Responses of wild freshwater fish to anthropogenic stressors in the Waikato River of New Zealand

A thesis
submitted in partial fulfilment
of the requirements for the degree
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THE UNIVERSITY OF
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Abstract

To assess anthropogenic impacts of point-source and diffuse discharges on fish populations of the Waikato River, compare responses to different discharges and identify potential sentinel fish species, we sampled wild populations of brown bullhead catfish (*Ameiurus nebulosus,* (LeSueur, 1819)), shortfin eel (*Anguilla australis* Richardson, 1848), and common bully (*Gobiomorphus cotidianus* McDowall, 1975) in the Waikato River. Sites upstream and downstream of: geothermal; bleached kraft mill effluent (BKME); sewage and thermal point-source discharges were sampled. At each site, the population parameters, relative abundance, age structure and individual indices such as: condition factor; and organ (gonad, liver, and spleen) somatic weight ratios; and number and size of follicles per female were assessed. Indicators of fish residence and in some cases exposure to contaminants in discharges were analyzed. Bile chemistry of brown bullhead and shortfin eel was assayed, liver and muscle metal levels were analyzed for brown bullhead and shortfin eel respectively, and stable isotopes of C and N in common bully were measured. Bile, metal and isotopic signatures gave strong evidence that fish had been resident at sites for some time before sampling. Signatures of bile and metal contaminants showed contamination was localised to discharge areas. Gradients in stable isotopes in common bully showed evidence of changes in water sources and anthropogenic effects along the river. Biochemical variables, hepatic ethoxyresorufin-*O*-deethylase (EROD) and plasma steroids indicated exposure and response of brown bullhead and shortfin eel to pulp and paper contaminants at the BKME site.

Physiological (blood) variables showed fish largely responded in a predictable way to elevated water temperatures at discharge sites at time of sampling, however total haemoglobin of brown bullhead and common bully blood failed to increase at the BKME site despite elevated temperatures and low dissolved oxygen. Growth rates, condition factor, age structure, and gonadosomatic index (GSI) suggest that discharges with significant heat or nutrients benefit brown bullhead despite physiological impairment at the BKME site. Shortfin eel individuals also benefited from heated water discharges. No consistent impacts on common bully health were obvious at individual discharge sites, or cumulatively along the river due to the gradual deterioration in water quality downstream. Common bully individuals also

benefited from heat in discharges but lack of juveniles at sites where numerous juvenile brown bullhead were found, suggest that unlike brown bullhead populations, common bully populations were not responding with significant recruitment. Although I found little evidence of toxic effects of discharges on shortfin eel, caution is required in assessing the potential of contaminants to impact eel populations due to the life history of shortfin eel, and exploited nature of populations. For example, reproductive damage suffered by adult eels may not immediately manifest itself in the effected population due to temporal delays in gonadal maturation, and recruitment, and single panmictic populations supplementing recruitment of impacted populations.

Distinct changes in population parameters at each of the paired sites and changes in individual variables showed that fish responded to discharges. The range of responses in species suggests different sensitivity to contaminants and amount of benefit which each species receives from heat in discharges. In these terms shortfin eel would be the most resistant, then brown bullhead and lastly common bully. Interpretation of population-level impacts at the geothermal and BKME discharge sites is made difficult due to benefits of additional heat. There is also the possibility that detection of sub-lethal or chronic effects on sensitive juvenile life-stages may be being hidden by compensatory density population responses. Responses and life history of common bully made them the preferred indicator species of the three species sampled, and supported overseas examples using small-bodied fish species as sentinels.

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Table of Contents

List of Figures

List of Tables

List of Plates

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Table of Contents

List of Figures

List of Tables

List of Plates

Chapter One: General Introduction

1.0 Introduction and study design

As long as humans impact freshwater environments there will be an interest and need to assess those impacts. Research on impacts on freshwater ecosystems now has tools to assess everything from water quality to fish. There are a number of ways that impacts and causal relationships can be investigated as summarized by Adams (2003) they are: (1) controlled laboratory exposures; (2) field microcosm and mesocosm exposures; (3) field observations of exposure and response including gradient studies at multiple levels of biological organisation; (4) mathematical simulation modelling; (5) statistical associations and approaches; (6) combinations of the previous approaches; and (7) weight or strength of evidence investigations. The approach taken in this thesis was to assess anthropogenic impacts of multiple anthropogenic discharges on wild populations of freshwater fish in a large New Zealand river using a comprehensive suite of biomarkers and bioindicators as described in (3) above. Strengths of this approach are that fish are assessed in the environment that they must live, feed, and reproduce in, so impacts on any aspect of their physiology and ecology should be reflected in captured fish. Captured fish will also be responding to all aspects of the discharge and any extraneous compounding or ameliorating anthropogenic or natural influences. However, it should be noted that the failure to consider extraneous influences, many of which are difficult to identify, can also be a major weakness of field studies. To maximise our ability to detect effects and consider extraneous influences I built on the experience gained in field assessments of fish health overseas for example Munkittrick (1992) and Munkittrick et al. (2000), and methods that have been refined in the national assessment of the pulp and paper environmental effects in Canada (Lowell et al., 2005) or Environmental Effects Monitoring (EEM). I also applied knowledge derived from previous assessment of fish health in the Waikato River (West et al., 1994, Richardson et al., 1996). By concurrently assessing the level of response of the same fish species to four types of discharges I hypothesised that I could infer the relative magnitude of the effects of respective discharges. It also allowed us to examine fish responses for consistent patterns to common components of the discharges, for example heat. Consistent responses of different fish species to discharges with common components would provide further evidence for establishing cause and effect. Key components of study design considered were: selection of indicator species; reference sites; timing of sampling; sample size; and sampling level (Munkittrick, 1992)

1.0.1 Selection of species

While the fish community of the Waikato River is quite diverse by New Zealand standards with 36 species being recorded as present in the New Zealand Freshwater Fish Database by 30 November 2005, only 15 species are present above the Karapiro Dam and of the 10 with more than 2 records, only 5 are native species. Brown bullhead catfish (*Ameiurus nebulosus,* (LeSueur, 1819)), shortfin eel (*Anguilla australis* Richardson 1848), common bully (*Gobiomorphus cotidianus* McDowall, 1975) and common smelt (*Retropinna retropinna* (Richardson, 1848)) were short-listed for investigation as indicator species. Sampling was subsequently carried out for all short-listed species except common smelt. There are a number of reasons smelt were not sampled, they: "are small, fragile fish" (Ward and Boubee, 1996) and have an extreme stress response to handling (Neilson, 1996). The main reason that became obvious in sampling other fish was the time taken to necropsy minimum numbers (20) of males and females. The length of time between capture and necropsy would have resulted in high mortality of smelt before sampling. The even smaller size of lake populations of smelt (Northcote and Ward, 1985) present at five of the sites in hydroelectric impoundments would have lengthened necropsy times and made viable blood sampling unlikely.

By choosing these three contrasting species I hypothesised that their range in size and ecology would alter their responses to discharges but that consistent patterns would emerge where sub lethal toxicity was significant. Individual values and results of each of the three species sampled are detailed in chapters 3-5 and responses compared in chapter 6.

1.0.2 Reference sites

To assess individual effects of any one type of discharge, it would be desirable to locate reference sites on an identical un-impacted river so effects can be assessed without confounding discharges or habitat effects. Significant wastewater discharges in New Zealand generally only occur on larger rivers, and the small and varied geography of New Zealand limits numbers of comparable large rivers. The potential for cumulative effects in large rivers is also increased due greater numbers of discharges. By sampling paired reference and discharge sites along the Waikato River I believe fish were captured from similar habitat and that I could also make some assessment of cumulative impacts at the downstream sites. As two discharges targeted for study were located in the lacustrine impoundments in the upper to mid reaches of the river we sampled a lacustrine reference site upstream of each discharge in the same impoundment. By sampling populations from the same impoundment any impoundment effects should be evident at both sites. Reference lacustrine populations of brown bullhead and common bully were also sampled from the unpolluted Lake Taupo. Two riverine reference sites were also sampled upstream of the sewage and thermal discharges. Municipal discharges upstream of the sewage discharge site and desire to get samples from a uncontaminated river reference population necessitated locating the reference site further upstream than was desirable and habitat was not as comparable as I would have liked.

1.0.3 Timing of sampling

Information from previous Waikato River studies on brown bullhead (Patchell, 1977) and common bully (Stephens, 1982) was used to target sampling to the period immediately preceding spawning so that reproductive endpoints could be measured. Because reproductive endpoints such as fecundity are not measurable in shortfin eel in freshwater, sampling for eel was undertaken at times of highest catches and potentially worst water quality (warm temperatures and low flows). This meant that brown bullhead were sampled in October-November 2002, shortfin eel in March 2003 and common bully in November-December 2003. All species could not be collected and processed at the same time due to: the importance of capturing respective species reproductive endpoints; different fishing gear types required for different species; and logistical difficulties of processing large numbers of fish from all sites within a limited time period. This has the potential to confound comparisons of shortfin eel responses with other fish to the same discharges and is discussed in the final chapter.

1.0.4 Sample size

Because the extraction of blood and necropsy of sub-sampled fish is time-critical and time consuming there is a limit to the numbers of fish that can be processed following capture. As sexes are treated separately there is also the need to sample sufficient numbers of each. I aimed to extract blood from 12 individuals of each sex and carry out necropsies on those individuals as well as an additional 18 fish of each sex. Where possible aging structures were collected from a selection of 10-20 male and female fish spanning the size range of all fish caught.

1.0.5 Sampling level

Assessment of impacts on fish in the wild can range in level from the presence and absence of fish species and communities to sensitive molecular responses of individual fish (Adams et al., 2000). The appropriateness of different assessment levels is often determined by society values, for example, abundance of sports fish, but where scientific defensibility is the priority, the use of relevant species and endpoints can often give better assessments. An excellent example of the latter is the Canadian Environmental Effects Monitoring http://www.ec.gc.ca/eem/ where specific guidance on selection of sampling level or endpoints is given. Detection of an effect solely at the biochemical or molecular level followed by inference of a significant ecological effect requires a level of causality which is largely absent (McCarty and Munkittrick, 1996). However, the power of using a combination of: sensitive biochemical; cellular; individual; population or community measurements has been shown by several field studies, for example (Adams et al., 2000, Siligato and Böhmer, 2001). From an assessment of 8 papers in Human and Ecological Risk Assessment 9(1) 2003, Collier (2003) proposed a set of 7 criteria that, if met, would provide strong evidence of cause and effect in field studies.

I tried to sample as wide a range of levels of biological organisation as possible so that our lowest level of sampling (for example hepatic mixed-function-oxygenase (MFO) enzyme activity) could help establish cause and effect but our highest level (for example population parameters) could attribute ecological significance to the results. This approach is illustrated by location of variables I measured in spectrums of biological organisation, response speed and ecological relevance shown in Figure 1.2.

Variables were chosen that largely reflect long-term (tertiary) responses of fish to their environments and discharges, rather than variables that reflect primary (for example cortisol) or secondary stress responses (for example mobilisation of glucose reserves) (Barton et al., 2002, Iwama et al., 2006). Inclusion of the analysis of blood samples from wild caught fish requires an acknowledgment that changes due to secondary stress responses may also be evident in those samples. Possible changes relevant to the variables I measured include: increased numbers of red blood cells from splenic release and red blood swelling from catecholamines (Nikinmaa, 1990). An investigation into the possibility of these changes was undertaken by comparing brown bullhead bled in the field and in the laboratory, and results are presented in Section 2.5 of the methods and discussed in the final chapter.

Ultimately the choice of variables to be sampled in fish is also constrained by access to specialised analytical equipment and techniques at the molecular level and ecological knowledge of fish species and communities at higher levels. The utility of variables will also change over time with new information allowing variables in the lower levels of biological organisation to be linked to changes at higher levels of organisation (Figure 1.2). Correspondingly, impacts on fish populations and communities can also be linked back to impacts at lower levels of biological organisation , for example, greater susceptibility of wild salmon populations from polluted estuaries to disease (Arkoosh et al., 1998).

In this study I endeavoured to use for the first time in New Zealand, sensitive biochemical endpoints, measurements of tissue contaminants and stable isotopes, and population modelling of a range of wild caught fish species relevant to New Zealand freshwaters. I also applied these and other methods according to experimental designs shown to be scientifically defensible and capable of attributing cause and effect. Comparisons of species responses to a variety of discharges, utility of variables measured and information on life history of sampled species will aid future assessment of effects of discharges in New Zealand.

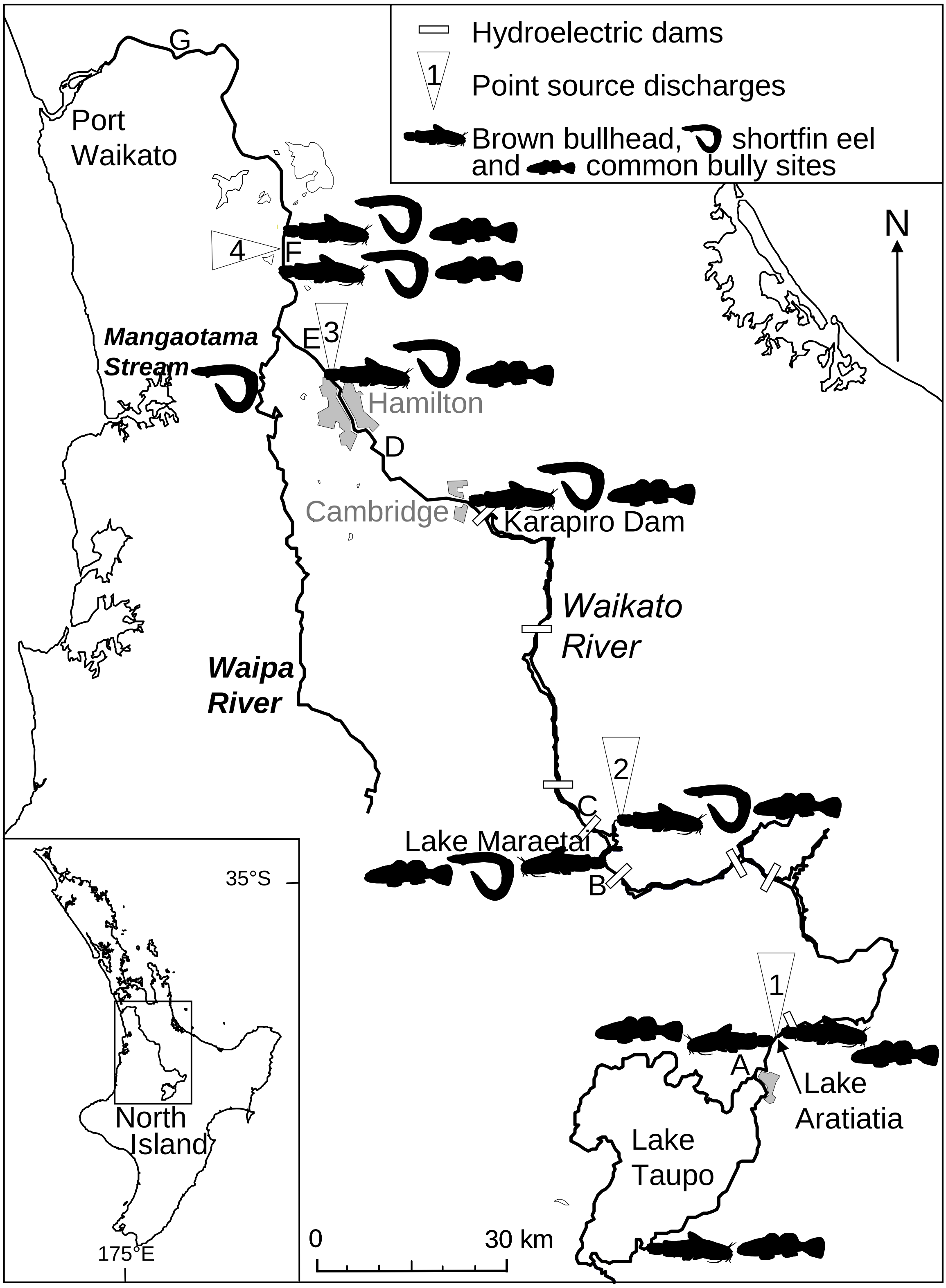

Figure 1.1. Waikato River point-source discharges, 1, Wairakei Geothermal Power Station; 2, Kinleith Pulp and Paper Mill; 3, Hamilton City Municipal Sewage; 4, Huntly Thermal Power Station. With sampling sites for species sampled indicated. Letters A-G are water quality monitoring sites referred to in Tables 3.1, 4.1 and 5.1.

