of mixed sex, age and length (46–61 cm) were captured
116 from Wyndham under the customary permits SI 01567 and SI 06305.   Eels were maintained in flow
117 through tanks at 10–12 °C in an eel fishery indoor tank facility for 3–4 weeks prior to caging to allow
118 depuration of potential chemical contamination. During the first week eels were treated with sea salt
119 (2.5 g/L) under static conditions for a period of 24 h to remove any traces of the rock snot algae
120 (*Didymosphenia geminata*) and fungal (white spot) or bacterial (fin rot) infections. No mortality or
121 disease was observed in the eels during this period. At the end of the decontamination period, five
122 eels were randomly allocated to one of 16 knotless nylon eel catch bags interspersed with rectangular
123 aluminium hoops (approximately 120×45×45 cm) [9-11]. Each bag was placed in a stainless-steel cage

124 (120×50×50 cm) for protection. Two cages were randomly allocated to each of the study sites.
125 Deployment of the cages was carried out over a two-day period and began with the upstream sites
126 followed by those further downstream.
127   Where feasible, cages were submerged at locations close to the riverbank where they could
128 benefit from natural shading and protection from strong currents. The cages were secured to a
129 stainless-steel fence post driven into the bank by a chain and secured with locks. Lengths of PVC
130 piping were inserted into the nylon nets to provide shelter habitat for the eels during daylight hours.
131   Eels were deployed in the field in late spring (November 2007) and late summer (March 2008)
132 for a period of 21 days. Previous research demonstrated this to be a sufficient time for triggering a
133 range of physiological responses resulting from acute (short term) or sub-chronic (medium term)
134 exposures to environmental pollutants [12]. The eel cages were physically and visually checked
135 weekly for damage or fouling due to river debris, and for evidence of abnormal fish behaviour.
136

137 2.2.2. Capture of Resident Eels

138 Eels naturally residing at each of the sampling sites–resident eels–were caught to be compared with
139 the caged eels. Fyke nets were set at each site a week prior to retrieving the cages and placed within
140 200 m either up or down stream of each cage site. Resident eels were captured using raw eggs as an
141 attractant placed inside plastic containers and secured inside 25 mm stretch mesh fyke nets. These
142 resident eels were caught and harvested using customary permits SI 01567 and SI 06305.
143

144 *2.3. Sampling*

145 2.3.1. Sediment Sampling

146   Samples were collected using a stainless-steel auger pushed to a depth of 10 cm, working
147 downstream to upstream from the cage sites. Approximately 500 g of sediment was collected as a
148 composite of subsamples collected over an area of about 1 m². Sediment was subsequently sub-
149 sampled into pre-cleaned 250 mL glass jars (100 g for metal analysis and 100 g for semi-volatile
150 organic compounds) and stored in the dark at 4 °C for transportation to the lab. Samples were then
151 frozen at -20 °C until analysis [13].

152 2.3.2. Tissue Sampling

153   After 21 days of exposure, the caged eels were retrieved from the field sites. Rain events in
154 summer produced high river flows that damaged some of the cages allowing the escape of some eels
155 and potentially adding additional stress to the fish. The eels were anesthetized using clove oil [14] to
156 minimize effects on blood chemistry [15], before being measured (weight and length) and killed by
157 spinal severance. Otoliths were collected to determine the age of the fish. The sex was not recorded
158 because non-migratory eels remain in a state of sexual immaturity [16,17] and reproductive organs
159 are regressed making it difficult to distinguish testis from ovary tissue.   A sample of fillet (60 g) from
160 the true right of the animal was removed, split into two and frozen at -20 °C for further chemical
161 analysis. Liver and gill samples collected for biomarker analysis were snap frozen and kept in liquid
162 nitrogen until use.

163 *2.4. Chemical Analysis*

164 2.4.1. Sediment Analysis

165   Chemical analysis of total recoverable metals in sediments was carried out by R J Hill
166 Laboratories Ltd (Hamilton, New Zealand) [18].
167   For organic contaminant analysis, sediments were thoroughly mixed and stored at -18 °C until
168 further analysis. A 10 g sample was dried at 50 °C for 24 h to determine sediment dry weight.

169 Organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in the sediment were
170 analysed using a single extraction and a three-stage clean-up method. A 40 g aliquot of sediment was
171 spiked with 50 ng of aldrin and α-BHC as internal recovery standards. The sediment was extracted
172 by sonication and shaking with a mixture of hexane/acetone solvent. The extract was filtered and
173 partitioned with water and a subsample of the upper hexane layer removed and concentrated. The
174 resulting crude extract was subjected to gel permeation chromatography (GPC) to remove lipids and
175 other high molecular weight co-extracted substances and isolate the fraction containing the OCs and
176 PCBs. This isolated fraction was concentrated and further purified and fractionated using Florisil®
177 column chromatography to produce two fractions: i) fraction A, containing all the PCBs, several OCs
178 such as DDT metabolites (p.p. DDE) and the internal recovery standard aldrin and ii) fraction B
179 containing the chlordanes, the remainder of the OCs including dieldrin and the internal recovery
180 standard α BHC.

181 Quantitative analysis was performed using high resolution gas chromatography with ion trap
182 MS/MS detection using a Varian Star 3400CX Gas Chromatograph fitted with a Varian SPI
183 temperature programmable injector and Varian Saturn 2000 ion-trap mass spectrometer. Retention
184 times and spectral matches were used to identify compounds. Calibration standard solutions were
185 prepared from pure analytical standards. Chlordanes were quantitated using standards of the cis-
186 and trans-chlordane isomers supplied by the United States Environmental Protection Agency (US-
187 EPA). A multiplication factor of 2.33 was applied to the sum of the isomers to derive equivalent
188 technical chlordane levels.

