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CUMULATIVE IMPACTS ASSESSMENT ALONG A LARGE RIVER, USING BROWN BULLHEAD CATFISH (*AMEIURUS NEBULOSUS*) POPULATIONS

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Abstract—The effects of point-source and diffuse discharges on resident populations of brown bullhead catfish (*Ameiurus nebulosus* (LeSueur, 1819)) in the Waikato River (New Zealand) were assessed at sites both upstream and downstream of point-source discharges. At each site, the population parameters, relative abundance, age structure, and individual indices, such as condition factor, organ (gonad, liver, and spleen) to somatic weight ratios, and number and size of follicles per female, were assessed. Physiological (blood), biochemical (hepatic ethoxyresorufin-*O*-deethylase [EROD] and plasma steroids), and other indicators (bile chemistry and liver metals) of exposure or response also were measured. No impacts on brown bullhead health were obvious at individual geothermal, municipal sewage, or thermal discharge sites or cumulatively along the river. Brown bullhead from the bleached kraft mill effluent site showed elevated levels of EROD, decreased numbers of red blood cells, increased numbers of white blood cells, and depressed levels of sex steroids. However, growth rates, condition factor, age structure, and gonadosomatic index suggest that discharges with significant heat or nutrients benefit catfish despite physiological impairment at one site. Consideration of brown bullhead population-level responses to discharges in a monitoring framework revealed three different population-level response patterns resulting from the point-source discharges.

Keywords—Fish health Discharges Steroids Blood Pulp and paper

INTRODUCTION

Freshwater fishes are widely used as indicators of contamination in waterbodies and are potentially powerful measures of cumulative or ecosystem stress. Fishes often integrate direct contaminant effects, bottom-up indirect effects on other biota, and changes in habitat. A significant scientific challenge remains in terms of teasing apart contaminant-mediated impacts from the multitude of other ecological variables. Despite the uncertainties surrounding population-level responses, effects-based monitoring methods are increasingly taking their place alongside more traditional, risk-based methods for the protection of the environment. Numerous examples of large-scale investigations of this type can be found [1,2], and effects-based tools employing adult fish surveys have been legislatively implemented in a national-scale Environmental Effects Monitoring Program [3].

In New Zealand, the use of macroinvertebrate communities for effects-based monitoring is well established [4]. However, data regarding environmental impacts on fish populations are scarce, largely because of a lack of research compounded by a poor diversity of highly migratory and mostly small diadromous native freshwater fish species. A limited number of effects-based studies have been used to assess impacts of discharges on fishes in New Zealand. These include efforts to monitor fish health using a combination of in situ exposure methods paired with biochemical and chemical indicators [5,6], mesocosm exposures [7,8], and wild-fish monitoring using fish community and health indices [9,10].

Monitoring of wild-fish populations has its roots in fisheries science. It has long been hypothesized that populations may respond in a predictable manner to environmental perturbations [11]. This concept was further expanded to include contaminant stress in the model of population-level response patterns [12]. This population monitoring framework was further refined to categorize fisheries variables into measures of age structure, energy storage, and energy allocation [13]. By recognizing specific patterns of response, such as to eutrophication, this framework can be semidiagnostic for the cause of population changes. By pairing such a population framework with biochemical and chemical measures of exposure and effect, potential contaminant effects either can be ruled out or can point the way to further diagnostic studies. The fish monitoring framework only recently has been used to study cumulative changes resulting from multiple environmental disturbances [1], and few studies have examined cumulative impacts along large rivers.

The purpose of the present study was to evaluate the impacts of anthropogenic stress using brown bullhead catfish (*Ameiurus nebulosus* (LeSueur, 1819)) in the longest river in New Zealand, the Waikato River. The brown bullhead was selected as the monitoring species because it is present at all sites; is a pest in New Zealand and, therefore, has no ethical restrictions regarding numbers able to be caught; and is larger than most native fish present at the sites. It also is a robust benthic species that does not migrate to spawn and is not commercially exploited to any significant extent. Brown bull-

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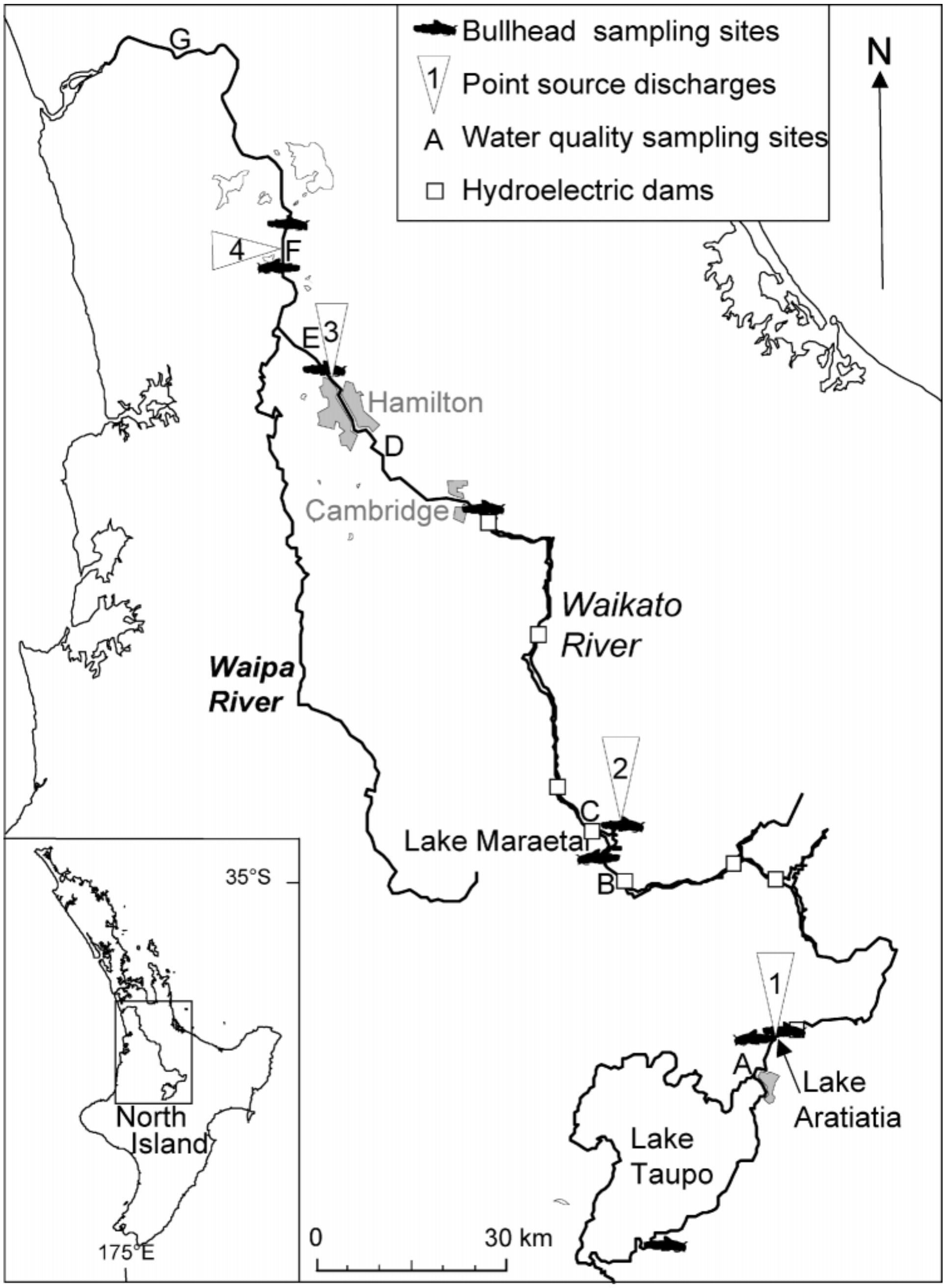

Fig. 1. Waikato River, New Zealand, brown bullhead sampling sites with point-source discharges (1 = Wairakei Geothermal Power Station; 2 = Kinleith Pulp and Paper Mill; 3 = Hamilton City Municipal Sewage; 4 = Huntly Thermal Power Station). White squares are dams for hydroelectric power generation. The letters A through F are water-quality monitoring sites referred to in Table 2.

head also have been studied in a large number of North American impact assessments [14,15].