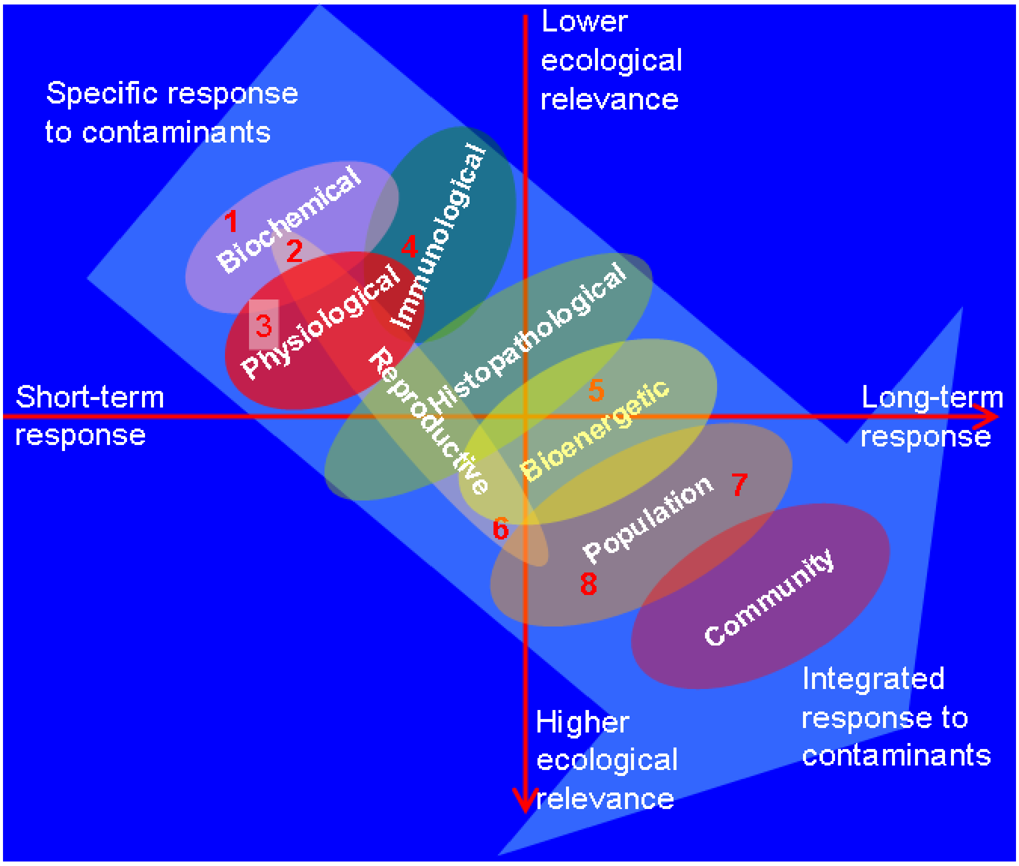

Figure 1.2. Variables sampled in fish captured from sites in the Waikato River, New Zealand. Variables are distributed across axes of response speed and ecological relevance and their relative specificity to contaminants: 1) 7-ethoxyresorufin-*O*-deethylase EROD; 2) sex steroids; 3) blood oxygen carrying capacity; 4) white blood cells; 5) liver somatic index (LSI) and condition factor; 6) fecundity; 7) population structure; and 8) growth (age). Modified from http://www.esd.ornl.gov/programs/bioindicators/whatare.htm , 17 October 2005.

Key indicators of fish population health identified by the Environmental Effects Monitoring (EEM) (Lowell et al., 2003) measured in this study were: age; size-at-age; relative gonad size; condition (body weight against length); and relative liver size (liver weight against body weight). These key indicators and measures such as fecundity were also classified in terms of fish population responses outlined in the sentinel monitoring framework of Gibbons and Munkittrick (1994).

1.1 History of fish health assessment in New Zealand

New Zealand's temperate maritime climate is characterised by high mean rainfall and a multitude of rivers which drain the mountain ranges that form the dominant landforms of all three major islands (Viner, 1987). Early industrial development utilized rivers as a convenient disposal conduit for water-borne waste and excess heat. Water quality reached a nadir in the mid 1970s but has improved in many rivers due to regulation by local government and efforts by industries to minimise their waste outputs and (Ministry for the Environment, 1997). Intensification of landuse since widespread European settlement in the 1800s has resulted in increased transport of nutrients and other land-based contaminants into rivers (Harding et al., 1999) but the diffuse and gradual nature of resulting changes in water quality means that these effects have only recently been recognised as significant impacts (Ministry for the Environment, 2004).

Sub-lethal impacts on fish still occur throughout New Zealand and compared to developed countries elsewhere, little research has been undertaken to understand the significance of these to native fish. Early investigations undertaken on impacts on native fish were largely undertaken by consultants retained by developers to evaluate environmental effects of substantial damming, extraction or discharge projects, for example, those associated with the establishment of the Huntly Power Station on the Waikato River (Boubée et al., 1990), or the Kinleith Pulp and Paper Mill discharge into Lake Maraetai on the Waikato River (Scrimgeour, 1989). Later studies for the Huntly Power Station also culminated in the publication of some fundamental studies on the biology of New Zealand native fish (for example Boubée et al. (1991) and Richardson et al. (1994)).

The introduction of the Resource Management Act (1991) (RMA) formalised impact assessment and provided a legislative structure under which effects-based assessment could be carried out. It also gave greater acknowledgement of the importance of water to Tangata Whenua and their role in managing it. A large number of assessments have been and continue to be carried out to assess impacts of activities in waterways that require resource consents under the RMA.

Published research using in situ exposure methods (caging) and feral shortfin eel has verified their ability to be used as indicators of contaminant loads (Jones et al., 1995) and as indicators of sub-lethal physiological effects (Tremblay, 2004, van den Heuvel et al., 2006). Mesocosm exposures using rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) (van den Heuvel et al., 2002, van den Heuvel and Ellis, 2002), have been used to test toxic properties of New Zealand pulp mill effluents. Biomarkers of metal exposure have been developed for common bully although further work is required before hepatic metallothione mRNA levels can be used as an indicator of metal exposure (Laurie, 2004). Effects on macro-invertebrate and fish communities of pulp mill discharges to the Waikato River have been documented by Scrimgeour (1989) and Sharples and Evans (1998), although mill modernisation over the period of the Sharples and Evans (1998) study resulted in improvements in fish communities. A predominance of larger common bully at sites receiving mill effluent was noted by Sharples and Evans which they said needed further investigation. Effects of pollution on important ecological functions such as the swimming ability of fish have begun to be investigated (Bannon and Ling, 2003, Landman et al., 2006). Fin erosion and associated pathology of goldfish exposed to pulp mill effluent in the Waikato River has been documented by Sharples et al. (1994) and Sharples and Evans (1996).

In addition to published research there is a large body of grey literature on sub-lethal impacts on New Zealand freshwater fish largely made up of regional council, consultancy and technical reports. Studies of effects of the Huntly Power Station on the lower Waikato River generated a large number of consultancy reports for example: Meredith et al (1987) on ichthyoplankton; Palmer et al. (1987) on impingement of fish and crustacean on intake screens; Stancliff et al. (1988), Boubée et al. (1990), Kusabs et al. (1990), on upstream migration and distribution of juvenile fish; Richardson et al. (1993) on temperature tolerances of aquatic fauna; and West et al. (1994) on health of fish exposed to thermal effluents. Pulp and paper mills on the Waikato and Tarawera Rivers have also generated a number of environmental effects assessment reports for example: Boubée et al. (1995) on effects of pulp mill effluent on Waikato River fish communities; Richardson et al. (1996) and Richardson and Boubée (1999) on effects on Waikato River fish health; Donald (1997), Richardson (1997b), Park and Wilding (1998) on effects on Tarawera River fish health.

Laboratory tolerance and preference studies that have relevance for sub-lethal impacts on New Zealand native fish include Richardson et al. (1994), Richardson (1997a), West et al. (1997), Rowe and Dean (1998), Dean and Richardson (1999) and Landman et al. (2005). The endocrine disrupting potential of a New Zealand pulp and paper mill effluent has also been assessed by mesocosm and laboratory exposures (Ellis, 2001) and the lethal and sub-lethal effects of pentachlorophenol on New Zealand native fish have also been researched (Hannus, 1998). Furthermore a standard laboratory method has been proposed for testing the acute toxicity of whole effluents to the New Zealand native freshwater fish (*Gobiomorphus cotidianus* McDowall, 1975) (Hall and Golding, 1998a).

1.2 Study site

From its origins on the western slopes of Mount Ruapehu (2,797 m above sea level) the Waikato River runs northwards for some 425 km to drain into the Tasman Sea on the west coast of the North Island of New Zealand. This makes it the longest river and second largest catchment (14,258 km^2) in New Zealand. The potential to generate electricity from the fast flowing waters of its upper reaches was quickly realised with a generating station being built at Hora Hora in 1913. A succession of eight dams (Figure 1.1) was subsequently constructed from 1929 to 1964 with the construction of the most downstream dam at Karapiro flooding the station at Hora Hora just upstream. Today these dams punctuate the top half of the river from its outlet at Lake Taupo to Karapiro. Land-use within the catchment ranges from exotic forestry to intensive dairy farming, and the lower Waikato basin is one of the most productive dairy areas in New Zealand. This study investigates the effects of the four largest (Table 1.1) of the approximately 80 point-source discharges to the Waikato River consented under the Resource Management Act (1991) on resident fish populations. The four point-source discharges chosen were: a geothermal power station; bleached kraft mill effluent (BKME) from a bleached kraft pulp and paper mill; 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. 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.

The location of the discharges from the upper reaches (Wairakei) to the lower reaches (Huntly thermal power station) allowed an assessment of cumulative effects in the Waikato River as a whole. However, the distance between the discharges and location of the Wairakei and Kinlieth Pulp and Paper Mill discharges in different impoundments lessons the likelihood of large confounding effects from upstream discharges.

Table 1.1. Characteristics of selected discharges including approximate volume, distance is approximate from the outlet of Lake Taupo, flow is mean river flow for October 2002 at nearest upstream Waikato Regional Council gauging site to sampling sites. Us indicates upstream. [a] (Timperley and Hill, 1997), [b] (Ray et al., 2001), [c] (Askey, 1994). [d] Mean figures for 2002, approximately half of BKME discharge volume is sourced from Kopakorahi Stream upstream of mill waste-stream.

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 & steam condensate	17.2	Heat (1000 MW), Hg (46.5 kg/a)[a], H_2S (17.2 g/s)[b], TN (1.4 g/s)[b]
Pulp & paper mill	117	253	BKME Us BKME	Bleached kraft mill effluents (BKME)	2.3[d]	Resin acids, chlorophenolic compounds, colour (38 t/d), TN (0.64 g/s), TP (55.6 kg/d), BOD (2.19 t/d), Heat
Hamilton City wastewater	227	266	Sewage Us Sewage	Sewage & municipal wastewaters	5	TP (293 kg/d), NH_4-N (871 kg/d), faecal Coliforms (<1000/ml), PAH (201 ppt), Cl_3-Cl_5 (1626 ppt)[c], heavy metals
Huntly 1000 MW thermal power station	255	471	Thermal Us Thermal	Cooling water	12	Heat[d] (742 MW)

1.2.1 Sampling sites

Photos of the nine main sampling sites selected for this study in an upstream to downstream direction follow. No photo of the tenth site (sampled for shortfin eel only) on the Mangaotama Stream (Figure 1.1) is presented. Discharge sampling sites were located in discharge plumes, mixing zones or more clearly “non-compliance zones” (Rutherford et al., 1994), to capture worse case impacts on resident fish. Effects greater than permitted by s107 of the Resource Management Act (1991) can occur in non-compliance zones.

Plate 1.1. Site named Taupo, Motuoapa Bay, southern Lake Taupo, August 2003. Map reference, NZMS260 T19 27590 62486.

Plate 1.2. Site named geothermal upstream (Us). Upstream site on true left hand bank of Lake Aratiatia, looking upstream in August 2003. Map reference, NZMS260 U17 27794 62807.

Plate 1.3. Site named geothermal, looking downstream of Wairakei Geothermal Power Station on true left bank of Lake Aratiatia, in August 2003. Map reference, NZMS260 U17 27817 62811.

Plate 1.4. Site named bleached kraft mill effluent upstream (BKME Us), on true right bank of Lake Maraetai, looking upstream in August 2003. Map reference, NZMS260 T17 27522 63093.

Plate 1.5. Site named bleached kraft mill effluent (BKME), in Kopakorahi Arm on true right bank of Lake Maraetai, looking downstream past 1st aerator towards Lake Maraetai in August 2003. Map reference, NZMS260 T16 27540 63143.

Plate 1.6. Site named sewage upstream true left bank looking downstream in August 2003. Note water level is approximately 1-2 m higher compared to when sampling was carried out. Map reference, NZMS260 T15 27332 63613.

Plate 1.7. Site named sewage true left bank in August 2003. Map reference, NZMS260 S14 27070 63834.

Plate 1.8. Site named thermal upstream, looking upstream on true right bank of island upstream of Tainui bridge in August 2003. Map reference, NZMS260 S13 27007 64003.

Plate 1.9. Site named thermal, looking upstream behind island on true left bank in August 2003. Map reference, NZMS260 S13 27008 64060.

Chapter Two: Methods

2.0 Site selection

Existing information on effluent mixing was used to ensure fish were caught from within discharge plumes. Counts of fish captured in nets 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.

2.1 Environmental variables

Water temperatures at each site (except the Mangaotama Stream shortfin eel reference site) were recorded at 2 hourly intervals using TidBit™ data loggers (Onset Computer Corporation, Pocasset, Massachusetts, USA) set in 1-2 m water depth as near as possible to where fish were captured. Loggers were deployed approximately 1 month prior to sampling and records from one year used to calculate degree days for each site. Due to 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. Summary information for water quality variables for relevant periods was obtained from Smith (2003) (Tables 3.1, 4.1 and 5.1). To compare site temperatures and the optimum growth temperature of brown bullhead (Keast, 1985), total degree days above 10°C were calculated for each site for the year prior to sampling (Table 3.1). Similarly to compare site temperatures with the preferred temperature of shortfin eel (Richardson et al., 1994) and minimum temperature for shortfin growth of 9°C (Graynoth and Taylor, 2000), total degree days above 9^{o}C were calculated for each site (Table 4.1). As optimum temperatures for growth are not known for common bully total degree days calculated as for brown bullhead and shortfin eel sites are reported (Table 5.1).

2.2 Capture

2.2.1 Brown bullhead

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 margins of lake or river and retrieved the following morning (16.8 ± 0.13 hours 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.

2.2.2 Shortfin eel

To capture shortfin eel, between 7 and 20 coarse mesh fyke nets (13 mm mesh with a single 2 m long leader) were set per site in the late afternoon between March 4 and March 17 2003 along the water margins and retrieved the following morning after an mean of 16.8 ± 0.13 hours fishing. In addition to coarse mesh fyke nets, eight and five fine mesh fyke nets (6 mm mesh with a single 2 m long leader) were set at BKME and BKME upstream sites respectively. On examination of catches from fine meshed nets it was found they contained three 25-28 cm shortfin eel that were smaller than eels from other sites so these small eels were excluded from further analysis. As shortfin eel populations could be influenced by densities of longfin eel, total number and estimated biomass (mean site shortfin eel weight multiplied by number of eel) of shortfin and longfin eel per net was used to give catch per unit effort (CPUE) of eel for comparison between sites. Numbers of other fish per net were recorded but fish were not measured.

2.2.3 Common bully

Beach seines after dark were carried out along the margins of lake or river sites as previous studies, for example Stephens (1982) have found common bully, especially larger individuals, to be most abundant along river and stream margins at night. Due to lack of suitable beaches, low water transparency, and presence of woody debris at the BKME site in the Kopakorahi arm of Lake Maraetai minnow traps were used to capture common bully from this site and its upstream reference site. Sampling for common bully was carried out at all sites between November 17 and December 9, 2003, with the exception of some additional common bully captured from the sewage upstream site on December 16, 2003. Sampling time was chosen to precede spawning so that reproductive endpoints could be measured. Counts of fish captured were used to calculate catch per unit effort (CPUE, fish per net per night) at the BKME sites.

2.3 Fish processing

Every effort was made to minimize time between removal of fish from nets and necropsy to lesson effect of further stress on variables measured. Handling of fish from paired sites was also kept consistent to ensure the validity of comparisons between paired sites. For brown bullhead we were able to process fish from Taupo, geothermal and BKME paired sites immediately on shore after collection from nets. The remaining brown bullhead, shortfin eel and common bully were transported back to the laboratory within 2 hours and processed.

2.3.1 Necropsy

Brown bullhead were killed by a blow to the head, weighed, measured (total length ± 1.0 mm), and blood extracted via a heparinised 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. Full necropsies for blood and all other biochemical and chemical analyses were conducted on the first 12 shortfin eel.

Shortfin eel were immobilized by immersion in an ice slurry and blood extracted via a heparinised syringe from the ventral caudal vein. Immediately after blood extraction, eel were killed by decapitation.