189 Polycyclic aromatic hydrocarbons (PAHs) and chlorophenols were analysed by removing
190 aliquots of the same crude hexane solvent extract. The aliquot of hexane for PAHs analysis was
191 concentrated and subjected to GPC followed by Alumina column chromatography clean-up. PAHs
192 in the purified sample extracts were analysed by high performance liquid chromatography (HPLC)
193 with fluorescence detection and quantitated against a mixed PAH standard (US-EPA). The sub-
194 sample of the raw hexane extract for chlorophenol analysis was subjected to acid/base partitioning
195 before the chlorophenols were acetylated with acetic anhydride. Quantitative analysis was carried
196 out by high resolution gas chromatography with ion trap MS/MS detection using a Varian Star
197 3400CX Gas Chromatograph fitted with a Varian SPI temperature programmable injector and Varian
198 Saturn 2000 ion-trap mass spectrometer. Retention times and spectral matches were used to identify
199 compounds. Calibration standard solutions were prepared from pure analytical standards.

200 The detection limits for OCs, PCBs, PAHs and chlorophenols 0.05, 0.05, 0.3 and 0.5 ng/g dw
201 respectively. Limits of quantitation (LOQ) were set at three times the detection limit (Table A1).
202 Results less than the LOQ are reported but the levels are in the region of uncertainty. Where
203 compounds were not detected, or below the limit, 'N.D' was used. Recovery experiments were
204 carried out for most compounds by spiking sub-samples of the sediment at two levels (0.3 and 3.2
205 ng/g dw) of each component and analysed through the method.

206 2.4.2. Tissue Analysis

207 Chemical analyses were carried out on composite samples of eel fillets prepared from animals
208 contained in the cage from each sample site together with the harvested resident eels. The first frozen
209 portion of fillet was analysed for total trace metals (mercury, lead, cadmium, copper, zinc, arsenic
210 and chromium) by Hill Laboratories (Hamilton, New Zealand) [18]. The second portion of the sample
211 was sent to HortResearch (Hamilton, New Zealand) and analysed for organic contaminants
212 (polychlorinated bi-phenyls, DDT, polycyclic aromatic hydrocarbons, organochlorine pesticides)
213 using the methods described above. Chemical analyses of PCBs, organochlorines and PAHs were not
214 carried out in eel sampled during summer since no chemical was detected in sediments.

215 *2.5. Biological Analysis*

216 2.5.1. Health Condition Indices and Age Determination

217 Health between individuals was assessed using the hepato-somatic index (HSI) [19] and the
218 Fulton (K) index, [20]. Sagittal otoliths were prepared using the crack-and-burn method [21]. Otolith
219 fragments were mounted in silicone rubber sealant on microscope slides and observed under a
220 binocular microscope using reflected light. Age was estimated by counting the number of black
221 winter rings [22].

222 2.5.2. Biomarker Analysis

223 Frozen tissues (gills and liver) of each animal were weighted and homogenized in phosphate
224 buffer (pH 7.4, 100 mM) (1:3 w:v) with a PYREX® Potter-Elvehjem homogenizer. Homogenates were
225 centrifuged for 30 minutes (4 °C) at 9000 g [23]. The sub-mitochondrial fractions (S9) were aliquoted
226 and stored at -80 °C before use for biomarkers measurement. Biomarkers were measured both in liver
227 and gills. Four biomarkers of exposure were measured: the ethoxyresorufin-O-deethylase (EROD)
228 [24], following the modified protocol for the microplate reader [25], glutathione-s-transferase (GST)
229 [26] and catalase [27] activities and the concentration of endogenous malondialdehyde expressing the
230 level of lipid peroxidation assessed with through the measurement of thiobarbituric acid reactive
231 species (TBARs) [28]. All data were expressed in relation to protein concentration determined using
232 the fluorescamine method [29]. Results were analysed by comparing biomarker responses between
233 resident and caged eels as well as differences between test sites.

234 *2.6. Statistical Analyses*

235 Statistical analyses were carried out using R [30]. A multiple comparison of biomarker values
236 between sites was carried out using a nonparametric Kruskal-Wallis test followed Dunn's test
237 adjusted for multiple comparing using a Bonferroni adjustment ('dunn.test' package [31]) with a
238 statistical difference determined at $P<0.05$.

239 **3. Results**

240 On both field sampling trips the Murihiku region received significant rainfall prior to the
241 recovery of the caged eels. While care was taken to locate the cages in locations away from main
242 current flows the contribution from these rain events produced high river flows which resulted in
243 some cage damage where animals escaped and could not be recovered (Table 1).

244 **Table 1.** Number of eels in cage recovered and number of resident eels caught.

		Piano Flat	Gore	Matāura	Tuturau	Wyndham	Gorge Rd	Niagara
Spring	Cage 1	5	0	2	5	5	5	5
	Cage 2	5	5	5	5	3	0	5
	Resident eels	3	9	10	10	4	0	10
Summer	Cage 1	2	5	4	5	4	0	5
	Cage 2	5	5	5	2	5	3	5
	Resident eels	5	10	10	10	5	4	4

245

246 *3.1. Chemical Analyses*

247 3.1.1. Sediments

248 The concentration of metals in sediment sampled in spring did not exhibit a consistent trend in
249 concentration between the different sampling sites, but in general the concentration was higher in
250 sediment downstream at Wyndham compared to that in the headwaters at Piano Flat (Table 2). The
251 concentration of Cu and Pb in sediment from Wyndham and Niagara sites was higher than the other
252 sites and Hg was only detected in sediment from the Wyndham site. In summer, the concentrations

253 of Pb and Zn generally increased in sediment at all the sampled sites compared to that in spring.
254 Notably, Hg was detected in sediment at all sites sampled in summer.
255

256   **Table 2.** Concentrations of trace metals in sediment (in mg/kg dry weight) in spring and summer
257   samples