The Waikato River provides an excellent testing ground to further investigate the relationship between stressors and feral fish responses to the diverse sources of pollution in the river. These pollution sources include industrial effluent, sewage effluent, storm-water runoff, geothermal fluid discharges, and dairy as well as other agricultural land uses. As with many large rivers in New Zealand and around the world, the Waikato River has numerous impoundments for the generation of hydroelectricity that create lacustrine habitats into the upstream reaches of the river and prevent access for migratory fish. Because of the number of impacts present along the length of the river, the present study allowed examination of the hypothesis that impacts may be cumulative as a result of additive degradation in environmental quality along the river. In addition, we aimed to distinguish individual discharge effects from cumulative effects by sampling brown bullhead at multiple levels of biological organization both upstream and downstream of the four largest discharges along the Waikato River.

MATERIALS AND METHODS

Experimental design and site descriptions

A number of sites were chosen along the Waikato River at which significant point-source discharges were known to occur (Fig. 1 and Table 1). Four point-source discharges were chosen: A geothermal power station, bleached kraft mill effluent

Table 1. Characteristics of selected discharges, including approximate volume, approximate distance from the outlet of Lake Taupo (New Zealand), and average river flow for October 2002 at nearest upstream Waikato Regional Council gauging site to sampling sites[a]

Discharge	Distance (km)	Flow (m^3/s)	Sites	Type	Volume (m^3/s)	Main contaminants (amount)
Wairakei 165-MW geothermal power station	9	194	Geothermal Us Geothermal	Cooling water and steam condensate	17.2	Heat (1,000 MW), Hg (46.5 kg/a),[b] H_2S (17.2 g/s),[c] and TN (1.4 g/s)[c]
Pulp and paper mill	117	253	BKME Us BKME	BKME	2.3[d]	Resin acids, chlorophenolic compounds, color (38 t/d), TN (0.64 g/s), TP (55.6 kg/d), BOD (2.19 t/d), and heat
Hamilton City wastewater	227	266	Sewage Us Sewage	Sewage and municipal wastewaters	5	TP (293 kg/d), NH_4–N (871 kg/d), fecal coliforms ($<$1,000/ml), PAH (201 ppt), Cl_3–Cl_5 (1,626 ppt),[e] and heavy metals
Huntly 1,000-MW thermal power station	255	471	Thermal Us Thermal	Cooling water	12	Heat[d] (742 MW)

[a] BMKE = bleached kraft mill effluent; BOD = biological oxygen demand; Ds = downstream; PAH = polycyclic aromatic hydrocarbons; TN = total nitrogen; TP = total phosphorous; Us = upstream reference.

[b] Timperley et al. [49].

[c] Ray et al. [50].

[d] Average figures for 2002. Approximately half of the BKME discharge volume is sourced from Kopakorahi Stream upstream of mill waste stream.

[e] Askey [28].

(BKME), a municipal sewage outfall, and a thermal power-generating station. Sampling at the discharge sites was undertaken in the mixing zones of the discharges to capture worse-case impacts if present. Each site with a point-source discharge had a paired upstream reference location that best reflected the physical nature of the discharge site. One additional reference site was chosen at the oligotrophic Lake Taupo, the source of the Waikato River. Statistical comparisons were only performed between fish from point-source discharge sites and paired upstream reference sites. In addition to the major discharges above, the river is modified by numerous hydroelectric impoundments, plantation forestry, intensive agriculture (particularly dairy), and numerous small communities and industry. Changes to fish populations along the length of the river were assessed qualitatively by comparison to the overall parameter means for the river.

Environmental variables

Water temperatures at each site were recorded at 2-h intervals using TidBit™ data loggers (Onset Computer, Pocasset, MA, USA) set in water to a depth of 1 to 2 m as near as possible to where fish were captured. Loggers were deployed approximately one month before sampling, and records from one year were used to calculate degree-days for each site. Because of fluctuating water levels and flooding, only partial records were obtained from the sewage upstream and thermal site, so Waikato Regional Council temperature logs were used to extrapolate site temperatures for the remainder of the year. To compare site temperatures and the optimum growth temperature of brown bullhead [16], total degree-days above 10°C were calculated for each site (Table 2). Summary information for water-quality variables was obtained from Smith [17] (Table 2).

Capture

To capture brown bullhead, between 7 and 20 fyke nets (13-mm mesh with a single 2-m leader) per site were set in the late afternoon along the lake margins or river and then retrieved the following morning (16.8 ± 0.13 h of fishing). Sampling for brown bullhead was carried out at all sites between October 20 and 29, 2002, with the exception of some additional brown bullhead captured from geothermal sites on November 5, 2002. Sampling time was chosen to coincide with maturation peaks so that reproductive endpoints could be measured. Existing information on effluent mixing was used to ensure that fish were caught from within discharge plumes. Counts of fish captured were used to calculate catch per unit effort (CPUE; fish per net per night). Slow-flowing areas and backwaters were targeted and, where possible, matched at upstream and downstream sites.

Fish processing

Captured fish were either processed on site or transported back to the laboratory within 2 h and processed immediately. The effect of transport stress on variables was not investigated but was thought likely to be insignificant. Fish from Taupo, geothermal, and BKME paired sites were bled immediately on shore after collection from nets and so were not affected by transport. Fish from sewage and thermal sites were transported, but previous studies that have examined the effect of transport on the brown bullhead steroids [18,19] have shown no significant changes. We cannot dismiss the likelihood of capture or transport stress affecting blood variables, but if it occurred, the fact that paired sites were subject to identical capture stress should ensure the validity of comparisons between paired sites. Brown bullhead were killed by a blow to the head, weighed, and measured (total length), and blood was extracted via a heparinized syringe from the caudal vein. Full necropsies for blood and all other biochemical and chemical analyses were conducted on the first 12 male and 12 female brown bullhead. When the numbers caught were sufficient, necropsies on a further 18 male and 18 female fish were carried out for somatic indices, age, and fecundity estimates. Dissected viscera, liver, spleen, and gonads were weighed (accuracy, 0.01 g). Portions of liver (1–2 g) from fully necropsied brown bullheads were stored in liquid nitrogen for 7-ethoxyresorufin-*O*-deethylase (EROD) and metal assays. Gallbladders (bile) also were frozen in liquid nitrogen when they could be dissected intact. Gonad development was assessed, and ovaries were split and preserved in 10% formalin. Sexually maturing female brown bull-

Table 2. River and site physicochemical characteristics[a]