Common bully were killed by an overdose of 0.1 g/l ethyl aminobenzoate (benzocaine), weighed (± 0.01 g), measured (total length ±1.0 mm), and blood was extracted from the caudal vein using a heparinised 0.5ml syringe. Full necropsies for blood and other variables were conducted on the first 12 adult male and 12 adult female common bully. Where numbers caught were sufficient, necropsies on a further 10 male and 10 female common bully were carried out for somatic indices, age, and fecundity estimates. Dissected viscera, liver, spleen, and gonads were weighed (± 0.0001g).

Dissected brown bullhead and shortfin eel viscera, liver, spleen, and gonads were weighed (± 0.01g). Portions of liver (1-2 g) from fully necropsied brown bullheads and shortfin eel were stored in liquid nitrogen for 7-ethoxyresorufin-*O*-deethylase (EROD). Gall bladders (bile) from necropsied brown bullhead and shortfin eel were also frozen in liquid nitrogen when they could be dissected intact.

2.3.2 Reproductive endpoints

In brown bullhead and common bully gonad development was assessed and ovaries were split and preserved in 10% formalin. Adult male common bully were observed to have large seminal vesicles or glands which were removed from testes before testes were weighed for calculation of gonadosomatic index (GSI). Sexually maturing female brown bullhead and common bully 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 further examination. Ovaries from adult female common bully with a GSI greater than 5 were chosen for comparisons of fecundity. For fecundity estimates, a 1.00 to 3.00g or 0.05 to 0.14 g slice of ovarian tissue was removed from the middle an ovary for brown bullhead and common bully respectively, follicles were separated (> 100 follicles), and photographed. Image analysis (Image-Pro® Plus, Media Cybernetics, Inc., Maryland, USA) was used to count and measure mean diameter of all follicles in sub-sample (n=400-1500 and 441-2530 follicles for brown bullhead and common bully respectively). Histograms of follicle sizes were then examined and where 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 follicles/g of body weight.

2.3.3 Aging

Fish carcasses were frozen and the fifth vertebra and otoliths were later removed from sub-sampled fish and a selection of smaller size classes if caught. Brown bullhead vertebrae were cleaned and aged as outlined in (Appelget and Smith, 1950). Sagittal otoliths from shortfin eel were aged by experienced staff at the National Institute of Water and Atmospheric Research in Christchurch, New Zealand using methods outlined in (Graynoth, 1999). Common bully sagittal otoliths were removed, cleaned, and stored dry until mounting in crystal bond, grinding (two sides for most adult fish) and counting of annuli.

To verify zones counted as annual growth increments on common bully otoliths were annuli, young-of-year (YOY) common bully with a mean total length of 29 mm and adult common bully with a mean total length of 76 mm were collected from the Waikato River at Hamilton. Captured adult bullies were either immersed in 125 mg/L or 250 mg/L of calcein blue or oxytetracycline respectively, or injected with 25 mg/kg of calcein blue or oxytetracycline in a bacteriostatic solution of 0.9% sodium chloride at a volume of 1 ml/kg. The small size of YOY common bully meant that they could not be injected so they were immersed in fluorophor solutions. Five YOY and adult fish were immersed and five adult fish injected with each of the fluorophors and placed into two separate outside 2000 L tanks, aerated and fed with rainwater. Water temperatures in tanks recorded at 2 hourly intervals using TidBit™ data loggers were almost identical, dropping to a minimum of 6-8°C in July to August (winter) and rising to a maximum of 24°C in February (summer). In addition to naturally occurring food from macrophytes in the tanks, fish were fed approximately every second day with frozen blood worms. After one year, 27 surviving fish out of the original 30 were captured from the tanks. Fish were measured, weighed and otoliths were extracted. Ground otoliths were examined for marks using 470 nm and 350 nm excitation for calcein blue and oxytetracycline respectively and compared to otoliths taken from fish of same initial size sacrificed at start of experiment.

After one year, YOY growth calculated from cohorts in fish from each tank were 41 and 45 mm. Adult growth was much slower with mean growth of 8 and 15 mm per year in the two tanks. Comparisons of otolith size from cohorts of fish followed the same pattern with young of the year fish otolith doubling in diameter and adult fish otoliths only increasing in diameter by 20-30%. Annuli of YOY and adult treatment fish aged at the start of the experiment matched position of inner annuli on fish sacrificed after one years growth. Otoliths of seven treated adult and one YOY common bully were found to have fluorescent marks. Only one common bully immersed in oxytetracycline as an adult showed a clear fluorescence under 350 nm UV light at 5x magnification. The almost complete band of fluorescence was aligned with the beginning of an opaque zone which corresponds with immersion of fish in late autumn, just before the onset of winter. The marks on the remaining marked otoliths were only clear at 10x or 20x magnification, but marks were aligned with onset of an opaque zone. No effect of type of fluorophor or exposure method on marking success was evident.

2.4 Tissue contaminant analysis

For metal analysis, four homogenized liver samples (each approximately 1.5 g wet weight) from male brown bullhead were analyzed per site. One gram skinless segments of flesh from the middle of the dorsal fillet of 12 eel necropsied per site were removed using clean plastic utensils, combined then homogenized for subsequent metal analysis. A suite of 33 metals were determined by nitric and hydrochloric acid digestion and ICP-MS (Inductively Coupled Plasma-Mass Spectrometer) or ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) following USEPA method 200.3 (except that HCl was used instead of H_2O_2) at a commercial laboratory (Hill Laboratories, Hamilton, New Zealand). Metal analysis was not preformed on common bully.

Pulp and paper-related organics in bile were determined according to methods in (van den Heuvel et al., 2002). Hydrolysed bile (ethanolic KOH digestion) was acidified and extracted with methyl tertiary-butyl ether, derivatised by silylation and analyzed by GC-MS (Gas Chromatograph-Mass Spectrometer). Samples were corrected for surrogate recovery and blank determinations. Bile samples (diluted 1:1600 with ethanol) were analysed for PAH (Polycyclic Aromatic Hydrocarbons) equivalent concentrations using fixed wavelength fluorescence (Aas et al., 2000) at excitation/emission wavelengths: pyrene 341/383, naphthalene 290/335, benzo[*a*]pyrene 380/430, and retene 302/372.

2.5 Biochemical and blood measurements

Brown bullhead and shortfin eel hepatic mixed-function-oxygenase (MFO) enzyme activity was estimated in post-mitochondrial supernatant (PMS) as 7-ethoxyresorufin-*O*-deethylase (EROD) activity using a modification of the fluorescence plate-reader technique outlined by (van den Heuvel et al., 1995). Liver extracts were homogenized in a cryopreservative buffer (0.1 M phosphate, 1 mM EDTA, 1 mM dithiothreitol, and 20% glycerol, pH 7.4) and spun at 9000 x g to obtain the supernatant. Total protein content was estimated from fluorescamine (Sigma) fluorescence (390 nm excitation, 460 nm emission filters) against bovine serum albumin (Sigma).The EROD reaction mixture contained 0.1 M HEPES buffer pH 7.8 (Sigma, St. Louis, MO, USA), 5.0 mM Mg^{2+}, 0.5 mM NADPH (Applichem, Darmstadt, Germany), 1.5 µM 7-ethoxyresorufin (Sigma), and about 0.5 mg/ml of PMS protein. The EROD activity was determined kinetically in 96-well plates using one reading every minute for 10 minutes on a BMG Polarstar Galaxy microplate fluorometer (BMG Labtechnologies, Offenburg, Germany). Resorufin was determined using 544 nm excitation and 590 nm emission filters.

Sex steroids in brown bullhead were determined using radioimmunoassay (RIA) according to methods of McMaster et al. (1992) . Blood plasma samples were thawed and steroid hormones were extracted with diethyl ether. The steroids, testosterone, estradiol and 11-ketotestosterone, were obtained from Sigma (St. Louis, MO, USA). 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, England) and tritiated 11-ketotestosterone was a custom synthesis purchased from the US Geological Service. The plasma extract from females was analyzed for estradiol and testosterone while that from males was analyzed for 11-ketotestosterone and testosterone using standard radioimmunoassay (RIA) procedures. Fish from sewage and thermal sites were transported to the laboratory before necropsy, but previous studies that have examined the effect of transport on the brown bullhead steroids I measured (Burke et al., 1984, Rosenblum et al., 1987) have shown no significant changes due to transportation.

Haematocrit (Hct, packed cell volume of red blood cells) and leucocrit (Lct, packed cell volume of white blood cells as represented by the "buffy" layer) were measured and expressed as per cent of total blood volume by the microcapillary method. Four µl of whole blood were added to 1 ml of Drabkin's solution and whole blood haemoglobin determined by absorbance at 540 nm using a Shimadzu UV1601 spectrophotometer. A volume of 2 µl of whole blood was mixed with 198 µl of red blood cell (RBC) diluting fluid (Dacie and Lewis, 1991) and placed on ice. Red blood cell counts were made using images of RBC on a hemocytometer captured (within one to two days of blood collection) at 10x magnification using AxioVision software and AxioCam HRc camera mounted on a Leica DMRD microscope. ImagePro Plus (Media Cybernetics, Washington, MD, USA) software was used to count cells after appropriate enhancement and filtering of images. Hematometric indices (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)) were calculated according to (Wintrobe, 1934). Some assessment of the effect of additional transport stress on blood-related variables was possible by comparing brown bullhead bled on the lake shore and those transported back to the laboratory. Comparisons of male and female brown bullhead spleen size, haematocrit, red blood cell counts, leucocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) from reference site, lake-bled fish and lab-bled fish were made using the non-parametric Mann-Whitney *U* Test with n=29 and 39 and n=30 and 39 for males and females respectively (Figure 2.1). A number of significant differences were found which are consistent with the additional stress of transport or increased time from removal from nets to blood sampling. For example transported fish had comparatively smaller spleens when processed (Figure 2.1) which could be explained by additional release of red blood cells from the spleen before blood extraction. These results are discussed in the final chapter.

2.6 Statistical analysis

The following indexes were calculated for graphical comparisons of all sub-sampled fish between sites: Fulton condition factor (*K*) = (fish weight (g)/(total length (cm)^3)) x 100, liver somatic index (LSI) = (liver weight (g)/(fish weight-liver weight)) x 100. Spleen and gonadosomatic indices (GSI) were calculated using the same formula as LSI but substituting spleen and gonad weights, respectively. Statistical testing of differences between paired site mean values of: liver size; spleen size; gonad size; fecundity; and follicle size were carried out by analysis of covariance with site as factor, and adjusted body weight (fish weight-organ weight) as covariate. Variables compared using analysis of variance (ANOVA) or analysis of covariance (ANCOVA) were tested for normality and homogeneity of variances, using Shapiro-Wilk's W and Hartley F-max statistic, Cochran C statistic, and the Bartlett Chi-square tests respectively (StatSoft, Inc. (2006). STATISTICA (data analysis software system), version 7.1. www.statsoft.com). Prior to statistical comparisons, fish variables were log transformed (except for EROD which required log10 transformation) where assumption of homogeneity of variance could not be met. Statistical comparisons of brown bullhead liver metal levels between paired sites were carried out using an ANCOVA with mean age as a covariate but mean age was never a significant covariate. 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 male brown bullhead from paired sites were also limited to mature males older than one year with 11-ketotestosterone levels higher than 0.36 ng/mL. For organ weights and condition factor, for which ANCOVA was used, interactions between variables were investigated in order to test the assumption of homogeneity of slopes. As seen in a previous study of brown bullhead (Rosenblum et al., 1987) positive relationships between plasma testosterone and GSI, and 17b-estradiol levels and GSI were found, and GSI was included as a covariate in statistical comparisons of sex steroids. Where samples were unsuitable for comparison using parametric tests, the non-parametric Mann-Whitney *U* Test was used.

Length at age relationships were modeled using a modified von Bertalanffy equation of the following form: Length = L_∞ x (1 – 0.96 x e$^{(-k\ Age\^{}1.2)}$) where L_∞ 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 of (Chen et al., 1992).

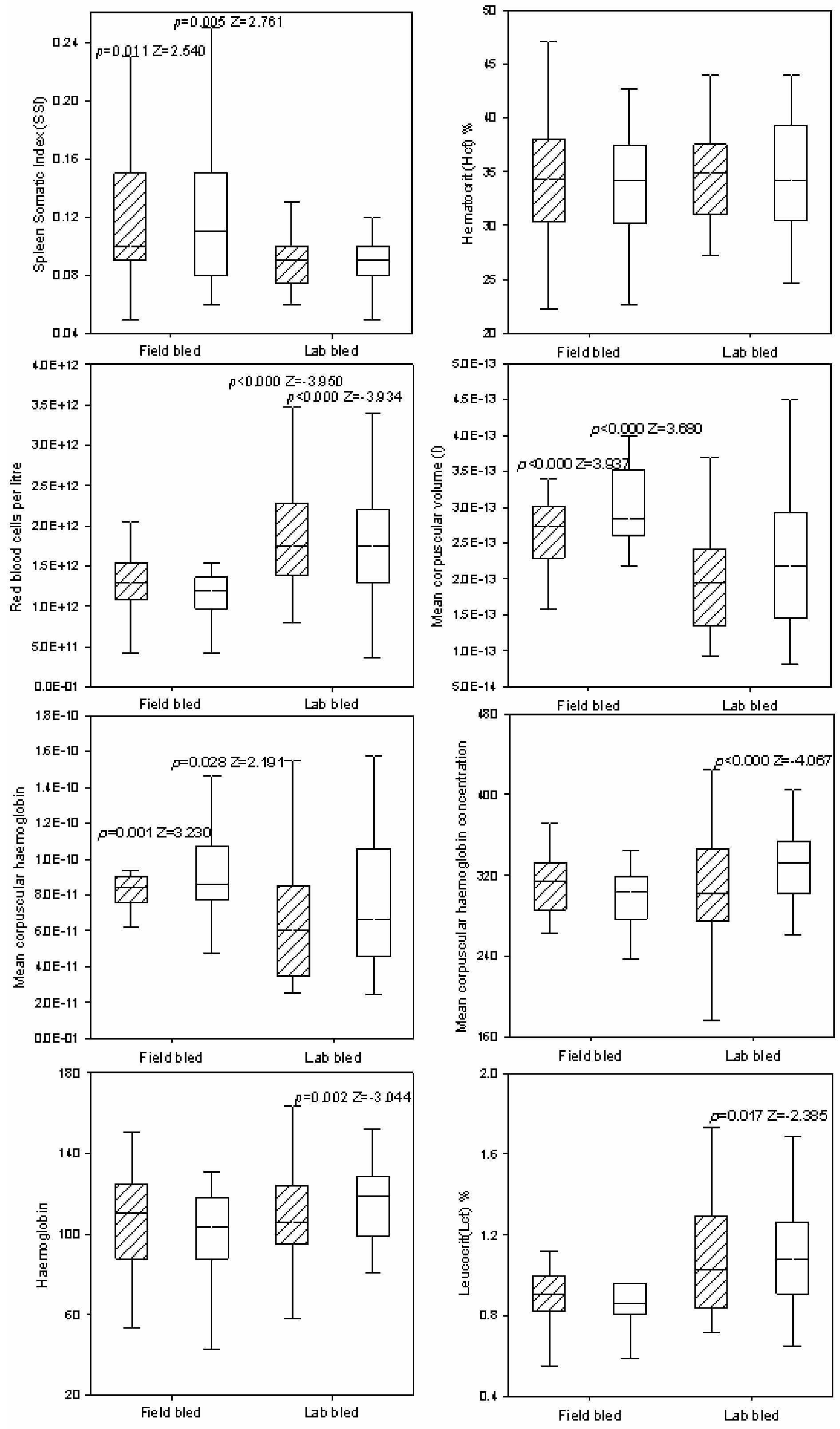

Figure 2.1. Comparison of blood variables from field and lab bled brown bullhead. Hashed bars are males and blank are females. Whiskers are non-outlier range, boxes inter-quartile range and line is the median. Significant Mann-Whitney *U* Test comparisons are labelled with *p* and Z values.

Chapter Three: Cumulative impacts assessment along the Waikato River, using brown bullhead catfish (*Ameiurus nebulosus*) populations[1]

3.0 Abstract

The effects of point-source and diffuse discharges on resident populations of brown bullhead catfish (*Ameiurus nebulosus,* (LeSueur, 1819)) in the Waikato River were assessed at sites upstream and downstream of point-source discharges. At each site, the population parameters, relative abundance and age structure and individual indices such as condition factor, and 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 indicators (bile chemistry and liver metals) of exposure or response were also 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 (BKME) site showed elevated levels of EROD, decreased and increased numbers of red and white blood cells respectively, and depressed levels of sex steroids. However, growth rates, condition factor, age structure, and gonadosomatic index (GSI) suggest that discharges with significant heat or nutrients benefit brown bullhead despite physiological impairment at one site. Consideration of brown bullhead population responses to discharges in a monitoring framework revealed three different population response patterns due to the point-source discharges.