		Piano Flat	Gore	Matāura	Tuturau	Wyndham	Gorge Rd	Niagara
Spring	Cd	0.053	0.038	0.043	0.039	0.069	N/A[A]	0.04
	Cu	6.7	7.4	6.2	7.8	12	N/A	15
	Pb	6.3	5.9	5.2	6.4	9.1	N/A	9.7
	Hg	N.D[B]	N.D	0.011	N.D	0.028	N/A	N.D
	Zn	46	32	30	31	47	N/A	53
Summer	Cd	0.04	0.03	0.04	0.07	0.06	0.38	0.13
	Cu	12.4	12	8.5	5.8	8.9	0.46	17.9
	Pb	9.43	5.09	9.16	9.24	9.3	0.76	15.7
	Hg	0.07	0.03	0.06	0.04	0.05	0.13	0.13
	Zn	44.9	25.2	45.1	105	76.8	226	64.4

258   [A]N/A: not analysed, [B]N.D: not detected (below limit of detection).
259
260   Recoveries of the α BHC and aldrin surrogate recovery standards added to each sample were
261   consistent with mean recoveries of 88±13% and 62±14% respectively at the 95% confidence level,
262   and CVs within 21% (Table A1). The results are not corrected for recovery.

263   Resides of PCBs were not detected in any of the sediment samples and are not discussed
264   further. In comparison residues of DDT were detected in all sediment samples and together
265   with other organochlorine pesticides their concentration in sediment increased going
266   downstream (Table 3), with the highest concentrations at Gorge Rd. Residues of
267   chlorophenols were only detected in sediment at the Tuturau site and organochlorine
268   pesticides in sediment from the Matāura and Tuturau sampling sites.. Interestingly the
269   sediment from the Niagara sampling site on the Waikawa River had a similar concentration
270   of DDT residues to sediment from the Wyndham site on the Matāura River.

271   **Table 3.** Total concentration of chlorophenol, DDT and other organochlorines residues in sediment
272   samples (μg/kg dry weight) in spring.

	Piano Flat	Gore	Matāura	Tuturau	Wyndham	Gorge Rd	Niagara
ΣChlorophenols	N.D[A]	N.D	N.D	1.6	N.D	6.2	N/A[B]
Total DDT[C]	0.61	0.46	0.27	0.51	1.02	9.64	1.09
Other organochlorines	N.D	N.D	0.05	0.47	0.65	3.88	N.D

273   [A]N.D: not detected (below limit of detection). [B]N/A: not analysed. [C]Sum of six individual isomers of
274   DDT, DDE and DDD
275

276 The concentration of PAHs (Table 4) were higher in sediment sampled in Spring than in Summer.
277 The concentration of PAHs in sediment from Gorge Road in the lower Mataura River was one to two
278 order of magnitude higher in concentration than sediment from the other sites in Spring, and three
279 orders of magnitude greater than sediment sampled from the same site in Summer.

280   **Table 4.** Sum[A] of polycyclic aromatic hydrocarbons (PAHs) residues in sediments in Spring and
281   Summer (μg/kg dry weight)

	Piano Flat	Gore	Matāura	Tuturau	Wyndham	Gorge Rd	Niagara
Spring	119	145	46	88	124	5939	79
Summer	N.D[B]	N.D	N.D	19	26	7	N.D
TOC[C]	0.35	0.19	0.30	0.19	1.6	N/A	0.94

282   [A] Sum of USEPA 16 priority PAHs. [B]N.D.: not detected (below limit of detection). [C]TOC: total organic
283   content (g/100 g dw)

284

285   3.1.2 Eels

286

287   Copper (Cu) and zinc (Zn) were the metals most frequently detected in the flesh of eel and at
288   higher concentrations than Cadmium (Cd), lead (Pb) and mercury (Hg) (**Table 5.** Residue of trace
289   metals in caged (average ± standard deviation) and resident eels' fillet (mg/kg dry weight) in Spring
290   and Summer.). Lead was found in caged eels' fillets in Spring but was not detected in Summer.

291   **Table 5.** Residue of trace metals in caged (average ± standard deviation) and resident eels' fillet
292   (mg/kg dry weight) in Spring and Summer.

			Piano Flat	Gore	Matāura	Tuturau	Wyndham	Gorge Rd	Niagara
Spring	Caged	Cd	0.07±0.005	0.07	0.07±0.02	0.08±0.01	0.06±0	0.094	0.09
		Cu	8.9±1.6	9.5	10.6±1.9	9.9±0.1	8.9±0	12	7.1±5.5
		Pb	1.0±0.1	1.2	1.3±0.1	1.2±0	1.05±0.07	1.5	0.8±0.9
		Hg	1.5±0.3	1.7	2.5±1.4	1.8±0.2	1.3±0.1	2.7	1.4±0.1
		Zn	125±7	110	125±21	155±7	110±0	190	115±7
	Resident	Cd	N.D	N.D	0.11	N.D	N.D	N/A	0.05
		Cu	2.8	3.9	4.5	4.6	5	N/A	6.7
		Pb	N.D	N.D	0.25	N.D	N.D	N/A	N.D
		Hg	1.4	1.6	3.1	2.1	2.5	N/A	8.6
		Zn	120	120	200	200	140	N/A	230
Summer	Caged	Cd	N.D	0.05	0.04±0	0.04	N.D	N.D	0.09±0.08
		Cu	6.6±6.2*	2.4±0.3	2.8±0.4	2.6±0.5	2.2±0.1	2.2±0.1	2.9±0.4
		Pb	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Hg	2.6±0.21	2.8±0.1	2.7±0.1	1.6±0.3	2.3±0.4	1.9±0.4	2.1±0.9
		Zn	225±78*	180±14	205±21	200±57	130±0	140±0	170±0
	Resident	Cd	0.05	0.03	0.05	N.D	N.D	N.D	N.D
		Cu	7.5	6.7	8.6	9.6	5	12	15
		Pb	N.D	N.D	0.31	N.D	0.15	1.9	0.38
		Hg	2.9	1.9	3.6	2.4	1.6	1.8	9.8
		Zn	210	140	220	230	150	160	260