	Water temperature (°C)								
Site name[b]	Degree	Max	Min	Median	DO (%)	N (g/m^3)	P (g/m^3)	Chlorophyll *a* (g/m^3)	Black disk
Taupo	1,847	23.7	8.2	14.0[c]	98.9[c]	—	—	—	—
A, control gates	—	—	—	14.3	101.4	0.07	0.006	0.002	>5.41[d]
Geothermal Us	1,868	20.7	11	12.1[c]	121.2[c]	—	—	—	—
Geothermal	2,816	26.1	12.5	15.2[c]	121.0[c]	—	—	—	—
B, Whakamaru	—	—	—	16.4	103.8	0.19	0.021	0.008	2.6
BKME Us	2,173	23.7	9.7	13.5[c]	124.0[c]	—	—	—	—
BKME	2,709	23.0	11.5	18.0[c]	22.0[c]	—	—	—	—
C, Waipapa	—	—	—	16.3	103.0	0.25	0.026	0.008	2.6
Sewage Us	2,198	21.4	10.5	13.5[c]	110.0[c]	—	—	—	—
Sewage	2,243	21.6	10.5	13.6[c]	108.8[c]	—	—	—	—
D, Narrows	—	—	—	16.2	100.5	0.35	0.032	0.010	1.6
E, Horotiu	—	—	—	16.3	99.6	0.41	0.043	0.012	1.4
Thermal Us	2,187	22.0	9.7	14.1[c]	107.4[c]	—	—	—	—
Thermal	2,862	23.5	11.8	16.1[c]	104.3[c]	—	—	—	—
F, Huntly	—	—	—	16.3	97.1	0.55	0.059	0.013	0.9
G, Tuakau	—	—	—	17.0	100.8	0.60	0.068	0.019	0.7

[a] Degree is total degree-days above 10°C for year from 24/9/02. Us = upstream reference, dissolved oxygen (DO), nitrogen (N), phosphorus (P). BKME = bleached kraft mill effluent.

[b] A–G = five-year median values for selected water-quality measurements recorded by the Waikato Regional Council at Waikato River locations (sites A–G are defined in Fig. 1) [17].

[c] Temperature at time of sampling.

[d] Value not available for this site; the value shown here is from 30 km downstream.

head had two obvious size classes of ovarian follicle: Those undergoing vitellogenesis, and immature follicles (presumably for the subsequent year). The two classes of follicles are not spatially separated, and the large size of the immature follicles makes it difficult to distinguish immature from maturing ovaries without detailed examination. For fecundity estimates, 1 to 3 g of follicles (>100 follicles) were removed from the middle of ovaries, separated, and photographed. Image analysis (Image-Pro® Plus; Media Cybernetics, Washington, MD, USA) was used to count and measure mean diameter of all follicles. Histograms of follicle sizes were then examined, and when the ultimate size class of follicles was discernable as a separate peak, counts of follicles in the ultimate size class were made and fecundity extrapolated as the number of follicles per kilogram of body weight. Carcasses were frozen, and the fifth vertebra was later removed from subsampled fish and a selection of smaller size classes if caught. Vertebrae were cleaned and aged as outlined by Appelget and Smith [20].

Tissue contaminant analysis

For metal analysis, four homogenized liver samples from male brown bullhead were analyzed per site. A suite of 33 metals were determined by nitric and hydrochloric acid digestion and inductively coupled plasma–mass spectrometer or inductively coupled plasma–optical emission spectrometer following U.S. Environmental Protection Agency method 200.3 [21], except that HCl was used instead of H_2O_2, at a commercial laboratory (Hill Laboratories, Hamilton, New Zealand).

Pulp and paper–related organics in bile were determined according to the methods described by van den Heuvel et al. [7]. Hydrolyzed bile (ethanolic KOH digestion) was acidified and extracted with methyl tertiary-butyl ether, derivatized by silylation, and analyzed by gas chromatography–mass spectrometry. Samples were corrected for surrogate recovery and blank determinations. Bile samples (diluted 1:1,600 with ethanol) were analyzed for polycyclic aromatic hydrocarbon (PAH) equivalent concentrations using fixed wavelength fluorescence [22] at the following excitation/emission wavelengths: pyrene, 341/383 nm; naphthalene, 290/335 nm; benzo[*a*]pyrene, 380/430 nm; and retene, 302/372 nm.

Biochemical and blood measurements

Hepatic mixed-function oxygenase enzyme activity was estimated in postmitochondrial supernatant (PMS) as EROD activity using a modification of the fluorescence plate-reader technique outlined by van den Heuvel et al. [23]. Liver extracts were homogenized in a cryopreservative buffer (0.1 M phosphate, 1 mM ethylenediaminetetra-acetic acid, 1 mM dithiothreitol, and 20% glycerol; pH 7.4) and spun at 9,000 *g* to obtain the supernatant. The EROD reaction mixture contained 0.1 M hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid buffer (pH 7.8; Sigma, St. Louis, MO, USA), 5.0 mM Mg^{2+}, 0.5 mM nicotinamide adenine dinucleotide phosphate (Applichem, Darmstadt, Germany), 1.5 μM 7-ethoxyresorufin (Sigma), and approximately 0.5 mg/ml of PMS protein. The EROD activity was determined kinetically in 96-well plates using one reading every minute for 10 min on a BMG Polarstar Galaxy microplate fluorometer (BMG Labtechnologies, Offenburg, Germany). Resorufin was determined using 544-nm excitation and 590-nm emission filters. Protein content was estimated from fluorescamine (Sigma) fluorescence (excitation filter, 390 nm; emission filter, 460 nm) against bovine serum albumin (Sigma).

Sex steroids were determined using radioimmunoassay according to the methods described by McMaster et al. [24]. Plasma samples were thawed, and steroid hormones were extracted with diethyl ether. The steroids testosterone, estradiol, and 11-ketotestosterone were obtained from Sigma. Testosterone and estradiol antibodies were obtained from ICN (Costa Mesa, CA, USA) and 11-ketotestosterone antibody from Helix Biotech (Vancouver, BC, Canada). Tritiated testosterone and estradiol were obtained from Amersham Biosciences (Little Chalfont, Buckhamshire, UK), and tritiated 11-ketotestosterone was a custom synthesis purchased from the U.S. Geological Service (Gainesville, FL). The plasma extract from females was analyzed for estradiol and testosterone, whereas that from males was analyzed for 11-ketotestosterone and testosterone using standard radioimmunoassay procedures.

Hematocrit (packed cell volume of red blood cells) and leukocrit (packed cell volume of white blood cells as represented by the ''buffy'' layer) were measured and expressed as a percentage of total blood volume by the microcapillary method. Four microliters of whole blood were added to 1 ml of Drabkin's solution, and whole-blood hemoglobin was determined by absorbance at 540 nm using a Shimadzu UV1601 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Two microliters of whole blood were mixed with 198 μl of red blood cell–diluting fluid [25] and placed on ice. Red blood cell counts were made using images of red blood cells on a hemocytometer captured (within 1–2 d of blood collection) at ×10 magnification using AxioVision software (Carl Zeiss, Oberkochen, Germany) and an AxioCam HRc camera mounted on a Leica DMRD microscope (Wetzlar, Germany). Image-Pro® Plus software was used to count cells after appropriate enhancement and filtering of images. Hematometric indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) were calculated according to the method described by Wintrobe [26].