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3.1 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 to tease apart contaminant-mediated impacts from the multitude of other ecological variables. Despite the uncertainties surrounding population responses, effects-based monitoring methods are increasingly taking their place alongside more traditional, risk-based methods for the protection of the environment. There are numerous examples of large scale investigations of this type (Munkittrick et al., 2000, Schmitt, 2002), and effects-based tools employing adult fish surveys have been legislatively implemented at a national-scale Environmental Effects Monitoring Program (Lowell et al., 2003).

In New Zealand, the use of macroinvertebrate communities for effects-based monitoring is well established (Hickey, 1995). However, there is a paucity of data on environmental impacts on fish populations. This is largely due to 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 (Jones et al., 1995, Tremblay, 2004), mesocosm exposures (van den Heuvel et al., 2002, van den Heuvel and Ellis, 2002), and wild fish monitoring using fish community and health indices (Scrimgeour, 1989, Richardson et al., 1996).

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 (Colby, 1984). This concept was further expanded to include contaminant stress in the model of population response patterns (Munkittrick and Dixon, 1989). This population monitoring framework was further refined to categorize fisheries variables into measures of age structure, energy storage, and energy allocation (Gibbons and Munkittrick, 1994). By recognizing specific patterns of response, e.g. to eutrophication, this framework can be semi-diagnostic for the cause of population changes. By pairing such a population framework with biochemical and chemical measures of exposure and effect, potential contaminant effects can either be ruled out, or can point the way to further diagnostic studies. The fish monitoring framework has only recently been used to study cumulative changes due to multiple environmental disturbances (Munkittrick et al., 2000) and there are few studies on cumulative impacts along large rivers.

The purpose of this study was to conduct an evaluation of 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 was known to be present at all sites, is a pest in New Zealand and therefore has no ethical restrictions on numbers able to be caught, and is larger than most native fish present at sites. It is also a robust, benthic species, that does not migrate to spawn, and is not commercially exploited to any significant extent. Brown bullhead have also been studied in a large number of North American impact assessments (Arcand-Hoy and Metcalfe, 1999, Pinkney et al., 2004).

The Waikato River provides an excellent testing ground to further investigate the relationship between stressors and feral fish responses due to the diverse sources of pollution in the river. These pollution sources include: industrial effluent; sewage effluent; storm water runoff; geothermal fluid discharges; dairy farm effluent; and other agricultural land use discharges. 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 in the upstream reaches of the river and prevent access for migratory fish. Due to the number of impacts present along the length of the river, this study allowed for the examination of the hypothesis that impacts may be cumulative due to additive degradation in environmental quality along the river. In addition, I aimed to distinguish individual discharge effects from cumulative effects by sampling brown bullhead at multiple levels of biological organisation upstream and downstream of the four largest discharges along the Waikato River.

3.2 Results

3.2.1 Site physicochemical characteristics

Total degree days above 10°C for the geothermal, BKME and thermal discharge sites were 25-50% higher than their respective upstream sites (Table 3.1). Increases in temperature were mostly evident through autumn, winter, and spring. Temperatures recorded during fish sampling (spring) were two to four degrees warmer than upstream sites (Table 3.1). These temperature increases were limited to the effluent plumes and though there was a change in the temperature of the river between Lake Aratiatia upstream and Lake Maraetai upstream, there was no overall temperature increase evident along the total length of river sampled. Wide fluctuations in water level were noted at the geothermal and sewage upstream sites during sampling due to hydroelectric flow management. There was a gradual increase in nutrients and primary productivity and a reduction in water clarity along most of the length of the river (Table 3.1).

3.2.2 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 3.2). 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. As and Hg had decreased to background levels from high levels at the geothermal site before reaching riverine sites (Table 3.2). Fish at the sewage site had metal loads typical of the sewage discharges (Askey, 1994). Resin acids typical of bleached kraft mill effluent (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 than fish from the main stem of the river, providing further evidence that there was little mixing occurring between reference and discharge fish (Table 3.2). Due to the number of diffuse inputs, metal contaminant data was 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 primarily sourced from this catchment. Other than metals noted above, contaminants in fish due to point-source discharges were only detectable near discharges and did not persist or accumulate with increasing distance downstream. Only males from the BKME site had significantly elevated EROD levels compared to the upstream reference site (Figure 3.1), EROD levels at all other sites were similar.

Table 3.1. River and site physicochemical characteristics. Degree is total degree days above 10°C for year from 24/9/02. Us = upstream reference. [a] Temperature at time of sampling. [b] A-G, 5 year median values for selected water quality measurements recorded by the Waikato Regional Council at Waikato River locations (A-G Figure 1.1) (Smith, 2003). [c] value not available for site, figure shown is from 30 km downstream.

Site name	Water temperature (°C)				DO	N	P	Chlorophyll	Black
	Degree	Max	Min	Median	(%)	(g/m^3)	(g/m^3)	*a* (g/m^3)	disc
Taupo	1847	23.7	8.2	14.0[a]	98.9[a]	-	-	-	-
A[b], control gates	-	-	-	14.3	101.4	0.07	0.006	0.002	>5.41[c]
Geothermal Us	1868	20.7	11	12.1[a]	121.2[a]	-	-	-	-
Geothermal	2816	26.1	12.5	15.2[a]	121.0[a]	-	-	-	-
B[b], Whakamaru	-	-	-	16.4	103.8	0.19	0.021	0.008	2.6
BKME Us	2173	23.7	9.7	13.5[a]	124.0[a]	-	-	-	-
BKME	2709	23.0	11.5	18.0[a]	22.0[a]	-	-	-	-
C[b], Waipapa	-	-	-	16.3	103.0	0.25	0.026	0.008	2.6
Sewage Us	2198	21.4	10.5	13.5[a]	110.0[a]	-	-	-	-
Sewage	2243	21.6	10.5	13.6[a]	108.8[a]	-	-	-	-
D[b], Narrows	-	-	-	16.2	100.5	0.35	0.032	0.010	1.6
E[b], Horotiu	-	-	-	16.3	99.6	0.41	0.043	0.012	1.4
Thermal Us	2187	22.0	9.7	14.1[a]	107.4[a]	-	-	-	-
Thermal	2862	23.5	11.8	16.1[a]	104.3[a]	-	-	-	-
F[b], Huntly-	-	-	-	16.3	97.1	0.55	0.059	0.013	0.9
G[b], Tuakau	-	-	-	17.0	100.8	0.60	0.068	0.019	0.7

Table 3.2. Mean trace metals (wet weights) in male brown bullhead livers, mean resin acids and pooled polycyclic aromatic hydrocarbons (PAHs); naphthalene (nap), pyrene (pyr), benzo[*a*]pyrene (B[*a*]P), and retene (ret) in bile of sub-sampled brown bullhead from sites in the Waikato River, New Zealand. Us = upstream reference. Also given is mean age of fish pooled for liver metal assay.

Site name	Age (yr)	Liver trace metals (mg/kg wet weight)										Bile				
												Resin acids (µg/g d.w.)	PAHs (µg/mL)			
		Ag	As	Cd	Co	Cu	Hg	Li	Pb	Se	Zn		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	1053	183.4	16.4	2.2	1266.9
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

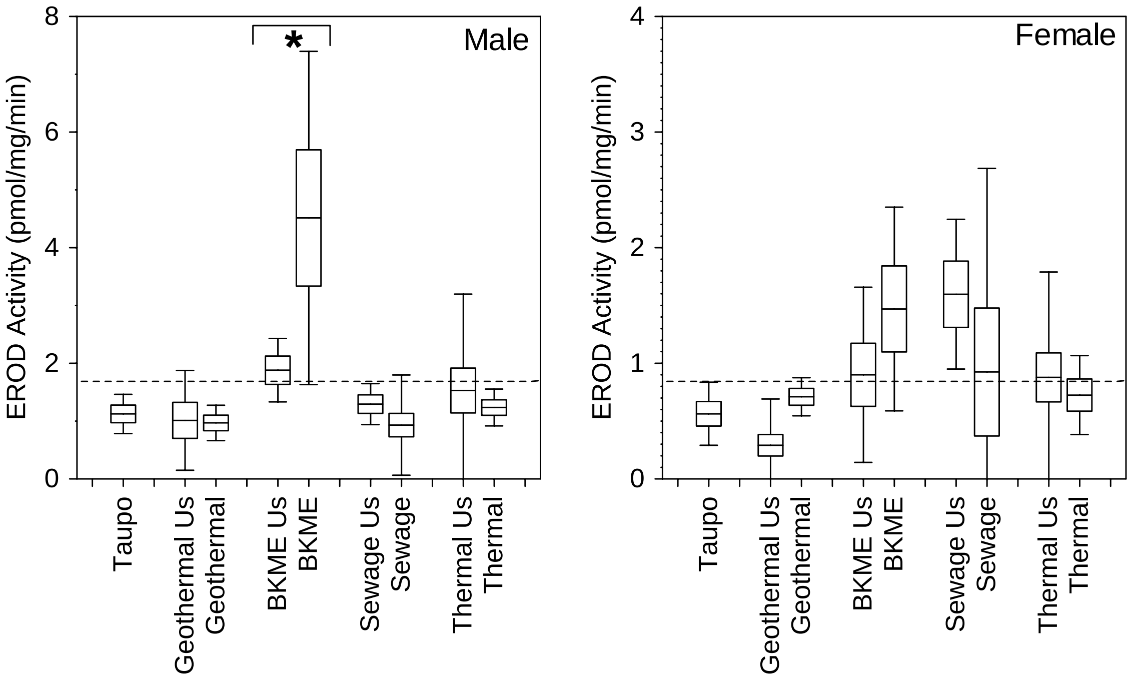

Figure 3.1. Liver detoxification enzymes (EROD) in mature male (left) and female (right) brown bullhead from the Waikato River, New Zealand. Boxes are mean ± SE and whiskers are 95% confidence intervals. Asterisk indicates pairs of values significantly different at * $p = 0.007$. Dashed horizontal line is river mean.

3.2.3 Blood variables

Maturity as determined by gonad size and sex steroid levels had no significant effect on blood parameters measured, so results from all sub-sampled fish are shown. Total haemoglobin in females was higher at all warmer sites except BKME (Table 3.3) where haematocrit (Hct) was significantly lower than the reference site. Leucocrit (Lct) values were significantly elevated in male and female brown bullhead at the BKME and sewage upstream sites (Table 3.3). 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 Hct, red blood cell counts, and Lct.

Table 3.3. Mean blood parameters, (Haematocrit (Hct), red blood cell count (RBC), mean cell volume (MCV), haemoglobin (Hb), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and leucocrit (Lct)) in brown bullhead from the Waikato River, New Zealand. Us = Upstream reference. Differences between paired sites ($p < 0.05$, ANOVA) are denoted by different superscript letters.

Site	No.		Hct (%)		RBC (cells/L x 10^{13})		MCV (L x 10^{-12})		Hb (g/L)		MCH (g/cell x 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

3.2.4 Catch rates

The only significant difference in catch per unit effort (upstream versus downstream) was at the thermal site ($p = 0.006$, Mann-Whitney *U* Test) (Table 3.4). Overall, Lake Taupo and both the BKME sites had higher CPUE than other sites and BKME sites were significantly higher ($p \leq 0.01$) than all sites except Lake Taupo and thermal sites. Catch per unit effort at most riverine sites was lower than upstream lacustrine sites.

3.2.5 Length frequency, age and growth

Large numbers of small brown bullhead were caught at the BKME and thermal sites (Figure 3.2) and the mean lengths of all fish were significantly different from the respective upstream sites ($p < 0.001$, Mann-Whitney *U* Test). This difference was strongly influenced by the smallest size class and indicates that these populations were skewed towards younger fish or conversely other sites were lacking young fish. Mean lengths of brown 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 mean. Lengths of sub-sampled fish were similar except for the thermal upstream site where the low number of large brown bullhead caught limits comparisons (Table 3.5). Ages of sub-sampled fish were also similar at paired sites except for thermal sites. Fish from the sewage sites were younger and geothermal fish older than the river mean (Table 3.5). Age-size relationships of males and females were not found to be significantly different for any of the nine sites compared using the residual sums of squares method of Chen et al. (1992) ($p = 0.408$ to 1.000). 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 3.5). This effect was largely due to the greater maximum length (L_∞, Table 3.5) at the BKME site and lower maximum length at the thermal site. Cumulative effects were not apparent over longitudinal range of sites we sampled.

Table 3.4. Total number of brown bullhead caught, mean catch per unit effort (CPUE, fish/net/night), fecundity/kg, follicle diameter, maximum length (L_{∞}) and growth constant (k) calculated using combined ages of males and females and a modified von Bertalanffy equation for sub-sampled fish from discharge and upstream reference (Us) sites. Values are given as mean ± SE. Numbers in brackets are total n where different to sub-sampled n. Differences between paired sites ($p < 0.05$, $^{A, B}$ residual sum of squares procedure (Chen et al., 1992), ^{C} Mann-Whitney *U* Test) are denoted by superscript letters.

	Total No.	CPUE	Fecundity/kg	Fecundity	Follicle diameter (mm)	L_{∞} (cm)	k
River average	1086	9.5 ± 3.3	13135 ± 402 (81)	4832 ± 183 (81)	2.17 ± 0.04	-	-
Taupo	158	19.8 ± 7.8	9728 ± 1134 (8)	2232 ± 290 (8)	1.72 ± 0.10	29	0.36
Geothermal Us	22	1.4 ± 0.4	11743 ± 1481 (7)	5798 ± 638 (7)	2.24 ± 0.11	33	0.68
Geothermal	39	2.4 ± 0.6	10079 ± 581 (14)	5904 ± 331 (14)	2.37 ± 0.08	35	0.82
BKME Us	443	21.1 ± 5.5	14417 ± 593 (14)	4796 ± 304 (14)	2.06 ± 0.10	31^{A}	0.52
BKME	212	23.6 ± 9.2	15863 ± 704 (17)	5357 ± 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	16284 ± 1250 (7)	5853 ± 629 (7)	2.17 ± 0.11	29	0.96
Thermal Us	8	0.9 ± 0.5^{C}	10189 ± 1914 (3)	3734 ± 315 (3)	2.49 ± 0.03	34^{B}	0.75
Thermal	146	13.3 ± 5.5^{C}	13343 ± 694 (11)	3631 ± 215 (11)	2.42 ± 0.12	30^{B}	0.75

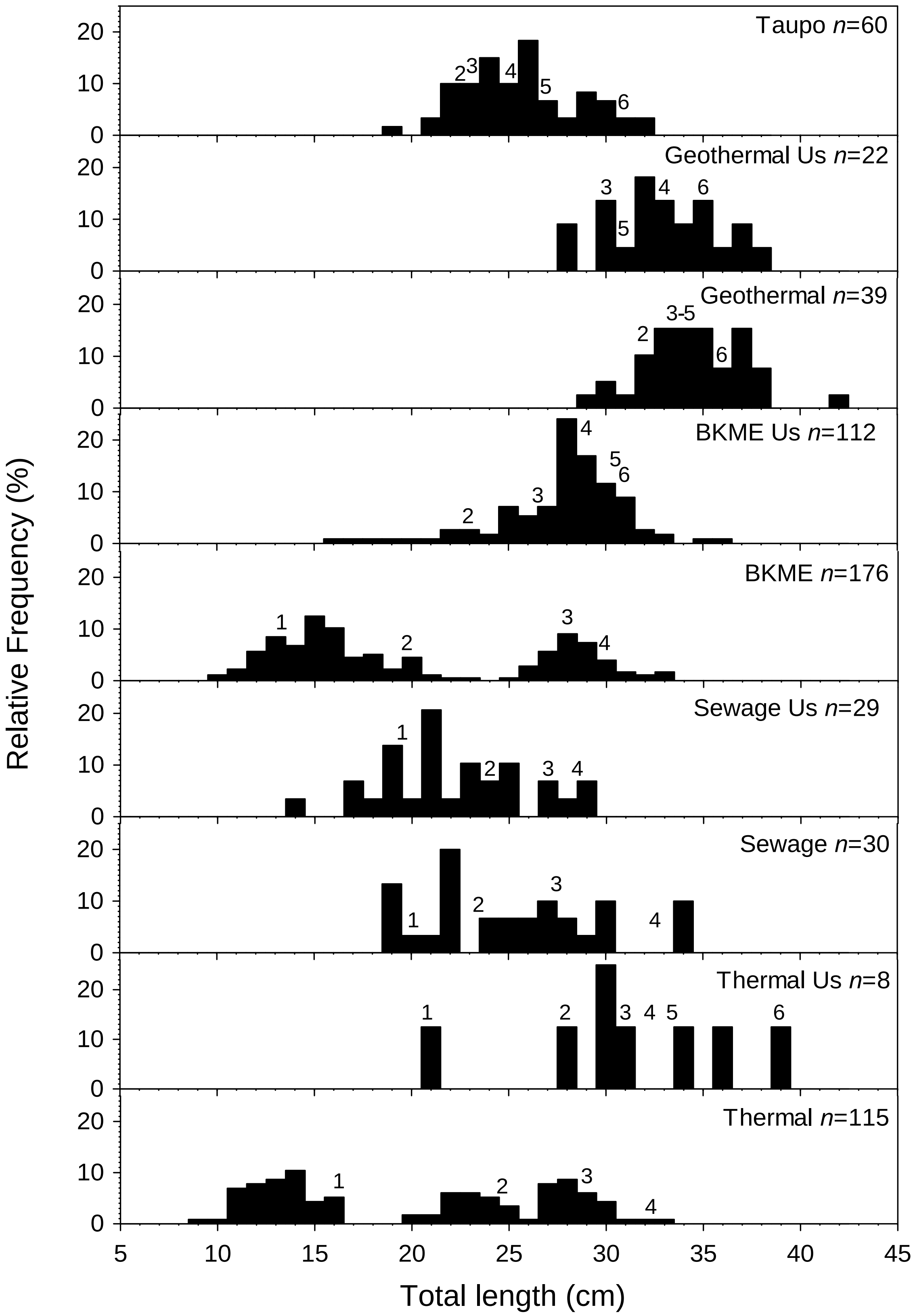

Figure 3.2. Lengths of brown bullhead at nine sites in the Waikato River, New Zealand. Numbers above bars are aligned with approximate mean lengths at age shown.