293   N.D.: not detected (below limit of detection). N/A not analysed. *indicates one of the values exceeds test limits
294

295   **Table 6.** Concentration of DDT pesticide residues (sum of DDT congeners) in eels' fillet (µg/kg dry
296   weight)

	Piano Flat	Gore	Matāura	Tuturau	Wyndham	Gorge Rd	Niagara
Spring	228	393	337	352	439	379	354
Summer	258	243	220	288	324	325	174

297

298 No residues of PCBs, chlorophenols or PAHs were detected above the reporting limit in the
299 tissue of eel at any of the sampling sites during the spring or summer sampling. In comparison,
300 residues of the organochlorine pesticide DDT were measured in all of the analysed eel samples from
301 both sampling times (Table 6). This reflects continued exposure of eels in Southland and other regions
302 of New Zealand to historical residues of DDT that were applied to pasture to control grass grub and
303 other grass and crop eating insect pests.

304 *3.2. Biological Analysis*

305 3.2.1. Age Determination and Health Condition Indices

306 The age, length and weight range of caged eels were similar within and between the two
307 sampling times (Table 7), so were the residents together. Bigger resident eels were captured upstream
308 of Matāura. Hepatosomatic indices were similar within and between the two sampling times except
309 for the resident eels at Piano Flat in Summer statistically different ($P<0.05$) from the others.

310

			Piano Flat	Gore	Matāura	Tuturau	Wyndham	Gorge Rd	Niagara
Spring	Caged	Age (years)	20 ± 3	22 ± 3	19 ± 2	20 ± 2	25 ± 5	21 ± 3	19 ± 4
		Length (cm)	52 ± 3	52 ± 2	53 ± 5	52 ± 5	53 ± 3	49 ± 1	53 ± 3
		Weight (g)	328 ± 48	345 ± 52	355 ± 138	361 ± 138	342 ± 53	257 ± 22	367 ± 78
		HSI	1.5 ± 0.3	1.5 ± 0.1	1.5 ± 0.2	1.5 ± 0.3	1.4 ± 0.3	1.8 ± 0.3	1.4 ± 0.3
	Resident	Age (years)	22 ± 1	21 ± 5	20 ± 4	17 ± 3	20 ± 6	n/a	24 ± 4
		Length (cm)	77 ± 21	60 ± 9	60 ± 5	52 ± 4	52 ± 8	n/a	50 ± 7
		Weight (g)	1529 ± 1323	605 ± 385	590 ± 204	378 ± 137	374 ± 231	n/a	313 ± 180
		HSI	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.4	n/a	1.5 ± 0.4
Summer	Caged	Age (years)	22 ± 6	21 ± 2	21 ± 3	20 ± 4	24 ± 3	21 ± 3	18 ± 4
		Length (cm)	57 ± 9	49 ± 3	54 ± 8	57 ± 11	52 ± 5	49 ± 4	52 ± 4
		Weight (g)	427 ± 301	230 ± 38	300 ± 130	332 ± 323	259 ± 83	213 ± 55	271 ± 72
		HSI	1.6 ± 0.4	1.5 ± 0.2	1.5 ± 0.4	1.4 ± 0.4	1.8 ± 0.7	1.8 ± 0.2	1.5 ± 0.2
	Resident	Age (years)	26 ± 3	18 ± 4	17 ± 3	19 ± 4	18 ± 3	17 (n=1)	18 (n=1)
		Length (cm)	73 ± 12	56 ± 5	53 ± 7	126 ± 199	64 ± 16	48 ± 3	52 ± 5
		Weight (g)	1120 ± 645	440 ± 121	339 ± 153	630 ± 170	268 ± 67	268 ± 67	326 ± 91
		HSI	1.8 ± 1.2	1.2 ± 0.2	1.2 ± 0.4	1.5 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	1.2 ± 0.3

311 **Table 7.** Average (± standard deviation) of age, length, weight and hepatosomatic (HSI) index for
312 caged and resident eels in spring and summer. (n/a: animals not available)

313 3.2.2. Biomarkers

314 GST and TBARs measured in eels' livers in spring did not show significant differences between
315 the seven sampling sites. Measurement of the EROD in the livers of caged eels in Wyndham and
316 Niagara was significantly higher when compared to that in eel from the most upstream site (Piano
317 Flat) (Figure 2B). In comparison, EROD activity in the liver of eels exhibited EROD activity in the
318 livers of resident eels was statistically different between the most upstream site (Piano Flat) and the
319 first township downstream at Gore. The EROD activity in eels' livers from Gore was statistically
320 higher than that in eel liver from Tuturau about 20 km downstream (Figure 2B). GST activity in the
321 gills from caged eels in Piano Flat (Figure 2D) was significantly higher than values measured in Gore,