Statistical analysis

We calculated the following indices for graphical comparisons of all subsampled fish between sites: Fulton condition factor (K) = (fish wt (g)/(total length (cm)^3))·100, and liver somatic index (LSI) = (liver wt (g)/(fish wt − liver wt))·100. Spleen somatic index (SSI) and gonadosomatic index (GSI) were calculated using the same formula as for LSI but substituting spleen and gonad weights, respectively. Statistical testing of differences between mean site values of liver size, spleen size, gonad size, fecundity, and follicle size were carried out by analysis of covariance (ANCOVA) with site as factor and adjusted body weight (fish wt − organ wt) as covariate. Variables compared using analysis of variance or ANCOVA were tested for normality and homogeneity of variances using the Shapiro-Wilk *W* and Hartley *F*-max statistic, Cochran C statistic, and the Bartlett chi-square test, respectively (Statistica®, Ver 6.1; StatSoft, Tulsa, OK, USA). Before statistical comparisons, fish variables were log-transformed (except for EROD, which required $\log_{10}$ transformation) when the assumption of homogeneity of variance could not be met. For female brown bullhead, statistical analysis of EROD, LSI, SSI, GSI, and sex steroids between paired sites was restricted to those individuals observed to have two distinct size classes of ovarian follicle. Statistical comparisons of EROD, LSI, SSI, GSI, and sex steroids between males from paired sites also were limited to mature males older than one year and with 11-ketotestosterone levels higher than 0.36 ng/ml. For organ weights and condition factor with which ANCOVA was used, interactions between variables were investigated to test the assumption of homogeneity of slopes. As seen in a previous study of brown bullhead [19], a positive relationship between plasma testosterone and 17β-estradiol levels and GSI was found, and GSI was included as a covariate in statistical comparisons of sex steroids. When samples were unsuitable for comparison using parametric tests, the nonparametric Mann-Whitney *U* test was used.

Length-at-age relationships were modeled using a modified von Bertalanffy equation of the following form: Length = $L_{\max}\cdot(1 - 0.96\cdot e^{(-k\cdot \mathrm{age}^{1,2})})$, where $L_{\max}$ is the length at infinite time (or maximum length) and k is the growth constant. This equation was empirically derived to minimize residual sums of squares and provide best fit for length-at-age data. Growth relationships were compared statistically using the residual sums of squares method described by Chen et al. [27].

RESULTS

Site physicochemical characteristics

Total degree-days above 10°C for the geothermal, BKME, and thermal discharge sites were 25 to 50% higher than their respective upstream sites (Table 2). Increases in temperature were mostly evident through autumn, winter, and spring. Temperatures recorded during fish sampling (spring) were 2 to 4°C warmer than upstream sites (Table 2). These temperature increases were limited to the effluent plumes, and though a change did occur in the temperature of the river between Lake Aratiatia upstream and Lake Maraetai upstream (New Zealand), no overall temperature increase was evident along the total length of river sampled. Wide fluctuations in water level were noted at the geothermal and sewage upstream sites during sampling because of hydroelectric flow management. Also noted were a gradual increase in nutrients and primary productivity and a reduction in water clarity along most of the length of the river (Table 2).

Chemical and biochemical indicators of exposure to contaminants

At discharge sites other than thermal, contaminants from respective discharges (geothermal, pulp and paper, and sewage waste) were found in fish tissues (Table 3). Background levels were found at upstream sites except for geothermally derived metals at the geothermal upstream site, where levels were higher than background levels in the upstream Lake Taupo. Arsenic and Hg had levels decreased to background from high levels at the geothermal site before reaching riverine sites (Table 3). Fish at the sewage site had metal loads typical of the sewage discharges [28]. Resin acids typical of BKME were found in bile from fish at the pulp and paper site. In addition, fish from the BKME site had markedly different patterns of metal exposure compared with fish from the main stem of the river, providing further evidence that little mixing occurred between reference and discharge fish (Table 3). Because of the number of diffuse inputs, metal contaminant data were examined for trends along the river. Some modest increases in metals, such as Ag, Co, Cu, and Cd, were observed, especially at the two most downstream sites. These sites are downstream of the confluence of the Waipa River, a major agricultural catchment. Additional Ag, Co, and Cd may be sourced primarily from this catchment. Other than metals noted above, contaminants in fish resulting from point-source discharges were only detectable near the discharges and did not persist or accumulate with increasing distance downstream. Only males from the BKME site had significantly elevated EROD levels compared to those from the upstream reference site (Fig. 2), and EROD levels at all other sites were similar.

Blood variables

Maturity, as determined by gonad size and sex steroid levels, had no significant effect on blood parameters measured, so results from all subsampled fish are shown. Oxygen-carrying capacity in females was higher at all warmer sites except BKME (Table 4), where hematocrit was significantly lower

Table 3. Average trace metals (wet wt) in male brown bullhead livers and average resin acids, pooled polycyclic aromatic hydrocarbons (PAHs), naphthalene (Nap), pyrene (Pyr), benzo[*a*]pyrene (B*a*P), and retene (Ret) in bile of subsampled brown bullhead from sites in the Waikato River, New Zealand[a]

		Liver trace metals (mg/kg wet wt)										Bile				
													PAHs (μg/ml)			
Site name	Age[b] (year)	Ag	As	Cd	Co	Cu	Hg	Li	Pb	Se	Zn	Resin acids (μg/g dry wt)	Nap	Pyr	B*a*P	Ret
Taupo	3.7	0.04	0.27	0.03	0.06	25.9	0.10	0.04	0.02	1.61	40.6	0	64.3	1.8	0.8	316.9
Geothermal Us	4.3	0.03	0.83	0.02	0.05	25.8	0.31	0.06	0.03	2.72	39.3	17	55.5	3.3	0.7	567.7
Geothermal	4.8	0.06	1.63	0.03	0.04	21.2	0.69	0.11	0.03	2.08	36.5	0	43.3	1.6	0.7	312.3
BKME Us	3.3	0.11	0.56	0.01	0.03	16.3	0.20	0.13	0.02	1.39	33.7	28	72.9	2.2	0.9	428.6
BKME	3.3	0.04	0.27	0.00	0.03	7.5	0.11	0.03	0.02	1.12	26.9	1,053	183.4	16.4	2.2	1,266.9
Sewage Us	1.8	0.10	0.24	0.02	0.05	30.6	0.07	0.14	0.01	1.75	41.8	302	70.6	1.7	0.9	325
Sewage	1.8	0.16	0.64	0.04	0.07	30.3	0.06	0.10	0.04	2.67	41.2	19	59.6	0.9	0.5	235.4
Thermal Us	4.0	0.18	0.10	0.09	0.13	34.9	0.12	0.08	0.02	1.97	36.0	0	67.2	1.9	0.7	289.2
Thermal	2.5	0.14	0.09	0.07	0.14	53.9	0.06	0.08	0.03	1.86	42.9	0	53.8	2.2	0.7	373.9

[a] BKME = bleached kraft mill effluent; Us = upstream reference.

[b] Average age of fish pooled for liver metal assay.

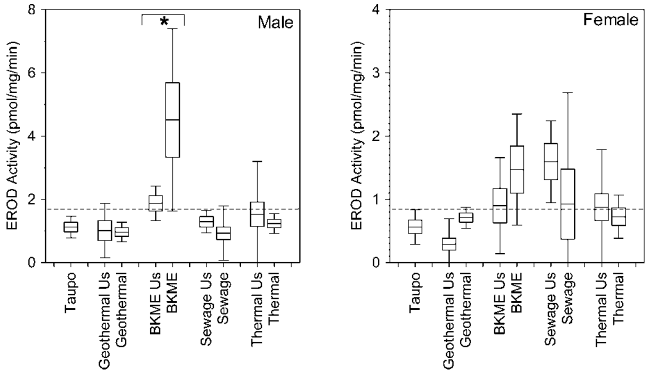

Fig. 2. Liver detoxification enzymes as measured by 7-ethoxyresorufin-*O*-deethylase (EROD) in mature male (left) and female (right) brown bullhead from the Waikato River, New Zealand. Boxes are the mean ± standard error, and whiskers are the 95% confidence intervals. Asterisks indicate pairs of values that are significantly different (*$p < 0.05$). The dashed horizontal line is the river average. BKME = bleached kraft mill effluent; Us = upstream.

than the reference site. Leukocrit values were significantly elevated in male and female bullhead at the BKME and sewage upstream sites (Table 4). No cumulative impacts are apparent in blood variables with increasing distance downstream, but changes from lacustrine to riverine habitat could explain the disjointed pattern seen in hematocrit, red blood cell counts, and leukocrit.