Table 3.5. Characteristics of sub-sampled brown bullhead from discharge and upstream reference (Us) sites. Values are given as mean ± SE.

No. sub-sampled		Length (cm)		Weight (g)		Age (year)		% > 2 yrs	
Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
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
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	97
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
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	92
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	92
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	97
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	8	9
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
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
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	27	17

3.2.6 Condition factor, liver somatic index (LSI) and spleen somatic index (SSI)

Sub-sampled male and female brown bullhead from the BKME site were in significantly better condition than those from the upstream site (Figure 3.3). Male and female fish from the sewage site were also in significantly better condition than at the upstream site but the lack of mature fish at the sewage upstream site made statistical comparisons impossible. Geothermal site females were also in significantly better condition than upstream females (Figure 3.3). Condition factors for both sexes of from Lake Taupo and sewage upstream sites fell below the overall river mean (Figure 3.3).

Statistical comparisons of liver size were limited to mature males and females. Males and females from the BKME site had significantly larger livers than those from the upstream site (Figure 3.3). In contrast to condition factor, females captured from the geothermal site had significantly smaller livers (Figure 3.3). 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 (Figure 3.3) 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 (Figure 3.3).

3.2.7 Gonadosomatic index (GSI), fecundity and follicle size and plasma sex steroids

To provide valid statistical evaluations between sites, only mature females where fecundity estimates were possible were compared. Males had uniformly small gonads so gonad size could not reliably be used to determine maturity. Therefore, mature males were taken to be fish greater than 1 year old and those having plasma 11-ketotestosterone values exceeding 0.37 ng/mL. Gonads of males and females from paired sites were not significantly different (Figure 3.4). Although sewage upstream fish were of equivalent size to fish downstream of the discharge and were of reproductive age, low GSI, lack of the ultimate follicle class, and low plasma steroid levels indicated that all fish at the reference location were non-reproductive. Gonad size (GSI) of mature females at other sites was consistent at about 10% of body weight, however, Lake Taupo reference and thermal site females had lower and higher GSIs than the river mean, respectively (Figure 3.4).

Fecundity estimates ranged from 5,660 to 22,001 follicles/kg of female body weight. Mean ultimate mean follicle diameters ranged from 1.33 to 3.11 mm. Unlike female gonad size, fecundity/kg varied widely (Figure 3.4). For the entire dataset of fish where estimates of fecundity were possible, there was a positive relationship between fecundity and follicle diameter and somatic weight (r =0.67, $p < 0.001$ and 0.36, $p = 0.001$ respectively). However, a negative relationship (r =-0.37, $p < 0.001$) between fecundity/kg and somatic weight indicates that larger fish had relatively fewer larger follicles. Steroid levels were only compared in mature males and females. There were significant relationships 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 analysis of covariance. Males and females from the geothermal site had significantly higher levels of testosterone and females from that site had significantly higher levels of 17β-estradiol (Figure 3.5). In contrast males and females from the BKME site showed significant reductions in levels of all steroids measured (Figure 3.5).

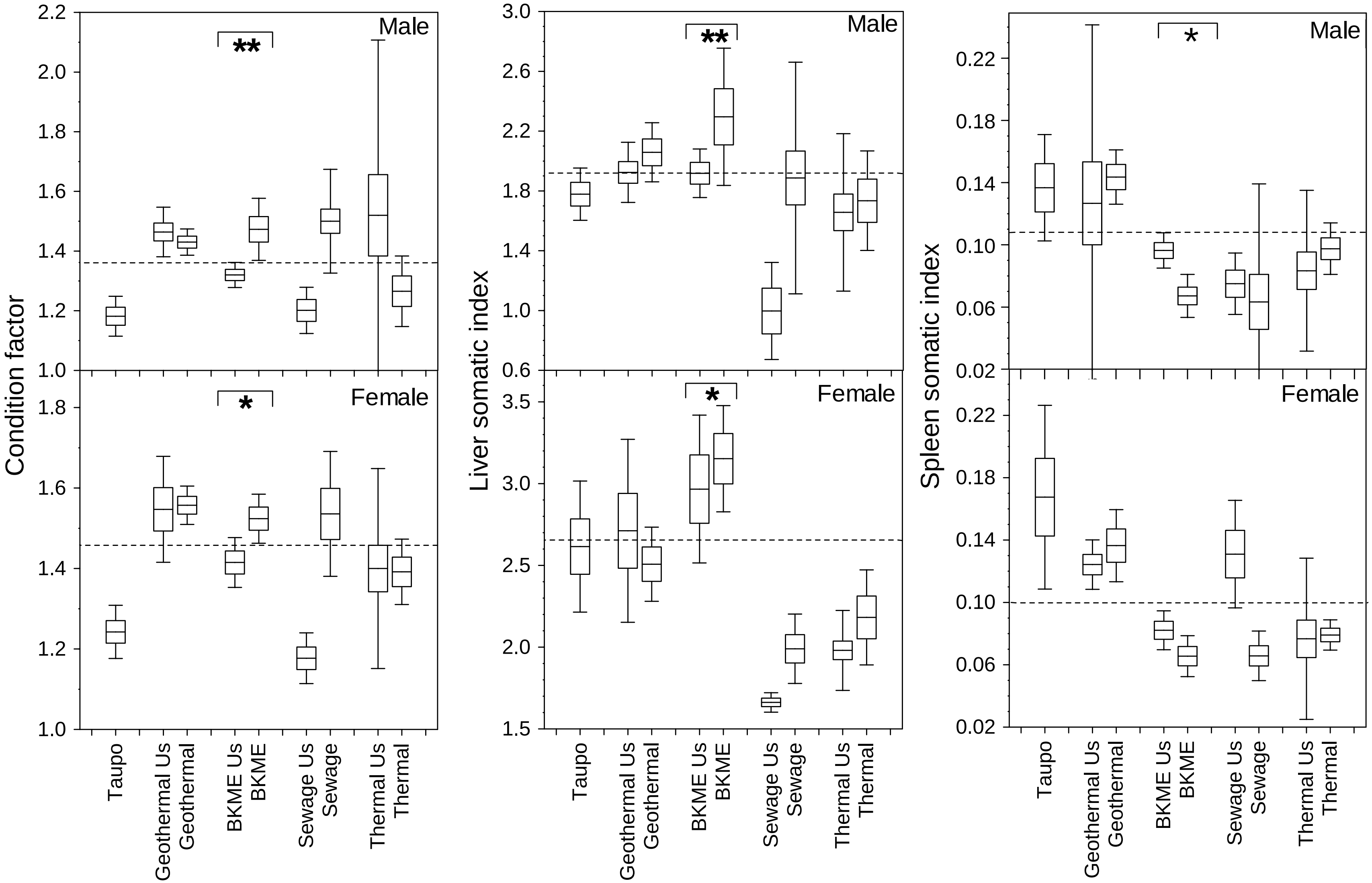

Figure 3.3. Somatic indices for male (top) and female (bottom) brown bullhead. Boxes are mean ± SE and whiskers are 95% confidence intervals. Asterisks indicate pairs of values significantly different at * $p < 0.05$ and ** $p < 0.01$. Dashed horizontal line is river mean.

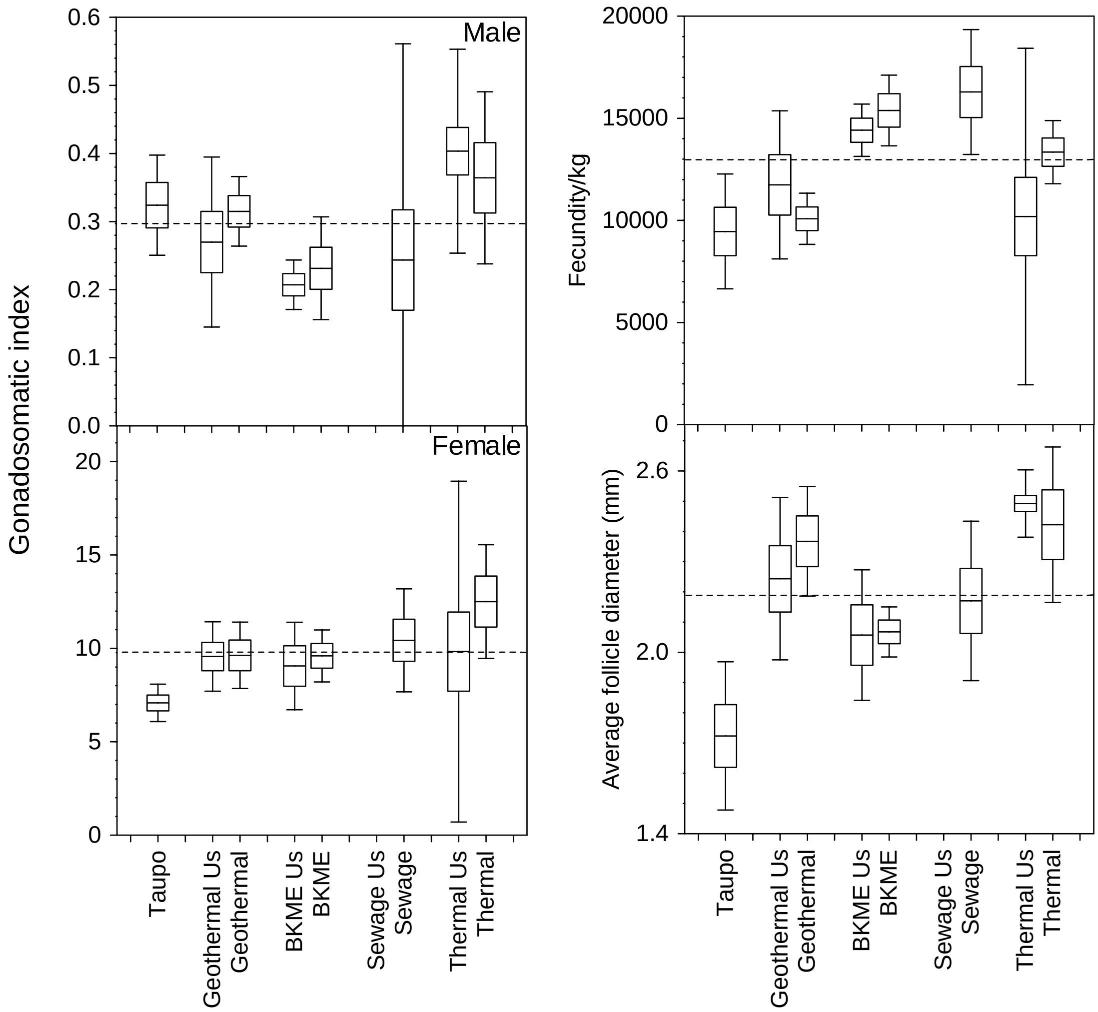

Figure 3.4. Gonadosomatic index (GSI), for mature male (top) and female (bottom) brown bullhead. Right hand graphs are fecundity per kilogram and mean follicle diameter for mature females. Boxes are mean ± SE and whiskers are 95% confidence intervals. Dashed horizontal line is river mean.

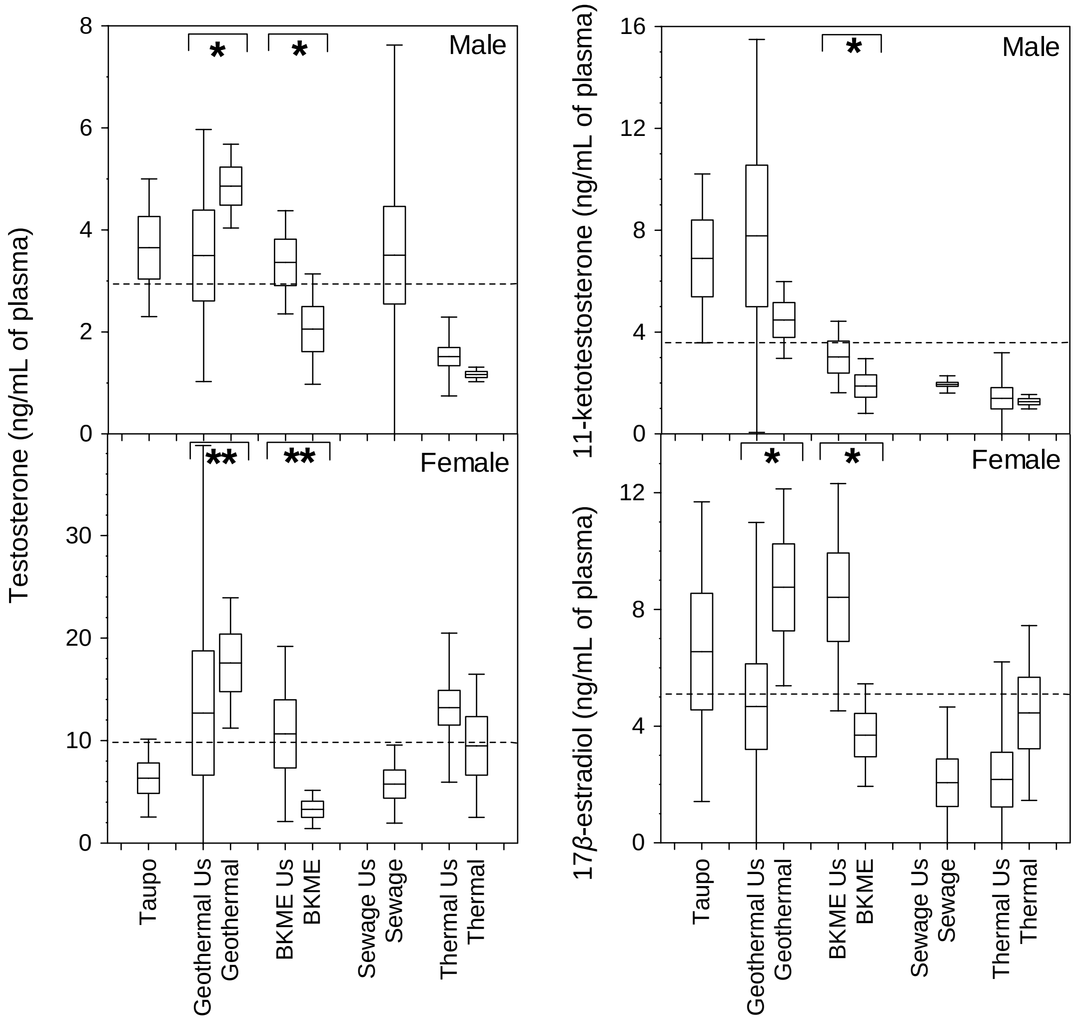

Figure 3.5. Sex steroid levels in blood plasma for mature male (top) and female (bottom) brown bullhead. Boxes are mean ± SE and whiskers are 95% confidence intervals. Asterisks indicate pairs of values significantly different at * $p < 0.05$ and ** $p < 0.01$. Dashed horizontal line is river mean.

3.2.8 Summary of population parameters

Population and physiological variables were categorized as being age structure, energy storage or energy allocation variables (Table 3.6). 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 while 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.

Table 3.6. Summary of population and physiological indicators in terms of age structure, energy storage, and energy. A + indicates a significant increase in at least one variable, a – indicates a significant decrease and a 0 indicates no change between impact and paired reference site. NC indicates that comparisons were not possible due to lack of mature fish. Bleached kraft mill effluent (BKME).