322   Tuturau, and Gorge Road (Figure 2D). GST, catalase and TBARs measured in gills of resident eels
323   showed a significant difference between Gore and the further downstream sites of Matāura, Tuturau
324   and Niagara (Figure 2D, 2E and 2F).
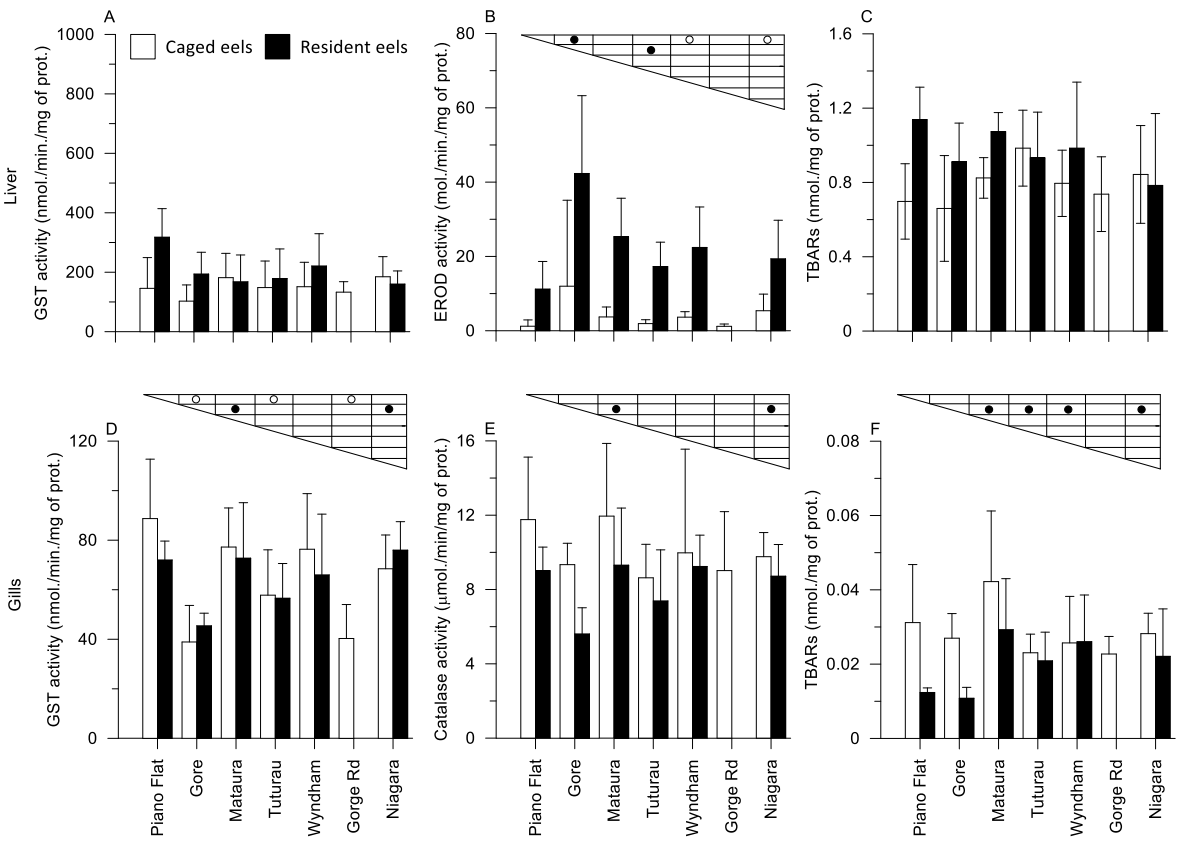
325

326
327   **Figure 2.** Biomarkers measured in caged and resident eels' livers and gills in spring at the seven
328   sites. (statistical significance (P<0.05) between sites indicated for caged ○ and resident ● eels)

329

330   In Summer, a statistical difference was observed in EROD activity in the liver of caged eels from
331   the upper catchment site at Piano Flat and the most downstream site at Gorge Road (Figure 3B).
332   EROD activity in resident eels' livers at the Matāura site was significantly lower than that at the Gore
333   (about 15 km upstream), and Wyndham (about 20 km downstream) sites (Figure 3B).
334   In spring and summer, the EROD activity is always higher in resident eels than in caged eels. The
335   EROD activity along the sampling sites between the two seasons seem to follow a similar pattern
336   with the lowest activity observed in eels from Piano Flat, followed by an increase in Gore and a
337   progressive decrease going downstream.
338   The TBARs in liver of resident eels' from the two most downstream sites on the Mataura river
339   (Wyndham and Gorge Rd) were significantly lower than those from the Tuturau site (about 15 km
340   upstream) (Figure 3C). With respect to gill biomarkers, only catalase from resident eels at the Gore
341   site were significantly different from those at the most upstream site (Piano Flat) (Figure 3E).
342   Qualitatively, GST activity in the liver and gills (slightly) of eel were elevated in summer
343   compared those in spring. In contrast, EROD, catalase and TBARS were notably lower in summer
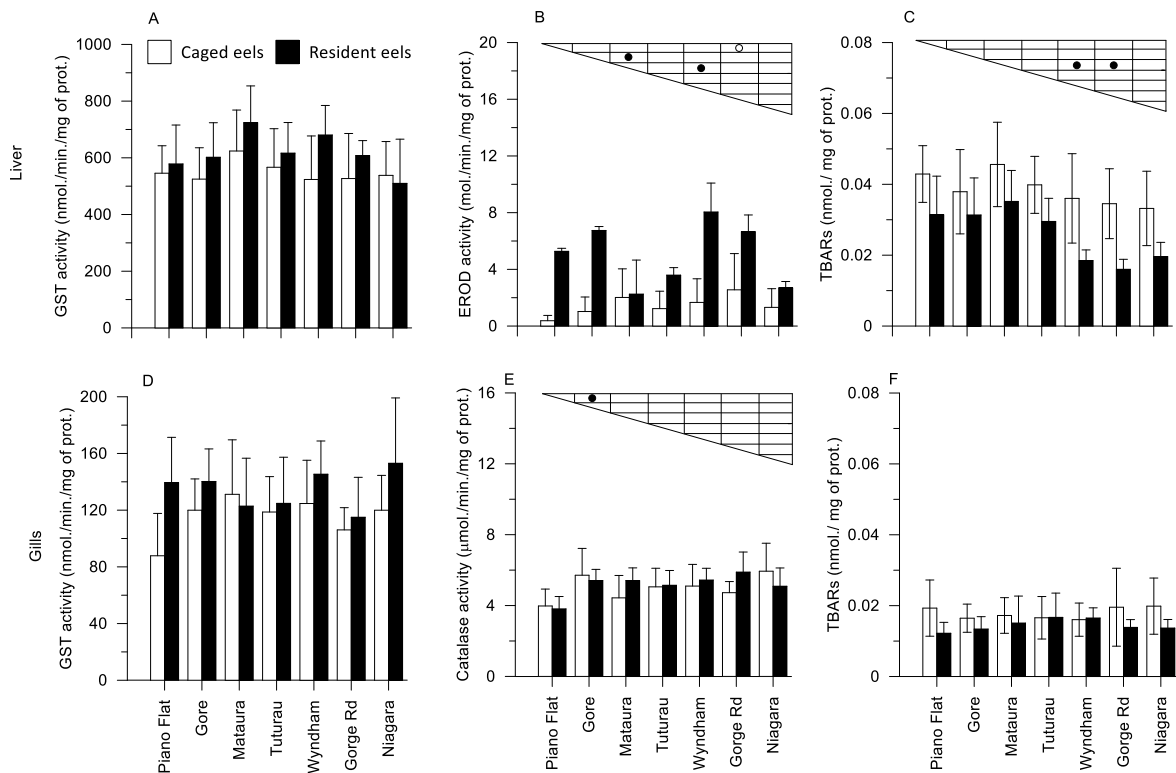344   compared to spring.
345