Catch rates

The only significant difference in CPUE (upstream vs downstream) was at the thermal site ($p < 0.01$, Mann-Whitney *U* test) (Table 5). Overall, Lake Taupo and both the BKME sites had higher CPUE than other sites, and BKME sites were significantly higher in terms of CPUE ($p \leq 0.01$) than all sites except Lake Taupo and thermal sites. The CPUE at most riverine sites was lower than that at upstream lacustrine sites.

Length frequency, age, and growth

Large numbers of small bullhead were caught at the BKME and thermal sites (Fig. 3), and the average lengths of all fish were significantly different ($p < 0.001$, Mann-Whitney *U* test) from the respective upstream sites. This difference was strongly influenced by the smallest size class and indicates that these populations were skewed toward younger fish or, conversely, that other sites were lacking young fish. Average lengths of bullhead caught at other paired sites were not significantly different. In comparison to all sites, fish from the geothermal and thermal upstream sites were larger than the overall river average. Lengths of subsampled fish were similar except for the thermal upstream site, where the low number of large bullhead caught limits comparisons (Table 6). Ages of subsampled fish also were similar at paired sites except for thermal sites. Fish from the sewage sites were younger, and geothermal fish were older, than the river average (Table 6). Age–size relationships of males and females were not found to be significantly different for any of the nine sites examined. Thus, males and females were pooled for subsequent growth modeling. Growth, as assessed by the modified von Bertalanffy function, was significantly different at the BKME and thermal sites (Table 5). This effect was largely a result of the greater maximum length ($L_{\max}$) (Table 5) at the BKME site and lower maximum length at the thermal site. Cumulative effects were

Table 4. Average blood parameters (hematocrit [Hct], red blood cell count [RBC], mean cell volume [MCV], hemoglobin [Hb], mean cell hemoglobin [MCH], mean cell hemoglobin concentration [MCHC], and leukocrit [Lct]) in brown bullhead from the Waikato River, New Zealand[a]

Site	Total fish (*n*)		Hct (%)		RBC (cells/L × 10^{13})		MCV (L × 10^{-12})		Hb (g/L)		MCH (g/cell × 10^{-10})		MCHC (g/L)		Lct (%)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Taupo	12	12	33.3	32.8	0.12	0.10	0.30	0.36	98.2	95.8	0.88	1.02	294	288	0.79	1.08
Geothermal Us	5	6	27.7	29.2 A	0.13	0.10	0.27	0.32	83.7	80.7	0.94	0.9	311	272	0.87	0.97
Geothermal	12	12	33.5	37.4 A	0.18	0.19	0.23	0.28	97.7	113.7	0.64	0.86	291	302	0.86	0.86
BKME Us	12	12	37.4	35.5 B	0.14	0.13	0.34	0.3	125.4	112.7	1.09	0.95	326	313	1.02 C	0.97 D
BKME	12	12	37.4	31.5 B	0.14	0.11	0.35	0.32	105.8	110.9	1.05	1.16	297	352	1.27 C	1.27 D
Sewage Us	12	11	36.7 E	34.4	0.30 F	0.24	0.13 G	0.17	101.7	108.3	0.34	0.54	270 H	309	1.26 I	1.30 J
Sewage	12	12	31.8 E	33.0	0.18 F	0.21	0.19 G	0.17	102.1	113.2	0.60	0.59	317 H	338	0.98 I	0.96 J
Thermal Us	3	4	33.1	29.5 K	0.12	0.11	0.29	0.29	127.6	106.2	1.12	1.04	384	356	0.99	1.05
Thermal	12	12	36.4	38.5 K	0.14	0.13	0.26	0.37	117	132.5	0.83	1.25	317	339	0.85	1.05

[a] Differences between paired sites ($p < 0.05$, analysis of variance) are denoted by different letters. BKME = bleached kraft mill effluent; Us = upstream reference.

not apparent over the longitudinal range of sites that we sampled.

Condition factor, LSI, and spleen somatic index

Subsampled male and female brown bullhead from the BKME site were in significantly better condition compared with those from the upstream site (Fig. 4). Male and female fish from the sewage site also were in significantly better condition compared with those from the upstream site, but the lack of mature fish at the sewage upstream site made statistical comparisons impossible. Females from the geothermal site also were in significantly better condition compared with upstream females (Fig. 4). Condition factors for both sexes of from Lake Taupo and sewage upstream sites fell below the overall river average (Fig. 4).

Statistical comparisons of liver size were limited to mature males and females. Males and females from the BKME site had significantly larger livers compared with males and females from the upstream site (Fig. 4). In contrast to condition factor, females captured from the geothermal site had significantly smaller livers (Fig. 4). With the exception of the Lake Taupo and sewage upstream sites, a trend for increased condition (condition factor and LSI) in lake compared to river sites was apparent (Fig. 4), but no cumulative impacts were apparent with increasing distance downstream. Spleen size showed consistent trends in males and females corresponding with observed changes in oxygen-carrying capacity, increasing at the geothermal and thermal sites but decreasing at the BKME site (Fig. 4).

GSI, fecundity, follicle size, and plasma sex steroids

To provide valid statistical evaluations between sites, only mature females for which fecundity estimates were possible were compared. Males had uniformly small gonads, so gonad size could not be used reliably to determine maturity. Therefore, mature males were taken to be fish older than one year and those having plasma 11-ketotestosterone values exceeding 0.37 ng/ml. Gonads of males and females from paired sites were not significantly different (Fig. 5). Although sewage upstream fish were of equivalent size to fish downstream of the discharge and were of reproductive age, the low GSI, lack of the ultimate follicle class, and low plasma steroid levels indicated that all fish at the reference location were nonreproductive. Gonad size (i.e., GSI) of mature females at other sites was consistent at approximately 10% of body weight; however, Lake Taupo reference and thermal site females had lower and higher GSIs, respectively, compared with the river average (Fig. 5).

Fecundity estimates ranged from 5,660 to 22,001 follicles/kg female body weight. Average ultimate mean follicle diameters ranged from 1.33 to 3.11 mm. Unlike female gonad size, fecundity per kilogram varied widely (Fig. 5). For the entire dataset of fish in which estimates of fecundity were possible, a positive relationship was found between fecundity and follicle diameter and somatic weight ($r = 0.67$, $p < 0.001$, and $r^2 = 0.36$, $p = 0.001$, respectively). However, a negative relationship ($r = -0.37$, $p < 0.001$) between fecundity per kilogram and somatic weight indicates that larger fish had relatively fewer larger follicles. Steroid levels were only compared in mature males and females. Significant relationships were found between the stage of maturity, as indicated by GSI, and steroid hormone levels. To account for this relationship, GSI was used as a covariate in an ANCOVA. Males and fe-

Table 5. Total number of brown bullhead caught, average catch per unit effort (CPUE; fish/net/night), fecundity/kg, follicle diameter, maximum length (L_{max}), and growth constant (k) calculated using combined ages of males and females and a modified von Bertalanffy equation for subsampled fish from discharge and upstream reference (Us) sites[a]