Impact Site	Age structure	Energy storage	Energy allocation
Geothermal	0	0	0
BKME	-	+	+
Sewage	0	NC	+
Thermal	-	0	-

3.3 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, though there appeared to be no recruitment in Lake Aratiatia as a whole. The pulp mill effluent caused increased energy allocation (growth) and storage and a proliferation of younger fish. Riverine fish upstream and downstream of sewage effluent were performing poorly as indicated by lack of reproductive maturity and low condition and comparisons were generally not appropriate. Thermal power generation resulted in younger age structure and decreased energy allocation (growth) but no differences in energy storage. There were numerous chemical and sub-organismal indications of exposure and some physiological impacts, 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 we sampled and changes in populations seemed more influenced 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 mercury was observed. Low and distinct metal signatures of brown bullhead livers from the BKME site corroborates bile resin acids and EROD results that indicate the residency and exposure to BKME of brown bullhead at that site. Metal concentrations were locally elevated and did not generally accumulate downstream. Some exceptions to this include Ag, Cd, Cu and Co. Phosphate fertiliser (predominantly superphosphate) is a possible primary source of the additional Ag and Cd in these samples. Superphosphate is the dominant source of Cd in pastoral New Zealand agriculture, with an estimated 8.3 t still being deposited on Waikato region farmland each year (Kim, 2005). Cu is likely to be 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 B12) 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 arsenic, cadmium, mercury and selenium were below toxic levels available for flesh (and some liver concentrations) (Beyer et al., 1996) although mercury at the geothermal site levels were approaching those levels. Overall levels of contamination (metals, PAH) in brown bullhead were low compared to brown bullhead sampled in North America (Arcand-Hoy and Metcalfe, 1999, Pinkney et al., 2004, Eufemia et al., 1997) 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 (Munkittrick et al., 1994, Kloepper-Sams and Benton, 1994). 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., 1994), and were 2-3 times those at the upstream site. In this study, brown bullhead showed approximately 5-fold induction as compared to other sites on the river. A study on highly contaminated regions of the Niagara River (Eufemia et al., 1997) showed a very comparable 6-fold level of induction when the most contaminated site was compared to the reference location. Injection experiments (Eufemia et al., 1997) 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 this 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, PCDs and PCDDs/PCDFs that are known to induce the *cyp1a* gene.

Red and white blood cells and the total haemoglobin of brown bullhead showed changes consistent with physiological response to the water temperature at all but one site. The exception was the BKME site where no increase in total haemoglobin of blood due to higher water temperatures and low dissolved oxygen was seen. In fact, there was a general decrease in most measures of blood oxygen carrying capacity, although only female haematocrit was significantly depressed. A decrease in Hct was also seen in five of the six pulp and paper effluent studies reviewed in (Folmar, 1993), and a corresponding drop in SSI, as seen in our study, was also seen in response to pulp mill effluents tested in the field and laboratory (Sepúlveda et al., 2004). The increase in white blood cells further suggests an immune response in BKME exposed brown bullhead, however, the literature reviewed in (Folmar, 1993) showed inconsistent patterns of response for leucocrit.

Differences in age structure were also strongly apparent 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 due to nutrient inputs. The geothermal site did not demonstrate this effect. However, this site and its upstream reference are dominated by older, larger fish, and no evidence of recruitment could be found. I 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 due to 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 (Munkittrick et al., 1994, McMaster et al., 1991, Karels et al., 1998), whereas poor condition of brown bullhead reflected the oligotrophic nature of Lake Taupo and the effects of fluctuating level and fast flowing waters at the sewage upstream site; this site was approximately 1 km downstream of the tailrace of a hydro dam, a very similar situation to (Barnes et al., 1984) 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 downstream riverine sections (Rubec and Quadri, 1982). This is consistent with the poorer condition of brown bullhead at riverine sites in our 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. BKME and thermal discharge showed opposite responses in ultimate length. Larger fish upstream of the thermal discharge may be due to the very low brown bullhead density, while increased growth in BKME may be due to increased productivity and temperature. When the river as a whole is examined there was a large range in ultimate length. Fast flowing riverine sites tended to produce poor growth whilst 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 non-reproductive. Despite a high frequency of external tumours and abnormalities, brown bullhead from the most contaminated site sampled by (Lesko et al., 1996) had the greatest number of follicles per female. The ability of brown 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 available for growth. This mechanism aptly called "capital spawning" by (Henderson et al., 1996) was also supported by the decrease in mature female brown bullhead liver size with increasing GSI. This strategy has inherent advantages for larger fish were larger ovarian follicles would be expected to produce larger larvae with a competitive advantage and higher survival (Einum and Fleming, 2000).

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 BKME site. Depression of sex steroids has been seen in other species exposed to pulp and paper effluents (Munkittrick et al., 1994, McMaster et al., 1991, Karels et al., 2001), however, I did not observe accompanying reductions in GSI or fecundity/kg. Thus it appears that out of all of the sites, the BKME exposed fish appear to experience some type of metabolic or endocrine disruption. However, as seen in previous studies (Munkittrick et al., 1992b, Munkittrick et al., 1992a) this effect was not manifest in adverse population responses. But 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 (Richardson et al., 1994) than brown bullhead (Cranshaw, 1974). These natives species may experience the combined stress of BKME and additional heat and be more sensitive to reductions in sex steroids than brown bullhead as seen with co-existing fish species elsewhere (Munkittrick et al., 1992a).

The overall patterns of population response in brown bullhead were difficult to attribute solely to point-source discharges. The BKME response was perhaps the most clear-cut, where increases in energy storage and allocation appear to have led to increased recruitment and thus can be classified as an eutrophication response (Gibbons and Munkittrick, 1994). 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 brown bullhead downstream of the discharges with a significant heat component suggest that brown bullhead benefit from the added heat as would be expected given their preferred temperature of approximately 30°C (Cranshaw, 1974).

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, there were no concomitant cumulative impacts observed in brown bullhead used to indicate river quality. Both contaminant exposure indicators, physiological and population responses were localized. However, it was also apparent that some of the population responses observed could not be fully explained. Both a better understanding of fish population responses and further study of the Waikato River is warranted. It is anticipated that ongoing comparative studies of other fish species to the same discharges will increase our understanding of brown bullhead responses.

This study documented relationships between the physiochemical nature of sites and variables assessed in individual fish, for example, 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, I also showed interrelationships between physiological variables, for example, between sex steroids and gonad size, and between female liver and gonad size. These relationships endorsed assessment of multiple levels of biological organisation (Munkittrick and Dixon, 1989, Adams et al., 1992). 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.

Chapter Four: Impact assessment along a large river, using shortfin eel (*Anguilla australis*) populations

4.0 Abstract

The effects of point-source and diffuse discharges on resident populations of shortfin eel (*Anguilla australis* Richardson, 1848) populations in the Waikato River, the longest river in New Zealand, were assessed. Eel were caught from sites upstream and downstream of pulp and paper, sewage and thermal point-source discharges. At each site, the population parameters relative abundance and age structure, and individual indices such as condition factor, and organ (gonad, liver, and spleen) to somatic weight ratios, were assessed. Physiological (blood), biochemical (hepatic ethoxyresorufin-*O*-deethylase (EROD)) and chemical indicators (bile chemistry and flesh metals) of exposure or response were also measured. Measures of exposure and response to point-source discharges confirmed residency of shortfin eel at sites sampled. Point-source discharges had few negative impacts on individual eel health and larger numbers of positive responses to those discharges with heat inputs were seen. Although I found little evidence of toxic effects of discharges on shortfin eel, caution is required in assessing the potential of contaminants to impact eel populations due to the life history of shortfin eel and exploited nature of populations. For example reproductive damage suffered by adult eels may not immediately manifest itself in the effected population due to temporal delays in gonadal maturation, and recruitment, and single panmictic populations supplementing recruitment of impacted populations.

4.1 Introduction

In New Zealand freshwaters, the shortfin eel *Anguilla australis* Richardson, 1848 and the endemic longfin eel *Anguilla dieffenbachii* Gray, 1842 are the top aquatic predators and the largest native fish species. Important traditional and commercial fisheries exist for the species (Jellyman and Todd, 1982). The shortfin eel is a widespread fish species in New Zealand and one of the few that persists in degraded environments. New Zealand eel scientists were amongst the signatories of the Quebec Declaration of Concern for the worldwide decline in eel resources in 2003, with particular concern regarding the level of recruitment of the endemic longfin eel.

The European eel *Anguilla anguilla* (Linnaeus, 1758) has frequently been used as an indicator species for environmental pollution, including exposure to: petroleum distillates (Pacheco and Santos, 2001); sewage; and bleached kraft mill effluent (BKME); and contaminated sediments (Maria et al., 2003). European eel have also had higher levels of contamination compared to other fish species caught from the same locations (Pointet and Milliet, 2000, Bordajandi et al., 2003). This is in part due to their high lipid content and the prevalence of lipophilic contaminants (van der Oost et al., 1996).

In New Zealand, shortfin eel have been used in impact assessments examining the effects of exposure to: the herbicide diquat (Tremblay, 2004); the extent of contamination of selected river systems across New Zealand (Jones et al., 1995); and the effects of caged exposure to pulp and paper effluent (van den Heuvel et al., 2006).

The use of population parameters to assess contaminant effects at higher levels of biological organisation is an approach advocated by (Munkittrick and Dixon, 1989, Adams et al., 1992) but one that is yet to be applied in New Zealand impact assessments using any species of eel in New Zealand. The population monitoring framework suggested by (Munkittrick and Dixon, 1989) can also be refined to categorize fishery variables into measures of age structure, energy storage, and energy allocation (Gibbons and Munkittrick, 1994). The fish monitoring framework has been used recently to study cumulative changes due to multiple environmental disturbances (Munkittrick et al., 2000). By pairing a population framework assessment with biochemical and chemical measures of exposure and effect, the goal is to either rule out potential contaminant effects or point the way to further diagnostic studies.

The Waikato River is the longest river (425 km) in New Zealand and provides a good testing ground to further investigate the relationship between stressors and feral fish responses due to its clean source at Lake Taupo and incremental introductions of diverse sources of pollution along the catchment. These pollution sources include: geothermal fluid discharges; industrial effluent; sewage effluent; urban storm water runoff; dairy farm effluent; and other agricultural land uses. As with many large rivers in New Zealand, and around the world, the Waikato River has been dammed for the generation of hydroelectricity. Eight impoundments create lacustrine habitats and restrict upstream access for migratory fish such as eel.

The purpose of this study was to evaluate the impacts of anthropogenic stress using shortfin eel populations in the Waikato River. By examining shortfin eel at multiple levels of biological organisation this study aimed to establish both the cause of any effects I observed, and their ecological relevance to this widespread and important native species. Due to the number of impacts along the length of the river, this study also examined the hypothesis that impacts may be cumulative due to increasing degradation of environmental quality along the river.

4.2 Results

4.2.1 Site physicochemical characteristics

Total degree days above 9°C for BKME and thermal discharge sites were 15-20% higher than their respective upstream sites (Table 4.1). Increases in temperature were mostly evident through autumn, winter, and spring. These temperature increases were limited to the effluent plumes and there was no overall temperature increase evident along the total length of river sampled. Fluctuations of up to a meter in water level during sampling were seen at the sewage upstream site due to hydroelectric flow management, this site also had higher water velocities and greater amounts of rock or bedrock substrate throughout the targeted reach compared to other sites therefore sampling was restricted to only three backwaters present. There was a gradual increase in nutrients and primary productivity indicated by an increase in chlorophyll *a* and a reduction in water clarity along most of the length of the river (Table 4.1).

4.2.2 Chemical and biochemical indicators of exposure to contaminants

Contaminants from pulp and paper and sewage waste discharges were found in eel bile and flesh at respective discharge sites (Table 4.2). Resin acids and PAHs typical of bleached kraft mill effluent were found in bile from eel at the BKME site. Overall eel flesh from the BKME site had lower levels of metal contamination than eel flesh from the main stem of the river, suggesting there was little movement of eel between reference and BKME sites (Table 4.2). Exposure of BKME eel to pulp and paper waste indicated by bile contaminants was confirmed by elevated EROD (Figure 4.1). Low EROD levels at all other sites suggests eel are exposed to negligible amounts of EROD inducing chemicals. Contaminants in eel due to point-source discharges were only detectable near discharges and did not persist or accumulate with increasing distance downstream.

Table 4.1. River and site physicochemical characteristics. Degree days are total degree days above 9°C for year from 24 September 2002 to 24 September 2003. Us = upstream reference. [a] (Davies-Colley and Smith, 2001). [b] A-G, 5 year median values for selected water quality measurements recorded by the Waikato Regional Council at Waikato River locations (Figure 1.1) (Smith, 2004). [c] Temperature and DO at time of sampling. [d] value not available for site, figure shown is from 30 km downstream.

Site name	Water temperature (°C)				DO	N	P	Chlorophyll	Black
	Degree days	Max	Min	Median	(%)	$(g.m^{-3})$	$(g.m^{-3})$	*a* $(g.m^{-3})$	disc[a]
A[b], control gates	-	-	-	14.3	100.8	0.07	0.006	0.002	>5.9[d]
B[b], Whakamaru	-	-	-	16.4	105.5	0.19	0.022	0.008	2.5
BKME Us	2538	23.7	9.7	22.5[c]	120.0[c]	-	-	-	-
BKME	3074	23.0	11.5	21.7[c]	73.5[c]	-	-	-	-
C[b], Waipapa	-	-	-	16.3	103.0	0.25	0.026	0.008	2.5
Sewage Us	2563	21.4	10.5	20.0[c]	114.0[c]	-	-	-	-
Sewage	2608	21.6	10.5	20.1[c]	101.2[c]	-	-	-	-
D[b], Narrows	-	-	-	16.0	100.6	0.34	0.033	0.012	1.4
E[b], Horotiu	-	-	-	16.1	100.0	0.40	0.043	0.013	1.4
Thermal Us	2552	22.0	9.7	20.4[c]	-	-	-	-	-
Thermal	3227	23.5	11.8	21.6[c]	-	-	-	-	-
Mangaotama	-	-	-	16.5[c]	74.4[c]	-	-	-	-
F[b], Huntly-	-	-	-	16.2	98.5	0.54	0.060	0.015	0.9
G[b], Tuakau	-	-	-	17.1	100.5	0.60	0.070	0.022	0.6

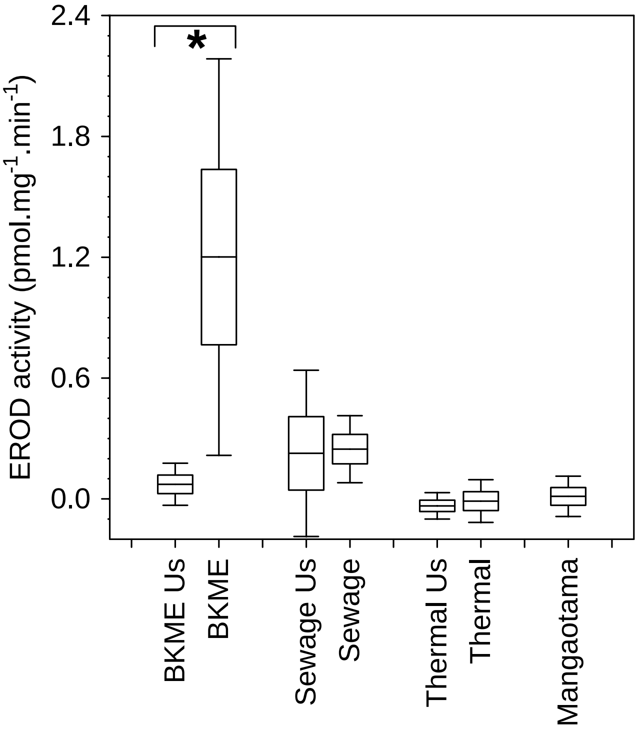

Figure 4.1. Liver detoxification enzymes (EROD) in shortfin eel from the Waikato River, New Zealand. Boxes are mean ± SE and whiskers are 95% confidence intervals. Asterisk indicates pairs of values significantly different at * $p < 0.05$.

Table 4.2. Mean age of eel pooled for flesh metal assay, trace metal concentrations (wet weights), mean resin acids and pooled Polycyclic Aromatic Hydrocarbons (PAHs); naphthalene (nap), pyrene (pyr), , benzo[*a*]pyrene (B[*a*]P) and retene in bile of sub-sampled shortfin eel from sites in the Waikato River, New Zealand. Us = upstream reference. < = below detection limit.