Figure 3. Biomarkers measured in caged and resident eels' livers and gills in summer at the seven sites. (statistical significance (P<0.05) between sites indicated for caged ○ and resident ● eels)

4. Discussion

This study was a first health assessment of the Matāura River that integrated physicochemical and biological-effect data to assess the potential adverse effects of anthropogenic stressors derived from land use activities in that catchment. The concentration of recognized anthropogenic contaminants (metals, organochlorines, PAHs) in the analysed sediments generally fell below the ANZECC sediment default guideline values [32] with the exception of the Gorge Road site. Sediment from the Gorge Road site was the only one in which contaminants exceeded the ANZECC sediment guideline default values for zinc in Summer (200 mg/kg of dry sediment), and for Total DDT in Spring (5 μg/kg of dry sediment). While the concentration of PAHs in Gorge road sediment sampled in spring did not exceed the ANZECC sediment default guideline value it was an order of magnitude higher than sediments at any other site. The absence of residues of PCBs, together with the infrequent detection of residues of chlorophenols and organochlorine pesticides other than DDT in sediment from the Matāura River, is explained by the absence of their production and use within the catchment. In comparison, the presence of DDT residues in all the analysed sediment samples reflects the previous widespread historical use of DDT on pasture within the Southland region and the continuing leakage of soil residues by erosion and their subsequent migration into riverine catchments in Southland.

When comparing the metal concentrations in the sediment samples, they varied between the spring and summer seasons, most notably for zinc at the Tuturau and Wyndham sites. While the concentration of Zn remained under guideline values, it suggests a seasonal effect with respect to potential anthropogenic inputs, which could pose a risk to these ecosystems. The concentrations of persistent organic contaminants in sediment at each site were consistent with those for metals with their concentrations falling below ANZECC guidelines. Regardless, the elevated concentration of PAHs in sediment from the Gorge Road site is of concern as there is strong evidence demonstrating

374 the PAHs in sediments are a main contributor to toxicity [33]. Overall, the risk posed by these
375 sediment-borne contaminants to aquatic organisms is low based on their respective guideline values.
376   Variations of chemicals concentration in sediments between spring and summer can be
377 explained by the physical characteristics of the river. The riverbed of the Matāura River contains
378 schistose sediment and weakly weathered gravel [34]. The region often experiences intense rain
379 events resulting in high river flows and flash floods that wash out and strip deposited sediment and
380 accumulated contaminants from the hard river bedrock further downstream and into the Mataura
381 River estuary at Fortrose.
382   However, measuring endpoints at differing levels of biological organisation in a sentinel animal
383 species (biological receptors) integrates realistic exposure to multiple, or mixtures of, contaminants.
384   All the tissue trace metal concentrations measured in eel were lower than those reported in other
385 overseas eel species [4,35]. In comparison to previous overseas studies no residues of PCBs,
386 chlorophenols or PAHs were detected in the tissues of eel in this study [35,36]. while DDT residues
387 were measured in all the eel tissue samples at comparable concentrations to previous measurements.
388 The results are particularly interesting as the elevated concentration of PAHs and DDT in sediment
389 from the Gorge Road site suggests *A. dieffenbachii* would similarly accumulate elevated residues of
390 PAHs and DDTs. This is not supported by the tissue data, which suggest that contemporary
391 concentrations of these contaminants are not high enough within the sediment to lead to elevated
392 biological accumulation, and/or, *A. dieffenbachii* is able to detoxify and depurate these contaminants.
393   This interpretation is supported by the biological results. Previous biomarker studies in the
394 European eel *Anguilla anguilla* [37] have demonstrated limited effects on GST and catalase response
395 following exposure to PAHs. However, EROD activity is a biomarker that generally responds to
396 PAHs exposures in European eels and other fish species [38,39]. Sublethal effects measured through
397 enzymatic biomarkers in *A. dieffenbachii* showed no clear evidence adverse effects. of anthropogenic
398 effects and natural variations [40,41]. Using contaminant tissue residues to assess   sublethal impacts
399 of pollution is ineffective   as effects will only emerge when fat is metabolized [42]. The accumulation
400 of residues is likely linked to historical rather than contemporary environmental conditions.   Further
401 investigations assessing the potential long-term implications of anthropogenic contaminants within
402 the Matāura River catchment may require the use of alternative bioindicator species, and/or,
403 biomarkers of exposure.
404

5. Conclusion

406   This study using *A. dieffenbachii* suggests that despite an increase in anthropogenic activity and
407 inputs down the length of the river there is limited evidence of increasing negative effects and
408 therefore negligible adverse outcomes occurring in the Matāura River system as it progresses towards
409 the sea . Despite this lack of evidence, future studies may be necessary to fully characterize the health
410 status of the Matāura River using alternative bioindicator species across trophic levels. For example,
411 alternative fish species like *Gobiomorphus cotidianus* (common bully) which are in more immediate
412 contact with prey habituating sediment may have a greater exposure to, and exhibit a heightened
413 sensitivity to the low levels of contamination that were present in the sediment of the Matāura River.