	Total fish (*n*)	CPUE	Fecundity/kg	Fecundity	Follicle diameter (mm)	L_{max} (cm)	k
River average	1,086	9.5 ± 3.3	13,135 ± 402 (81)	4,832 ± 183 (81)	2.17 ± 0.04	—	—
Taupo	158	19.8 ± 7.8	9,728 ± 1,134 (8)	2,232 ± 290 (8)	1.72 ± 0.10	29	0.36
Geothermal Us	22	1.4 ± 0.4	11,743 ± 1,481 (7)	5,798 ± 638 (7)	2.24 ± 0.11	33	0.68
Geothermal	39	2.4 ± 0.6	10,079 ± 581 (14)	5,904 ± 331 (14)	2.37 ± 0.08	35	0.82
BKME Us	443	21.1 ± 5.5	14,417 ± 593 (14)	4,796 ± 304 (14)	2.06 ± 0.10	31 A	0.52
BKME	212	23.6 ± 9.2	15,863 ± 704 (17)	5,357 ± 317 (17)	2.06 ± 0.04	34 A	0.45
Sewage Us	29	1.9 ± 0.7	—	—	—	27	1.16
Sewage	29	1.0 ± 0.3	16,284 ± 1,250 (7)	5,853 ± 629 (7)	2.17 ± 0.11	29	0.96
Thermal Us	8	0.9 ± 0.5 C	10,189 ± 1,914 (3)	3,734 ± 315 (3)	2.49 ± 0.03	34 B	0.75
Thermal	146	13.3 ± 5.5 C	13,343 ± 694 (11)	3,631 ± 215 (11)	2.42 ± 0.12	30 B	0.75

[a] Values are given as the mean ± standard error. Numbers in parentheses are total *n* where different to subsampled *n*. Differences between paired sites ($p < 0.05$, A and B, residual sum of squares procedure [26]; C, Mann-Whitney *U* test) are denoted by different letters. BKME = bleached kraft mill effluent.

males from the geothermal site had significantly higher levels of testosterone, and females from that site had significantly higher levels of 17β-estradiol (Fig. 6). In contrast, males and females from the BKME site showed significant reductions in levels of all steroids measured (Fig. 6).

Summary of population parameters

Population and physiological variables were categorized as being age structure, energy storage, or energy allocation variables (Table 7). Age structure was based on the mean age and by the relative distribution of the youngest year class versus mature brown bullhead. Energy storage was indicated by condition factor and liver size, whereas estimates of energy expenditure included gonad size, fecundity, and growth. The complete failure to develop reproductively at the sewage upstream site resulted in a relative increase in energy allocation at the sewage site.

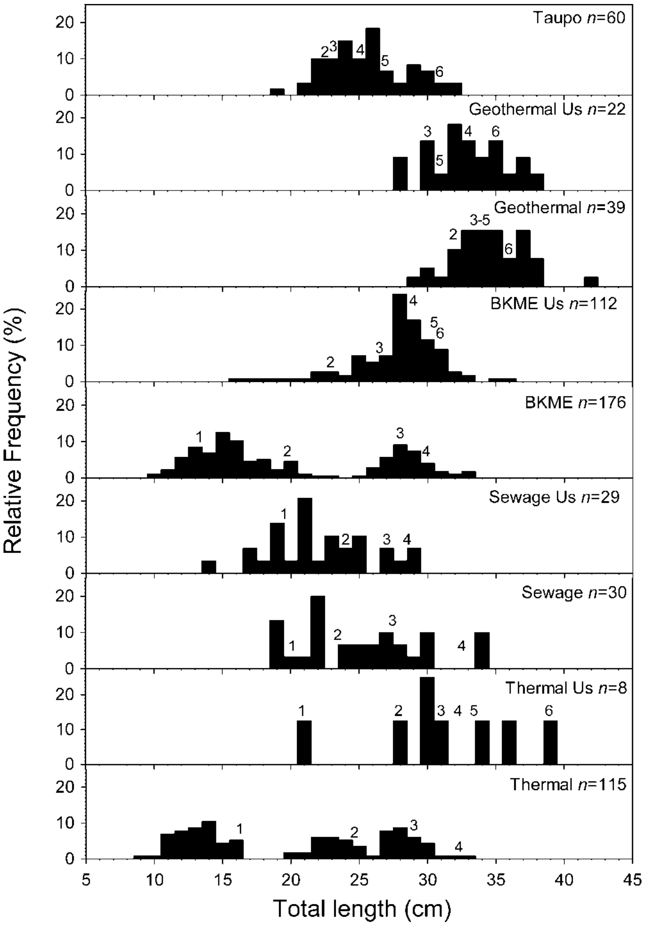

Fig. 3. Lengths of brown bullhead at nine sites in the Waikato River, New Zealand. Numbers above bars are approximate mean lengths at the age shown. BKME = bleached kraft mill effluent; Us = upstream.

DISCUSSION

Point-source discharges had a diverse range of impacts on the brown bullhead populations. The geothermal effluent had limited upstream/downstream impacts on population variables, but there appeared to be no recruitment in Lake Aratiatia as a whole. The pulp mill effluent caused increased energy allocation (growth) and storage as well as a proliferation of younger fish. Riverine fish both upstream and downstream of sewage effluent were performing poorly, as indicated by lack of reproductive maturity and low condition, and comparisons generally were not appropriate. Thermal power generation resulted in younger age structure and decreased energy allocation (growth) but no differences in energy storage. Numerous chemical and suborganismal indications of exposure as well as some physiological impacts were found, but none could be related to population-level effects. Physicochemical and habitat characteristics of sites had a significant influence on brown bullhead populations over the geographic range of sites that we sampled, and changes in populations seemed to be influenced more by local water quality and habitat variables, with little evidence of additive or cumulative changes along the river.

Responses observed in brown bullhead from discharge areas indicated that they were resident and subject to prolonged exposure to contaminants. This was particularly evident at the geothermal location, where an obvious bioaccumulation of Hg was observed. Low and distinct metal signatures of brown bullhead livers from the BKME site corroborate bile resin acids and EROD results that indicate the residency and exposure to BKME of brown bullhead at that site. Metal concentrations were elevated locally and generally did not accumulate downstream. Some exceptions to this include Ag, Cd, Cu, and Co. Phosphate fertilizer (predominantly superphosphate) is a possible primary source of the additional Ag and Cd in these samples. Superphosphate is the dominant source of Cd in pas-

Table 6. Characteristics of subsampled brown bullhead from discharge and upstream reference (Us) sites[a]

Site	No. subsampled		Length (cm)		Weight (g)		Age (year)		% >2 years	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
River average	186	179	27.5 ± 0.4	26.9 ± 0.3	319.0 ± 13.9	296.2 ± 11.8	3.1 ± 0.1	3.0 ± 0.1	72	70
Taupo	30	30	24.8 ± 0.5	23.7 ± 0.6	193.5 ± 12.2	169.4 ± 13.1	3.6 ± 0.1	3.5 ± 0.1	100	96.7
Geothermal Us	11	11	31.9 ± 0.8	31.3 ± 0.9	529.1 ± 48.4	484.8 ± 47.0	4.3 ± 0.3	3.6 ± 0.2	100	100
Geothermal	22	17	34.7 ± 0.6	33.5 ± 0.5	630.0 ± 35.7	582.4 ± 26.1	4.8 ± 0.3	4.2 ± 0.3	100	91.7
BKME Us	30	30	28.4 ± 0.5	27.8 ± 0.4	316.9 ± 17.9	304.3 ± 17.7	3.3 ± 0.1	3.2 ± 0.2	100	91.7
BKME	29	30	28.2 ± 0.3	28.3 ± 0.3	327.7 ± 11.4	333.8 ± 14.2	3.1 ± 0.1	3.1 ± 0.1	100	96.7
Sewage Us	17	11	22.3 ± 0.9	21.6 ± 1.0	145.2 ± 19.2	129.5 ± 21.8	1.8 ± 0.2	1.5 ± 0.2	7.7	9.1
Sewage	13	16	23.5 ± 1.5	26.3 ± 0.9	210.0 ± 48.7	274.8 ± 36.1	1.8 ± 0.3	2.7 ± 0.2	25	58.3
Thermal Us	4	4	33.8 ± 2.1	27.8 ± 2.6	605.3 ± 159.2	314.8 ± 77.9	4.0 ± 0.7	3.0 ± 0.9	100	50
Thermal	30	30	25.8 ± 0.7	24.8 ± 0.6	240.7 ± 17.8	216.0 ± 14.3	2.2 ± 0.1	2.1 ± 0.1	26.7	16.7