Site	Flesh trace metals (mg.kg^{-1} wet weight)											Bile				
												Resin acids (µg.g^{-1} d.w.)	PAHs (µg.mL^{-1})			
	Age (yr)	Ag	As	Cd	Co	Cu	Hg	Li	Pb	Se	Zn		nap	pyr	B[*a*]P	Retene
BKME Us	10.3	<	0.03	<	<	3.37	0.17	0.03	0.01	0.32	11.1	0.0	98.4	4.5	1.1	473.8
BKME	10.5	<	<	<	<	1.87	0.08	<	<	0.19	12.1	345.8	152.0	21.8	2.4	1105.0
Sewage Us	15.5	<	0.02	<	<	2.62	0.33	0.02	0.01	0.26	8.3	1.0	72.5	4.0	1.4	321.8
Sewage	15.6	<	0.04	<	<	1.32	0.24	0.02	0.01	0.31	7.9	95. 9	39.3	6.4	1.1	209.2
Thermal Us	20.3	<	0.03	<	<	1.24	0.15	<	<	0.74	9.6	115.3	43.0	6.7	1.2	230.4
Thermal	19.7	<	0.02	0.003	<	2.05	0.14	0.02	0.01	0.59	15.0	112.0	40.1	9.5	1.7	258.5
Mangaotama Stream	21.3	0.012	<	0.006	0.04	3.94	0.16	<	0.03	0.73	9.9	109.6	132.4	4.4	0.9	480.2

4.2.3 Blood variables

Total haemoglobin (haematocrit and RBC counts) in eel blood was higher at the two warmer discharge sites, although only the thermal site was significantly higher (Table 4.3). Leucocrit (Lct) values were significantly elevated in eel blood at the sewage upstream site and were also higher at BKME site compared to its upstream reference site (Table 4.3). No cumulative impacts are apparent in blood variables with increasing distance downstream.

4.2.4 Catch rates, length frequency, age and growth

The Mangaotama Stream was intended to be a reference site but was not comparable to other sites as it was a large stream rather than a river site; therefore I restricted comparisons to between other sites. Catch per unit effort (CPUE) of shortfin eel was significantly higher at the sewage upstream site compared to the sewage site ($p <$ 0.05, Mann-Whitney *U* Test) (Table 4.4). When comparing biomass of eel at sites the large number of longfin eel at the sewage upstream site (Figure 4.2) and large size of shortfin eel increased this site's biomass to almost as high as the thermal site where shortfin eel were more numerous (Figure 4.2 and Table 4.4). Overall, sites in the lower river (thermal upstream and thermal) had higher CPUE than other sites (Table 4.4).

Most eel caught were sexually immature although eel that had external morphological attributes of migrant eel and visible gonads were caught and noted as being migrant eels. Two migrant eels whose gonads were weighed at the thermal site had gonadosomatic indexes (GSI) of 2.55 and 2.46. Larger numbers of migrant eel were caught at the BKME and thermal discharge sites (Table 4.4). Effect of migrant status on other variables measured was examined but no effect was discernable.

Table 4.3. Mean blood parameters, (Haematocrit (Hct), red blood cell count (RBC), mean cell volume (MCV), haemoglobin (Hb), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC)) in shortfin eel from the Waikato River. Us = Upstream reference. Differences between paired sites ($p < 0.05$, ANOVA) are denoted by superscript letters.

Site	No.	Hct (%)	RBC (cells.L^{-1} x 10^{13})	MCV (L x 10^{-12})	Hb (g.L^{-1})	MCH (g.$cell^{-1}$ x 10^{-10})	MCHC (g.L^{-1})	Lct (%)
BKME Us	12	30.9	0.34	0.09	110.7	0.34	358	1.32
BKME	12	31.2	0.38	0.08	115.9	0.32	377	1.84
Sewage Us	12	28.9	0.28	0.11	96.9	0.36	334	1.93^{B}
Sewage	15	26.7	0.30	0.09	91.6	0.32	346	1.28^{B}
Thermal Us	12	28.3^{A}	0.38	0.08	102.5	0.28	368	1.33
Thermal	12	32.9^{A}	0.39	0.08	100.7	0.26	310	1.19
Mangaotama Stm	12	29.5	0.36	0.08	82.0	0.23	278	0.97

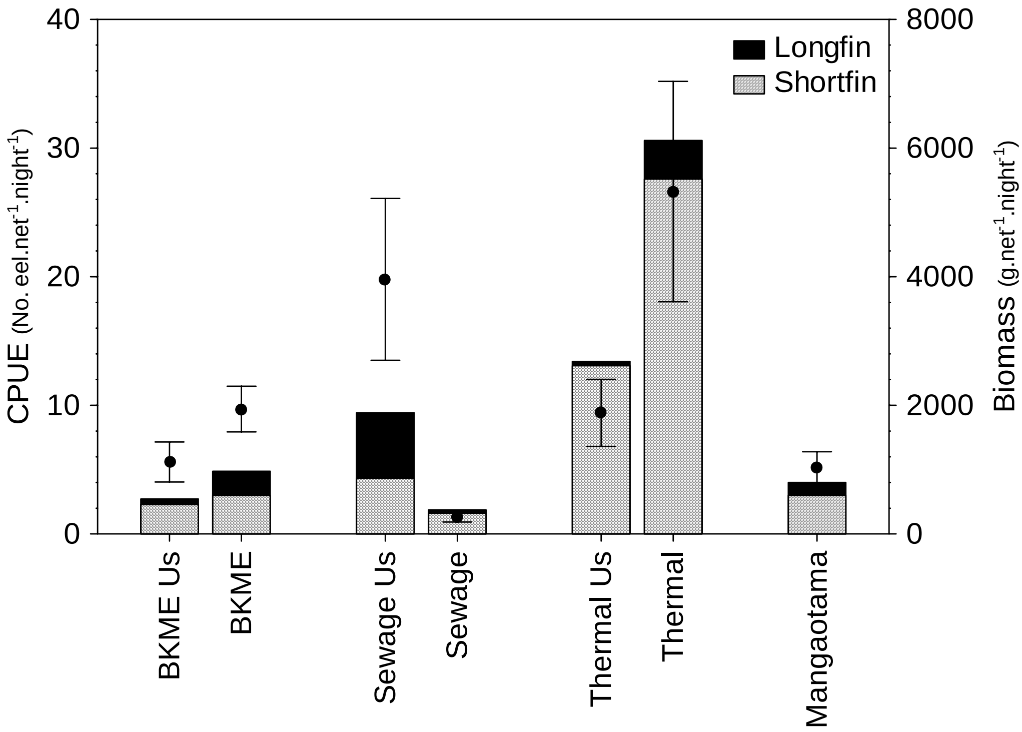

Figure 4.2. Bar graph of mean shortfin and longfin eel catch per unit effort (CPUE, number of eel/net/night) and whisker plot of mean biomass ± SE of both species of eel (gram of eel/net/night) at seven Waikato River, New Zealand sites.

Eel caught from the two Lake Maraetai (BKME) sites were the largest and youngest of eel caught at any site (Figure 4.3, Table 4.4). BKME eel L_∞ was higher but not significantly so than upstream reference eel. Eel catches from the lower Waikato River sites were dominated by small eel (Figure 4.3). I attribute the high L_∞ at the thermal site to the single largest 89.5 cm eel biasing growth modeling, when this eel was removed from the model; L_∞ was identical to the upstream reference site. Growth was comparatively slow at thermal sites where eel density and biomass was high (Table 4.4).

Table 4.4. Total number, mean catch per unit effort (CPUE, No. shortfin eel.net^{-1}.night^{-1}, biomass gram eel.net^{-1}.night^{-1}) of shortfin and longfin eel (Eel) and number sub-sampled, with attributes of migrants, maximum length (L_{∞}) and growth constant (k) calculated using size/age model of shortfin eel from discharge and upstream reference (Us) sites. Values are mean ± SE. Column significant differences ($p < 0.05$) are denoted by, *paired sites Mann-Whitney *U* Test, $^{A\text{-}F}$ Tukey post-hoc test any sites or $^{+}$modeled growth of paired sites residual sum of squares procedure (Chen et al., 1992).

Site	Eel			Shortfin eel						
	Total No. eel	CPUE No.	CPUE Biomass	No. sampled	No. migrants	Length (cm)	Weight (g)	Age (year)	L_{∞} (cm)	k
River	824	8.3 ± 1.6		302		48.1 ± 0.7	273.2 ± 14.1	16.0 ± 0.5	-	-
BKME Us	38	$3.7 \pm 1.1^{*}$	1237	34	1	56.2 ± 2.2^{B}	412.3 ± 60.1	10.5 ± 0.4^{ABC}	80.0	0.085
BKME	78	$5.5 \pm 1.5^{*}$	1957	50	9	54.2 ± 1.7^{C}	398.6 ± 38.1	10.5 ± 0.4^{ABC}	88.2	0.061
Sewage Us	97	$8.1 \pm 2.7^{*}$	3958	37	2	57.1 ± 1.6^{A}	489.7 ± 37.4	15.5 ± 0.7	67.1^{+}	0.069
Sewage	43	$1.9 \pm 0.6^{*}$	251	40	0	40.3 ± 1.0^{ABCF}	140.0 ± 15.1	15.7 ± 0.7	40.4^{+}	0.22
Thermal Us	161	13.4 ± 4.0	1882	57	2	41.1 ± 0.8^{DEF}	140.2 ± 12.0	20.3 ± 0.7^{B}	50.6	1.29
Thermal	367	31 ± 10.4	5324	57	11	43.2 ± 1.3^{ABCEF}	174.1 ± 24.2	19.7 ± 1.0^{C}	86.3	0.029
Mangaotama	40	4.0 ± 1.0	1024	27	1	50.7 ± 1.3^{ABCDF}	256.0 ± 24.8	21.3 ± 0.8^{A}	50.7	1.29

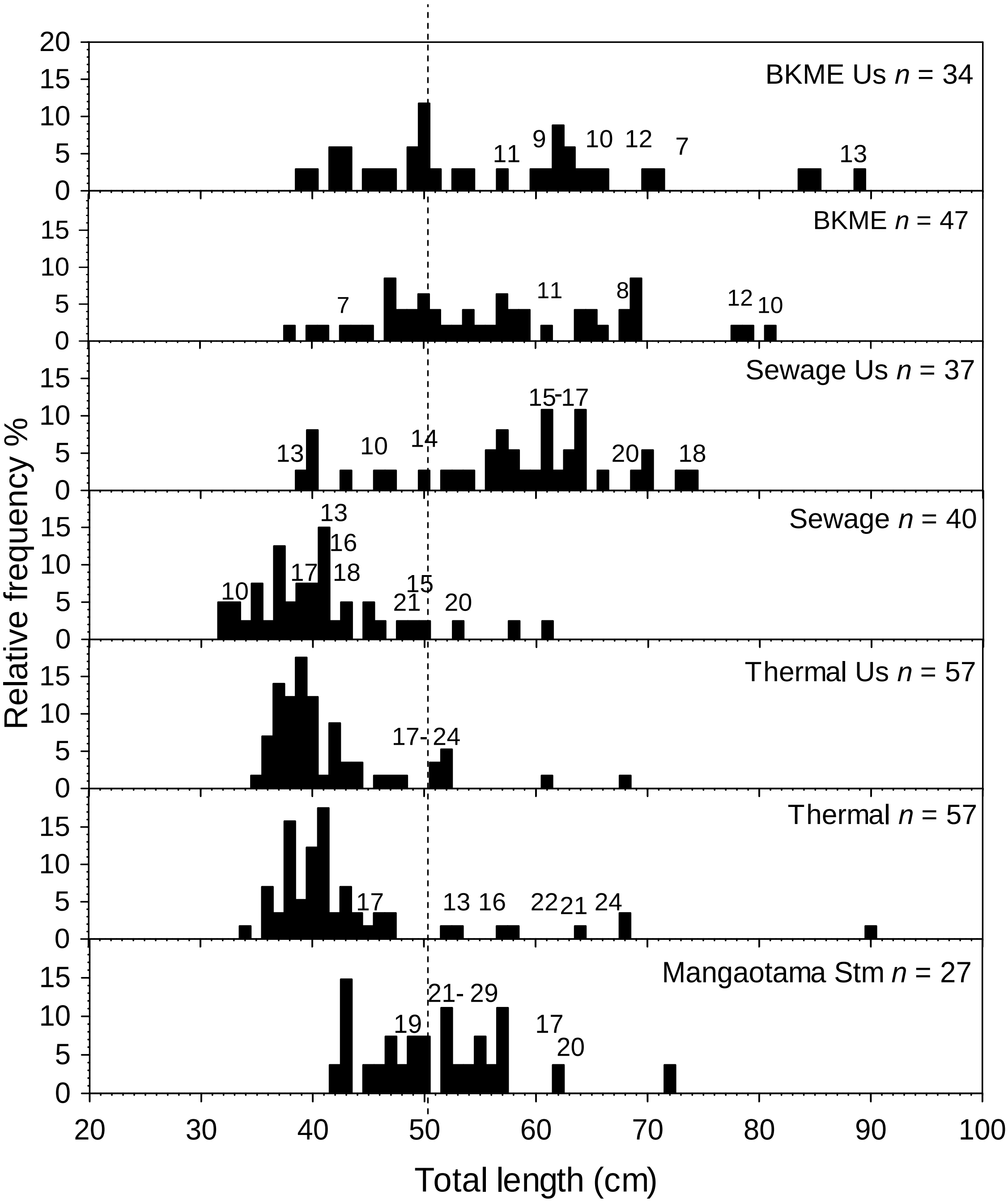

Figure 4.3. Lengths of shortfin eel at nine sites in the Waikato River, New Zealand. Numbers above bars are aligned with approximate mean lengths at age shown. Dashed vertical line is estimated minimum length of commercially harvested eels.

4.2.5 Condition factor, liver somatic index (LSI) and spleen somatic index (SSI)

The mean condition factor of eel caught from the BKME site was significantly higher than its upstream reference site and higher than mean condition factor for the river as a whole (Figure 4.4). Eel from the sewage upstream site had the highest condition factor of all sites, and mean condition factor was significantly higher than its paired sewage discharge site (Figure 4.4). Patterns in liver size mirrored condition factor except at the Thermal site (Figure 4.4). Shortfin eel at the BKME site also had larger livers than better conditioned sewage upstream eel (Figure 4.4). Livers and spleens from BKME and sewage upstream site eel were significantly larger than their respective paired sites (Figure 4.4).

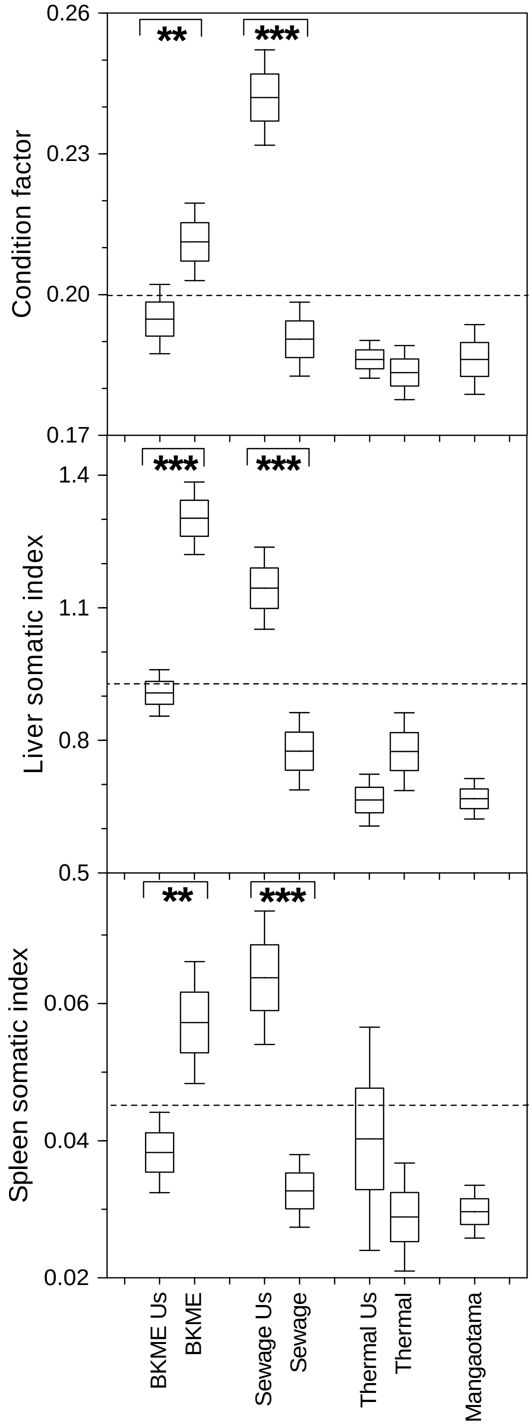

Figure 4.4. Somatic indices for shortfin eel. Boxes are mean ± SE and whiskers are 95% confidence intervals. Asterisks indicate pairs of values significantly different at ** $p < 0.01$ and *** $p < 0.001$. Dashed horizontal line is river mean.

4.2.6 Summary of population parameters

Population and physiological variables were categorized as being: age structure; energy storage; or energy allocation variables (Table 4.5). Age structure was based on the mean age and length frequency distributions. Energy storage was indicated by condition factor and liver size, while estimates of energy expenditure were limited to growth. Shortfin eel at the BKME site had the highest amount of stored energy. Eel caught at the sewage site had lower levels of energy storage and allocation than upstream reference site eel.

Table 4.5. Summary of population and physiological indicators in terms of age structure, energy storage, and energy. A + indicates a significant increase in at least one variable, a – indicates a significant decrease and a 0 indicates no change between impact site and paired upstream reference site.