414

415 **Author Contributions:** Conceptualization, J.A, L.T. and G.N.; methodology, J.A., G.N. and L.T.; formal analysis,
416 O.C.; investigation, J.A., O.C, G.K, G.N.; resources, J.A.; data curation, G.N and O.C.; writing—original draft
417 preparation, O.C , G.N. and A.B.; writing—review and editing, A.B., J.A, L.T, G.N; visualization, O.C.;
418 supervision, J.A and L.T; project administration, J.A.; funding acquisition, J.A and L.T. All authors have read
419 and agreed to the published version of the manuscript.

434   Appendix A

435

436   Table A1. Analytical method detection limits (mg/kg) for metals and organic compounds

	Sediment	Biota
Total recoverable metals		
Cadmium	0.010	0.020
Chromium	0.20	N/A
Copper	0.20	0.050
Lead	0.040	0.010
Mercury	0.010	0.010
Zinc	0.40	0.50
Organic compounds		
Organochlorine pesticides	0.001	0.001
Equivalent technical chlordane	0.002	0.003
Chlorinated phenols	0.001	N/A
Polychlorinated biphenyls	0.001	0.001
Sum of 35 PCB congeners	0.04	0.04
Polycyclic aromatic hydrocarbons	0.001	0.001
Sum of 16 PAHs	0.02	0.02

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References

1. Witherow, W.D.; Scott, D. *The Mataura trout fishery*. Acclimatisation Societies of Southland and Otago, New Zealand: 1984; p 71.

2. Ryder, G. *Matāura catchment: water quality review.*; the Southland Regional Council: 1995; p 250.

3. Gaw, S.; Harford, A.; Pettigrove, V.; Sevicke-Jones, G.; Manning, T.; Ataria, J.; Cresswell, T.; Dafforn, K.A.; Leusch, F.D.; Moggridge, B., *et al.* Towards sustainable environmental quality: priority research questions for the australasian region of Oceania. *Integr. Environ. Assess. Manage.* **2019**, *15*, 917-935.

4. Maes, G.E.; Raeymaekers, J.A.M.; Pampoulie, C.; Seynaeve, A.; Goemans, G.; Belpaire, C.; Volckaert, F.A.M. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: Relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquat. Toxicol.* **2005**, *73*, 99-114.

5. Patey, G.; Couillard, C.M.; Pierron, F.; Baudrimont, M.; Couture, P. Biotransformation, antioxidant and histopathological biomarker responses to contaminants in European and American yellow eels from the Gironde and St. Lawrence estuaries. *Chemosphere* **2017**, *188*, 292-303.

6. Robinet, T.T.; Feunteun, E.E. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? *Ecotoxicology* **2002**, *11*, 265-277.

7. Belpaire, C.; Goemans, G. The European eel *Anguilla anguilla*, a rapporteur of the chemical status for the Water Framework Directive. In *GIS GRISAM EEL Meeting (Perpignan 2006-04-24)*, Laboratoire Arago, Université Pierre et Marie Curie, Banyuls-sur-Mer: 2007; Vol. 57, pp 235-252.

8. NZ Fisheries. South Island Customary Fishing, Regulations 1999 - Declaration of Mātaitai Reserve at Mataura River and Appointment of Tangata Tiaki/Kaitiaki - Notice 2005-go5185 (No. F329). 2005; 123, p 3010.

9. Fenet, H.; Casellas, C.; Bontoux, J. Laboratory and field-caging studies on hepatic enzymatic activities in European eel and rainbow trout. *Ecotoxicol. Environ. Saf.* **1998**, *40*, 137-143.

10. Fenet, H.; Gomez, E.; Rosain, D.; Casellas, C. Polycyclic aromatic hydrocarbon metabolites and 7-ethoxyresorufin O-deethylase activity in caged European eels. *Arch. Environ. Contam. Toxicol.* **2006**, *51*, 232-236.

11. van den Heuvel, M.R.; Landman, M.J.; Tremblay, L.A. Responses of shortfin eel (*Anguilla australis*) exposed *in situ* to pulp and paper effluent. *J. Toxicol. Environ. Health, A* **2006**, *69*, 1763-1779.

12. Oikari, A. Caging techniques for field exposures of fish to chemical contaminants. *Aquat. Toxicol.* **2006**, *78*, 370-381.

13. Buckland, S. *Sampling protocols and analytical methods for determining petroleum products in soil and water. Report prepared by the Oil Industry Environmental Working Group*; 1999; p 59.

14. Walsh, C.T.; Pease, B.C. The use of clove oil as an anaesthetic for the longfinned eel, *Anguilla reinhardtii* (Steindachner). *Aquacult. Res.* **2002**, *33*, 627-635.

15. Altun, T.; Hunt, A.O.; Usta, F. Effects of clove oil and eugenol on anaesthesia and some hematological parameters of European eel *Anguilla anguilla*, L., 1758. *J. Appl. Anim. Res.* **2006**, *30*, 171-176.

16. Lokman, P.M.; Young, G. Gonad histology and plasma steroid profiles in wild New Zealand freshwater eels (*Anguilla dieffenbachii* and *A. australis*) before and at the onset of the natural spawning migration. II. Males. *Fish Physiol. Biochem.* **1998**, *19*, 339-347.