[a] Values are given as the mean ± standard error. BKME = bleached kraft mill effluent.

toral New Zealand agriculture, with an estimated 8.3 tons still being deposited on Waikato region farmland each year [29]. Copper likely is indicative of increased industrial activity in the lower reaches, and Co is an element widely used in New Zealand pastoral agriculture, partly to offset a Co deficiency in some New Zealand soils. A total of 472 Co compounds (including vitamin B_{12}) are registered for animal use in New Zealand as antibiotics, antidotes, bloat remedies, cardiovascular agents, endoparasiticides, nutrient/electrolytes, and vaccines (http://www.nzfsa.govt.nz/acvm/registers-lists/acvm-register/index.htm). Levels of problematic metals in brown bullhead livers, such as As, Cd, Hg, and Se were below toxic levels available for flesh (and some liver concentrations) [30], although Hg at the geothermal site levels were approaching those levels. Overall levels of contamination (metals and PAH) in brown bullhead were low compared to brown bullhead sampled in North America [14,15,31], and no overt signs of contamination, such as lip papilloma, were seen.

Increases in EROD in brown bullhead from the BKME site confirmed that they were responding to compounds in the discharge as predicted from responses to BKME documented in other species [32,33]. No EROD response was seen at the other sites. This related well to elevated bile PAH metabolites, suggesting that PAH-like biotransformation products of resin acids (e.g., retene) were responsible for this induction. The magnitude of response was highest in males, as found by Munkittrick et al. [32], and were two- to threefold those at the upstream site. In the present study, brown bullhead showed approximately fivefold induction as compared to other sites on the river. A study on highly contaminated regions of the Niagara River (USA) [31] showed a very comparable, sixfold level of induction when the most contaminated site was compared to the reference location. Injection experiments [31] indicated that the contaminated-site EROD levels were very comparable to the maximum level of induction that could be obtained with β-naphthoflavone. Thus, in both the present study and the Niagara River study, it would appear that the EROD induction of brown bullhead may be approaching the maximum levels exhibited by this species. Overall, these results indicate that exposure to *cyp1a*-inducing chemicals at the BKME site was significant in light of the known response of this species, and that the remainder of the sites are relatively free of contaminants, such as PAHs, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans, that are known to induce the *cyp1a* gene.

Red and white blood cells and the oxygen-carrying capacity of brown bullhead showed changes consistent with physiological adaptation to the water temperature at all but one site. The exception was the BKME site, where no increase in the oxygen-carrying capacity of blood because of higher water temperatures and low dissolved oxygen was seen. In fact, a general decrease was observed in most measures of blood oxygen-carrying capacity, although only female hematocrit was significantly depressed. A decrease in hematocrit also was seen in five of the six pulp and paper effluent studies reviewed by Folmar [34], and a corresponding drop in SSI, as observed in the present study, also was seen in response to pulp mill effluents tested in the field and laboratory [35]. The increase in white blood cells further suggests an immune response in BKME-exposed bullhead; however, the literature reviewed in Folmar [34] showed inconsistent patterns of response for leukocrit.

Differences in age structure also were strongly apparent

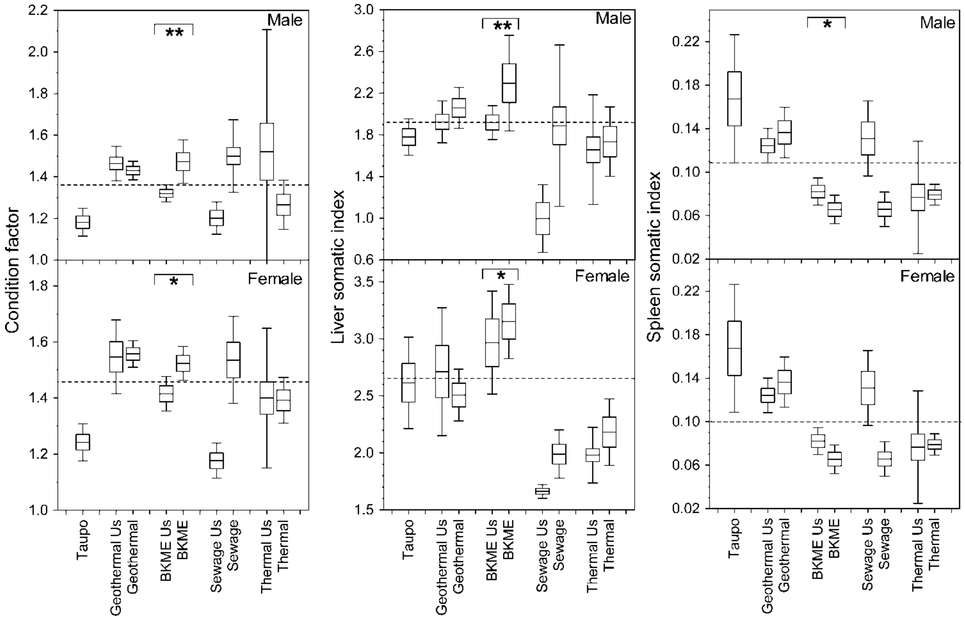

Fig. 4. Somatic indices for mature male (top) and female (bottom) brown bullhead. Boxes are the mean ± standard error, and whiskers are the 95% confidence intervals. Asterisks indicate pairs of values that are significantly different (*$p < 0.05$, **$p < 0.01$). The dashed horizontal line is the river average. BKME = bleached kraft mill effluent; Us = upstream.

throughout the river. In some cases, this appeared to be strongly influenced by point-source discharges. The BKME and thermal sites were both dominated by young age classes, presumably in response to increased survival of progeny. This may have been influenced by the warmer temperatures and, in the case of BKME, increased food supply resulting from nutrient inputs. The geothermal site did not demonstrate this effect. However, this site and its upstream reference were dominated by older, larger fish, and no evidence of recruitment could be found. We postulate that this population is maintained by immigration. The primary reason for the recruitment failure is unclear, but geothermally derived chemicals, limited littoral habitat, and rapidly fluctuating water levels resulting from power-generating requirements may all play a role.

The indicators of energy storage (condition factor and liver size) appeared to respond positively to lacustrine habitats, heat, and nutrients and negatively to riverine habitat (fast-flowing river sections and fluctuating water levels). Increased energy storage at the BKME site matches observations in other fish species [32,36,37], whereas poor condition of bullhead reflected the oligotrophic nature of Lake Taupo and the effects of

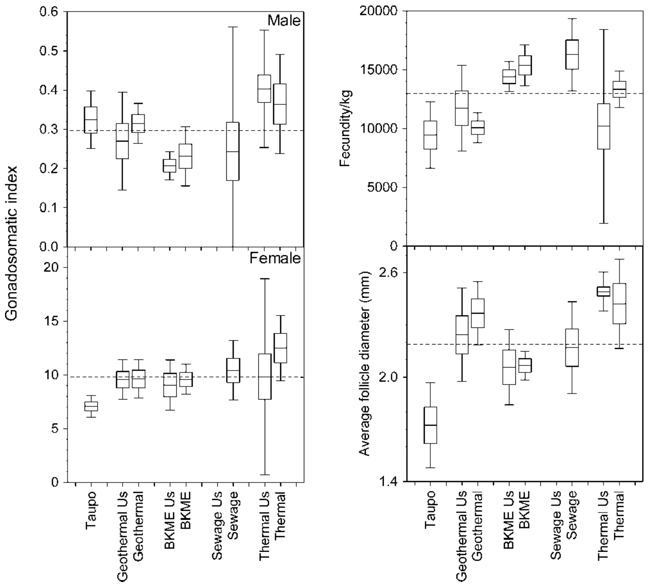

Fig. 5. Gonadosomatic index for mature male (top) and female (bottom) brown bullhead. Right-hand graphs are fecundity per kilogram and average follicle diameter for mature females. Boxes are the mean ± standard error, and whiskers are the 95% confidence intervals. The dashed horizontal line is the river average. BKME = bleached kraft mill effluent; Us = upstream.

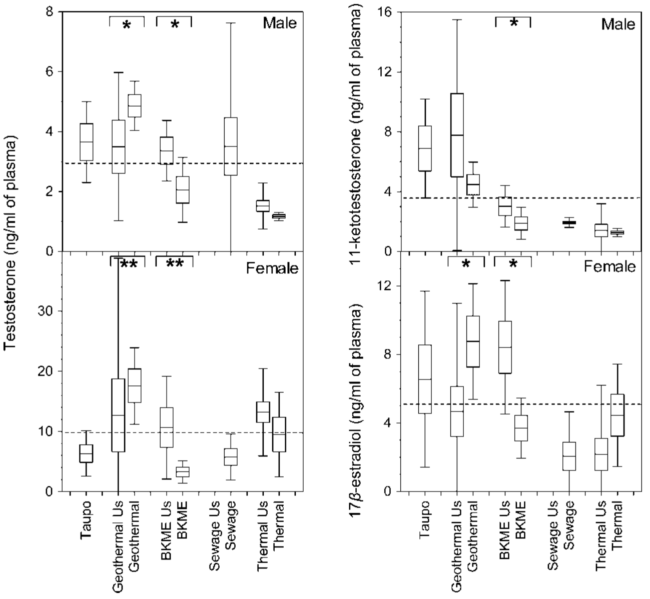

Fig. 6. Sex steroid levels in blood plasma for mature male (top) and female (bottom) brown bullhead. Boxes are the mean ± standard error, and whiskers are the 95% confidence intervals. Asterisks indicate pairs of values that are significantly different (*$p < 0.05$, **$p < 0.01$). The dashed horizontal line is the river average. BKME = bleached kraft mill effluent; Us = upstream.

Table 7. Summary of population and physiological indicators in terms of age structure, energy storage, and energy[a]

Impact site	Age structure	Energy storage	Energy allocation
Geothermal	0	0	0
BKME	−	+	+
Sewage	0	NC	+
Thermal	−	0	−

[a] The plus symbol (+) indicates a significant increase in at least one variable; the minus symbol (−) a significant decrease, and 0 no change. BKME = bleached kraft mill effluent; NC = comparisons were not possible because of the lack of mature fish.

fluctuating level and fast-flowing waters at the sewage upstream site, which was approximately 1 km downstream of the tailrace of a hydroelectric dam. This is a very similar situation to that described by Barnes et al. [38], where Lake Whitefish (*Coregonus clupeaformis*) downstream of a hydroelectric control structure were in much poorer condition than a reference population. Despite a similar increase in river productivity in the Ottawa River (Canada), brown bullhead populations in upstream lake sections were in better condition than those in downstream riverine sections [39]. This is consistent with the poorer condition of brown bullhead at riverine sites in the present study, which suggests that brown bullhead are best adapted to lentic conditions.

Reproduction and growth were the two measures of energy allocation examined. Growth was best determined by the nature of the age–size relationships. Brown bullhead at the BKME and thermal discharge sites showed opposite responses in ultimate length. Larger fish upstream of the thermal discharge may be the result of the very low bullhead density, whereas increased growth in BKME may be the result of increased productivity and temperature. When the river as a whole is examined, a large range in ultimate length is found. Fast-flowing riverine sites tended to produce poor growth, whereas sites with low density produced large fish.

Unlike many of the other variables measured, reproductive energy allocation, as indicated by gonad size, was remarkably consistent and showed no detectable effect of point-source pollution or habitat. The exception to this is the fast-flowing riverine site (sewage upstream), where females were nonreproductive. Despite a high frequency of external tumors and abnormalities, bullhead from the most contaminated site sampled by Lesko et al. [40] had the greatest number of follicles per female. The ability of bullhead to maintain constant investment in reproduction at a wide range of sites says much for the robustness of the species. With respect to stored energy, the reproductive allocation appears to be constant, with the remainder being available for growth. This mechanism, aptly called ‘‘capital spawning’’ by Henderson et al. [41], also was supported by the decrease in mature female brown bullhead liver size with increasing GSI. This strategy has inherent advantages for larger fish, in which larger ovarian follicles would be expected to produce larger larvae with a competitive advantage and higher survival [42].

Changes in sex steroid levels followed patterns seen in oxygen-carrying capacity, being generally elevated at the warm-water geothermal and thermal discharges but depressed at the warm-water BKME site. Depression of sex steroids has been seen in other species exposed to pulp and paper effluents [32,36,43]; however, we did not observe accompanying reductions in GSI or fecundity per kilogram. Thus, it appears that of all the sites, the BKME-exposed fish appear to experience some type of metabolic or endocrine disruption. However, as seen in previous studies [44,45], this effect was not manifest in adverse population-level responses. However, the potential exists that beneficial effects of other components of the discharge, such as heat, may offset or mask other impacts. New Zealand native species, except eels, have lower preferred temperatures [46] compared with those of brown bullhead [47]. These native species may experience the combined stress of BKME and additional heat and be more sensitive to reductions in sex steroids compared to bullhead, as seen with coexisting fish species elsewhere [45].

The overall patterns of population-level response in bullhead were difficult to attribute solely to point-source discharges. The BKME response was, perhaps, the most clear-cut, in which increases in energy storage and allocation appear to have led to increased recruitment and, thus, can be classified as an eutrophication response [13]. No effect of the sewage effluent was observed, possibly because of the overriding influence of unsuitable riverine habitat at both sites. The geothermal and thermal power stations contributed considerable thermal energy to the river, but populations responded differently. Upstream and downstream geothermal sites appeared to have recruitment failure, whereas thermal effluent responses included increased recruitment. Size range, length at age, and CPUE of bullhead downstream of the discharges with a significant heat component suggest that bullhead benefit from the added heat, as would be expected given their preferred temperature of approximately 30°C [47].

Overall, brown bullhead populations appear not to be negatively affected by any of the major point-source discharges to this large river system. By contrast, some of these discharges appear to enhance conditions for this species. Despite the gradual deterioration in water-quality downstream, particularly nutrient enrichment and increased turbidity, no concomitant cumulative impacts were observed in brown bullhead used to indicate river quality. Both contaminant exposure indicators, physiological and population-level responses, were localized. However, it also was apparent that some of the population-level responses observed could not be fully explained. Both a better understanding of fish population-level responses and further study of the Waikato River are warranted. It is anticipated that ongoing comparative studies of other fish species to the same discharges will increase our understanding of brown bullhead responses.

We documented relationships between the physicochemical nature of sites and variables assessed in individual fish, such as the effect of water temperature and dissolved oxygen on blood oxygen-carrying capacity and the effect of habitat on condition indices. At an individual fish level, we also showed interrelationships between physiological variables, such as between sex steroids and gonad size and between female liver and gonad size. These relationships endorsed assessment of multiple levels of biological organization [12,48]. They also illustrated the integrated response of fish to all aspects of discharges and habitat and showed that cause and effect in wild-fish populations can be established when relationships between variables are included in assessments and appropriate reference sites sampled.

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