17. Haro, A. Downstream migration of silver-phase anguillid eels. In *Eel Biology*, Aida, K.; Tsukamoto, K.; Yamauchi, K., Eds. Springer: Tokyo, 2003; pp 215-222.

482 18. US EPA. Method 200.2, Revision 2.8: sample preparation procedure for spectrochemical determination
483   of total recoverable elements. 1994; p 13.

484 19. Ricker, W.E. Computation and inter-pretation of biological statistics of fish populations. *J. Fish. Res.*
485   *Board Can.* **1975**, *191*, 1-382.

486 20. Fulton, T.W. The rate of growth of fishes. *22nd Annual Report of the Fishery Board of Scotland* **1904**, *3*, 141-
487   241.

488 21. Hu, L.C.; Todd, P.R. An improved technique for preparing eel otoliths for aging. *N. Z. J. Mar. Freshwat.*
489   *Res.* **1981**, *15*, 445-446.

490 22. Graynoth, E. Improved otolith preparation, ageing and back-calculation techniques for New Zealand
491   freshwater eels. *Fish. Res.* **1999**, *42*, 137-146.

492 23. Aarab, N.; Champeau, O.; Mora, P.; Daubèze, M.; Garrigues, P.; Narbonne, J.-F. Scoring approach based
493   on fish biomarkers applied to French river monitoring. *Biomarkers* **2004**, *9*, 258-270.

494 24. Pohl, R.J.; Fouts, J.R. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal
495   subcellular fractions. *Anal. Biochem.* **1980**, *107*, 150-155.

496 25. Kennedy, S.W.; Jones, S.P. Simultaneous measurement of cytochrome P4501A catalytic activity and
497   total protein concentration with a fluorescence plate reader. *Anal. Biochem.* **1994**, *222*, 217-223.

498 26. Habig, W.H.; Pabst, M.J.; Jakoby, W.B. Glutathione S-transferase. The first step in mercapturic acid
499   formation. *J. Biol. Chem.* **1974**, *249*, 7130-7139.

500 27. Clairborne, A. Catalase activity. In *Handbook of methods for oxygen radical research*, Greenwald, R.A., Ed.
501   CRC Press: Boca Raton, Florida, 1985; pp 283-284.

502 28. Buege, J.A.; Aust, S.D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302-310.

503 29. Lorenzen, A.K.S.W. A fluorescence-based protein assay for use with a microplate reader. *Anal. Biochem.*
504   **1993**, *214*, 346-348.

505 30. R Core Team *R: A language and environment for statistical computing. R Foundation for Statistical Computing,*
506   *Vienna, Austria.*, 2020.

507 31. Dinno,   A.   *Dunn's   test   of   multiple   comparisons   usig   rank   sums*   https://cran.r-
508   project.org/web/packages/dunn.test/dunn.test.pdf, 2017.

509 32. ANZECC. Australian and New Zealand guidelines for fresh and marine water quality.
510   https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/sediment-quality-toxicants

511 33. McGrath, J.A.; Joshua, N.; Bess, A.S.; Parkerton, T.F. Review of polycyclic aromatic hydrocarbons
512   (PAHs) sediment quality guidelines for the protection of benthic life. *Integr. Environ. Assess. Manage.*
513   **2019**, *15*, 505-518.

514 34. McIntosh, P.D.; Eden, D.N.; Burgham, S.J. Quaternary deposits and landscape evolution in northeast
515   Southland, New Zealand. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **1990**, *81*, 95-113.

516 35. Oliveira Ribeiro, C.A.; Vollaire, Y.; Sanchez-Chardi, A.; Roche, H. Bioaccumulation and the effects of
517   organochlorine pesticides, PAH and heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature
518   Reserve, France. *Aquat. Toxicol.* **2005**, *74*, 53-69.

519 36. De Meyer, J.; Belpaire, C.; Boeckx, P.; Bervoets, L.; Covaci, A.; Malarvannan, G.; De Kegel, B.; Adriaens,
520   D. Head shape disparity impacts pollutant accumulation in European eel. *Environ. Pollut.* **2018**, *240*,
521   378-386.

522 37. van der Oost, R.; Goksøyr, A.; Celander, M.; Heida, H.; Vermeulen, N.P.E. Biomonitoring of aquatic
523   pollution with feral eel (*Anguilla anguilla*) II. Biomarkers: pollution-induced biochemical responses.
524   *Aquat. Toxicol.* **1996**, *36*, 189-222.

525   38.   van der Oost, R.; Beyer, J.; Vermeulen, N.P.E. Fish bioaccumulation and biomarkers in environmental
526   risk assessment: a review. *Environ. Toxicol. Pharmacol.* **2003**, *13*, 57-149.

527   39.   Pacheco, M.; Santos, M.A. Induction of EROD activity and genotoxic effects by polycyclic aromatic
528   hydrocarbons and resin acids on the juvenile eel (*Anguilla anguilla* L.). *Ecotoxicol. Environ. Saf.* **1997**, *38*,
529   252-259.

530   40.   Barrick, A.; Châtel, A.; Marion, J.M.; Perrein-Ettajani, H.; Bruneau, M.; Mouneyrac, C. A novel
531   methodology for the determination of biomarker baseline levels in the marine polychaete *Hediste*
532   *diversicolor. Mar. Pollut. Bull.* **2016**, *108*, 275-280.

533   41.   Burgeot, T.; Gagné, F.; Forget-Leray, J.; Bocquené, G. In *Acethylcholinesterase : Methodology development*
534   *of a biomarker and challenges of its application for biomonitoring*, ICES CM Code: F-25, 2010; p 15.

535   42.   Belpaire, C.; Pujolar, J.M.; Geeraerts, C.; Maes, G.E. Contaminants in eels and their role in the collapse
536   of the eel stocks In *Biology and Ecology of Anguillid Eels*, Arai, T., Ed. CRC Press: Boca Raton, FL, USA,
537   2016; pp 225-250.

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539