Impact Site	Age structure	Energy storage	Energy allocation
BKME	0	+	0
Sewage	0	-	-
Thermal power	0	0	0

4.3 Discussion

With the exception of the eel population exposed to pulp and paper mill effluent that showed an eutrophication response, there was little evidence of other population level impacts due to point-source effluent exposure. Levels of contaminants, and measures of exposure to contaminants due to exposure to point sources of pollution were observed. However, those measures did not persist far from the exposure areas. Marked differences in shortfin eel populations were apparent over the reach of the river sampled, and while some can be potentially attributed to habitat (impoundment), differences between similar riverine sites (e.g. upstream and downstream sewage site) suggest other factors such as commercial exploitation are having a major effect on eel populations. Despite the significant impacts of human activity along the length of the Waikato River, and the associated degradation in water quality in general, there was no evidence of cumulative impacts on eel populations.

Measures of contaminants and exposure variables due to point-source pollution were localized and suggested shortfin eel sampled had been resident at the sites for some time. Retene metabolite content of bile and induction of hepatic *cyp1a* (EROD) in eel from the BKME site is consistent with their exposure and reaction to known pulp and paper contaminants in this type of discharge (Jones et al., 1995, Teles et al., 2004). Levels of metals of concern in eel flesh such as arsenic, cadmium, mercury and selenium were below toxic levels available for flesh (Beyer et al., 1996). With the exception of copper, overall levels of metals in shortfin flesh were low, and in many cases an order of magnitude lower than seen in similar scale studies in other countries (Bordajandi et al., 2003, Schmitt, 2004, Ribeiro et al., 2005). No discharge or eel age induced pattern was obvious in levels of copper which were only marginally higher than studies cited above.

Blood parameters showed a number of changes due to site conditions. No decrease in Hct or spleen size was seen at the BKME site, although most pulp and paper studies reviewed by (Folmar, 1993) showed reductions in Hct, and a reduction in spleen size was seen by (Sepúlveda et al., 2004). Brown bullhead catfish (*Ameiurus nebulosus* (Lesueur, 1819)) caught from this site showed a significant reduction in Hct and spleen size (West et al., 2006). The increase in Lct suggests an immune response in BKME exposed eel, but the literature reviewed in (Folmar, 1993) showed inconsistent patterns of Lct response. Increased Lct was also seen in brown bullhead caught from the BKME site (West et al., 2006). The increased Lct in sewage upstream eel is harder to explain, although a similar increase was seen in brown bullhead caught at this site (West et al., 2006). However unlike brown bullhead, eel were in very good condition. I consider that differences between the sewage site and its upstream reference site reflect differences in food availability and density rather than any direct impact of the sewage discharge.

Considerable differences in eel growth and energy storage were noted along the river. The fast growth of shortfin eel at the Lake Maraetai hydroelectric impoundment sites (BKME upstream and BKME) is probably due to densities that are below carrying capacity due to restricted recruitment at these sites. These populations are sustained by limited releases of elvers caught and transported from the base of the downstream Karapiro dam (Chisnall et al., 1998). Very high growth rates for longfin and shortfin eel from Lake Maraetai were also reported in (Chisnall and Hicks, 1993, Chisnall et al., 1998). Due to the limited recruitment of elvers into this lake it might be interpreted that density is a primary factor leading to enhanced growth. However, measures of relative density and biomass did not adequately explain the differences. Further evidence indicates that habitat modification and density alone may not entirely explain differences in growth as eel captured at the sewage upstream site (just below the last dam) showed the best growth with age of the site samples. This was the fastest flowing river site and supported the second highest estimated biomass. Inter- and intra-specific competition, habitat, and food availability would all be expected to impact on growth and the results do not reflect changes in one of these factors alone. Food was certainly abundant in the hydro lake as lacustrine species such as rudd, goldfish and brown bullhead thrive there. However, the upstream sewage site did not share these attributes.

Condition factor and liver size of eel along the river generally followed what was observed for growth. The increased energy storage of eel as measured by those variables at the BKME site matches observations in other fish species and is consistent with an enrichment effect often observed at pulp and paper mill-influenced sites (McMaster et al., 1991, Munkittrick et al., 1994, Karels et al., 1998). In this study, liver size at the BKME site was the highest in the river and some of this increase may be due to the metabolic demand or disruption of effluent exposure.

I can not attribute any of the population responses of shortfin eel primarily to point-source discharges, the only eel to show discharge related impacts are BKME exposed eel which appear to experience minor metabolic disruption, but this effect was not manifested in adverse individual or population responses as has been noted in other fish populations (Munkittrick et al., 1992a, Munkittrick et al., 1992b). In comparison to brown bullhead from the three upstream sites (West et al., 2006) shortfin eel were in better condition and appear more resistant to BKME exposure. Increases in energy storage and allocation of eel at the BKME site can be classified as an eutrophication response (Gibbons and Munkittrick, 1994). Better condition of eel downstream of the discharges with a significant heat component suggests that eel benefit from added heat. This is consistent with their preferred temperature of approximately 27°C (Richardson et al., 1994). The higher numbers of migrant eel may also indicate earlier maturation of eel at these sites.

The marked differences in eel populations in the lower Waikato River compared to the hydroelectric impoundment and upper-most river site may be due to a number of factors. Fast growth of hydroelectric impoundment (Lake Maraetai) eel as seen at BKME sites was noted by (Chisnall and Hicks, 1993, Chisnall et al., 1998). Previous studies on eel populations in the lower Waikato River have cited habitat characteristics, density and food availability as possible causes of varied growth rates (Chisnall and Hayes, 1991). Later surveys of eel populations in Lower Waikato lakes have also revealed catches dominated by high densities of small shortfin eel and reductions in numbers of large eel from historical (1987) catches (West et al., 1993, West et al., 2000). Reductions in mean lengths of shortfin eel in areas extensively commercially fished elsewhere in New Zealand has also been documented (Todd, 1980, Jellyman and Todd, 1998, Beentjes et al., 2006) but concomitant reductions in habitat quality blur the role of commercial fishing in that reduction. Simulated eel harvest of a Waikato stream resulted in an increase in density of small shortfin eel (Chisnall et al., 2003). I consider that the high density of small shortfin eel in the lower Waikato River has a major role in poor condition and growth of those eel. High densities of small eel may be due to individual factors such as; harvest of large eel reducing numbers and piscivorous role of large eel. River channelisation removing, preferred juvenile eel habitat in backwaters (Chisnall, 1989) and access to important feeding areas during floods (Jellyman, 1989). Habitat degradation due to high densities of competing exotic fish species and declines in water quality is also a pervasive impact. In reality all the above factors are likely to be contributing to poor condition and growth of shortfin eel in the Waikato River.

Although we found little evidence of toxic effects of discharges, the life history of shortfin eel and exploited nature of populations we sampled warrant caution in assessing potential of contaminants to impact eel populations (Barnthouse et al., 1990). For example reproductive damage suffered by adult eels may not immediately manifest itself in the effected population due to temporal delays in gonadal maturation and recruitment and single panmictic populations (Smith et al., 2001) supplementing recruitment of impacted populations.

Chapter Five: Cumulative impacts assessment along a large river, using populations of a small-bodied fish species, the common bully (*Gobiomorphus cotidianus*)

5.0 Abstract

The effects of point-source and diffuse discharges on resident populations of common bully (*Gobiomorphus cotidianus* McDowall, 1975) in the Waikato River were assessed at sites upstream and downstream of geothermal, bleached kraft mill effluent (BKME), sewage and thermal point-source discharges. At each site, population parameters such as age structure and individual indices such as condition factor, and organ (gonad, liver, and spleen) to somatic weight ratios, and number and size of follicles per female were assessed. Physiological (blood) parameters and stable isotopes of C and N were also measured. Isotopic signatures gave strong evidence that fish were resident at sites, and showed a gradient along the river. A wide size range of common bully was collected from all sites. A lack of large males limited comparisons of physiological variables, but the capture of a representative size range of individuals in the population allowed assessment of population responses. No consistent impacts on common bully health were obvious at individual discharge sites, or cumulatively along the river, due to the gradual deterioration in water quality downstream, particularly nutrient enrichment and increased turbidity. Distinct changes in population parameters at each of the paired sites and changes in individual variables suggested that fish responded to discharges. Population-level responses at the geothermal and BKME discharge sites raise the possibility that detection of sub-lethal or chronic effects on sensitive juvenile life-stages may be being hidden by compensatory population responses. Responses and life history of common bully validated their use as an indicator species and supported overseas examples using of small-bodied fish species as sentinels.

5.1 Introduction

The effective use of freshwater fishes as bio-indicator or sentinel organisms requires fulfillment of a number of criteria, especially that fish caught from a site are resident or at least exposed to site conditions and contaminants long enough to elicit biological responses. Small size and benthic habitat of sentinel fish contributes to meeting this criterion as the home ranges and migrations of these fish are correspondingly smaller than large fish. Sampling of small-bodied fishes in tandem with more widely used large fish species have verified their potential (Gibbons et al., 1998a). Small-bodied fishes also allow studies of smaller and more diffuse discharges in low order streams (Gray et al., 2005) where reproduction usually occurs in adult habitat. The ability to rapidly capture fish of all life stages from a population also facilitates assessment of important population responses (Gibbons and Munkittrick, 1994). Sampling small-bodied fish does, however, pose challenges. For example, measuring biochemical endpoints is more difficult due to limitations in the quantity of tissue available, although this can be surmounted by use of in vitro techniques (Tetreault et al., 2003). Smaller fish species are also less often studied than large bodied commercial or recreational species. The use of small bodied fish aids the study of population responses, and helps effects-based monitoring methods take their place alongside more traditional, risk-based methods for the protection of the environment. An increase in the successful use of fish in assessments of pulp mill effects between cycle 1 and 2 of the Environmental Effects Monitoring (EEM) program has been partly attributed to the increased use of small bodied fish (Lowell et al., 2003). Already there are numerous examples of large scale investigations of this type (Munkittrick et al., 2000, Schmitt, 2002), and effects-based tools employing adult fish surveys have been legislatively implemented with a national-scale EEM program (Lowell et al., 2003).

In New Zealand, the use of macroinvertebrate communities for effects-based monitoring is well established (Hickey, 1995). However, there is a paucity of data on environmental impacts on fish populations. This is largely due to 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 (Jones et al., 1995, Tremblay, 2004); mesocosm exposures (van den Heuvel et al., 2002, van den Heuvel and Ellis, 2002); and wild fish monitoring using fish community and health indices (Scrimgeour, 1989, Richardson et al., 1996, West et al., 2006, Tremblay et al., 2005). Common bully (*Gobiomorphus cotidianus* McDowall, 1975) have been used as a indicator species in two of these studies (Richardson et al., 1996, Tremblay et al., 2005) and are included in a manual on assessing fish health in New Zealand (Richardson, 1998). Common bully, habitat (Jowett and Richardson, 1995), tolerance to low dissolved oxygen (Dean and Richardson, 1999, Landman et al., 2005) and ammonia (Richardson, 1997a) have also been described, so responses to these variables in field impact assessment can be inferred. Common bully are in the Order Perciformes and Family Eleotridae, and grow to 150 mm total length, though 70-80 mm is a more common size for adults especially in lakes (McDowall, 1990). Males are territorial, taking on black nuptial coloring and defending basic nest sites on or under almost any hard surface that females can lay eggs on (McDowall, 1990). Although they can be diadromous, common bully readily establish populations in waterbodies without upstream access from the sea. In this study, I used the common bully as a monitoring species because it: was known to be present at all sites; is a benthic species; spawns in adult habitat; and is not commercially exploited.

Due to the diverse sources of pollution in the Waikato River, this river provides an excellent testing ground to further investigate the relationship between stressors and wild fish responses. These pollution sources include: industrial effluent; sewage effluent; storm water runoff; geothermal fluid discharges; dairy farm effluent; and other agricultural land use discharges. At 425 km the Waikato River is the longest river in New Zealand and this study will contribute to the few on cumulative impacts along large rivers. 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 in the upstream reaches of the river and prevent access for migratory fish. Due to the number of impacts present along the length of the river, this study allowed for the examination of the hypothesis that impacts may be cumulative due to additive degradation in environmental quality along the river. By examining stable isotopes of carbon and nitrogen I also aimed to examine bully residency at sites, and investigate if changes and increases in nitrogen sources (for example increased applications of nitrogen fertilizers and discharges of sewage through the catchment) are reflected in increased $^{15}N/^{14}N$ ratios in fish. In addition by sampling population, physiological and biochemical endpoints in the common bully upstream and downstream of the four largest discharges along the Waikato River I aimed to delineate individual discharge effects from cumulative effects. By pairing a population framework assessment (Gibbons and Munkittrick, 1994) with biochemical and chemical measures of exposure and effect, I sought to either rule out potential contaminant effects or point the way to further diagnostic studies.

Table 5.1. River and site physicochemical characteristics. Degree is total degree days for year from 16 December 2002 to 16 December 2003. Us = upstream reference. [a] Temperature at time of sampling. [b] A-G, 5 year median values for selected water quality measurements recorded by the Waikato Regional Council at Waikato River locations (A-G Figure 1.1) (Smith, 2003). [c] value not available for site, figure shown is from 30 km downstream.

Site name	Water temperature (°C)				DO	N	P	Chlorophyll	Black
	Degree	Max	Min	Median	(%)	(g/m^3)	(g/m^3)	*a* (g/m^3)	disc
Taupo	5564	23.7	8.2	15.1[a]	-	-	-	-	-
A[b], control gates	-	-	-	14.3	101.4	0.07	0.006	0.002	>5.41[c]
Geothermal Us	5598	20.7	11	15.5[a]	-	-	-	-	-
Geothermal	6507	26.1	12.5	19.4[a]	-	-	-	-	-
B[b], Whakamaru	-	-	-	16.4	103.8	0.19	0.021	0.008	2.6
BKME Us	5908	23.7	9.7	18.6[a]	-	-	-	-	-
BKME	6406	23.0	11.5	19.6[a]	-	-	-	-	-
C[b], Waipapa	-	-	-	16.3	103.0	0.25	0.026	0.008	2.6
Sewage Us	5892	21.4	10.5	17.5[a]	-	-	-	-	-
Sewage	5923	21.6	10.5	17.6[a]	-	-	-	-	-
D[b], Narrows	-	-	-	16.2	100.5	0.35	0.032	0.010	1.6
E[b], Horotiu	-	-	-	16.3	99.6	0.41	0.043	0.012	1.4
Thermal Us	5896	22.0	9.7	19.7[a]	-	-	-	-	-
Thermal	6550	23.5	11.8	21.0[a]	-	-	-	-	-
F[b], Huntly-Tainui	-	-	-	16.3	97.1	0.55	0.059	0.013	0.9
G[b], Tuakau	-	-	-	17.0	100.8	0.60	0.068	0.019	0.7

5.2 Results

5.2.1 Site physicochemical characteristics

Total degree days for the geothermal, BKME and thermal discharge sites were 8-14% higher than their respective upstream sites (Table 5.1). Increases in temperature were mostly evident through autumn, winter, and spring. These temperature increases were limited to the effluent plumes and though there was a change in the temperature of the river between Lake Aratiatia upstream (geothermal Us) and Lake Maraetai upstream (BKME Us), there was no overall temperature increase evident along the total length of river sampled. Wide fluctuations in water level were noted at the geothermal and sewage upstream sites during sampling due to hydroelectric flow management. There was a gradual increase in nutrients and primary productivity, and a reduction in water clarity along most of the length of the river (Table 5.1).

5.2.2 Stable isotopes

Significant differences in $\delta^{13}C$ and $\delta^{15}N$ were found between common bully from all paired sites except the sewage sites (Figure 5.1a). In particular a strong shift in $\delta^{13}C$ towards a more terrestrial signature was seen in common bully from the BKME site (Figure 5.1b). Downstream trends of increasing $\delta^{15}N$ and decreasing $\delta^{13}C$ were apparent (Figure 5.1a).

5.2.3 Blood variables

An increase in total haemoglobin in both sexes at all warmer sites was seen, and male RBC counts and female Hct were positively correlated ($p < 0.05$) with site degree days. Females from the thermal site had significantly higher Hct, MCV and MCH than paired reference site (Table 5.2). Leucocrit (Lct) values in male bully from the sewage site were significantly higher than its upstream paired reference site (Table 5.2). No cumulative impacts are apparent in blood variables with increasing distance downstream.

5.2.4 Catch rates

On average three times as many common bully were caught per minnow trap at the BKME upstream site compared to the BKME discharge site ($p < 0.001$, Mann-Whitney *U* Test). Although difficult to compare numbers caught in beach seines at other sites, six beach seines (approximately 27 fish per seine) were required at the geothermal upstream site to catch comparable numbers of large bullies to those caught in two beach seines (approximately 79 fish per seine) at the geothermal discharge site. Considerable effort was also undertaken beach seining at the Motuoapa Bay, Lake Taupo site with the more than 10 beach seines carried out over a large expanse of lake shore failing to capture sufficient large common bully to extract blood from.

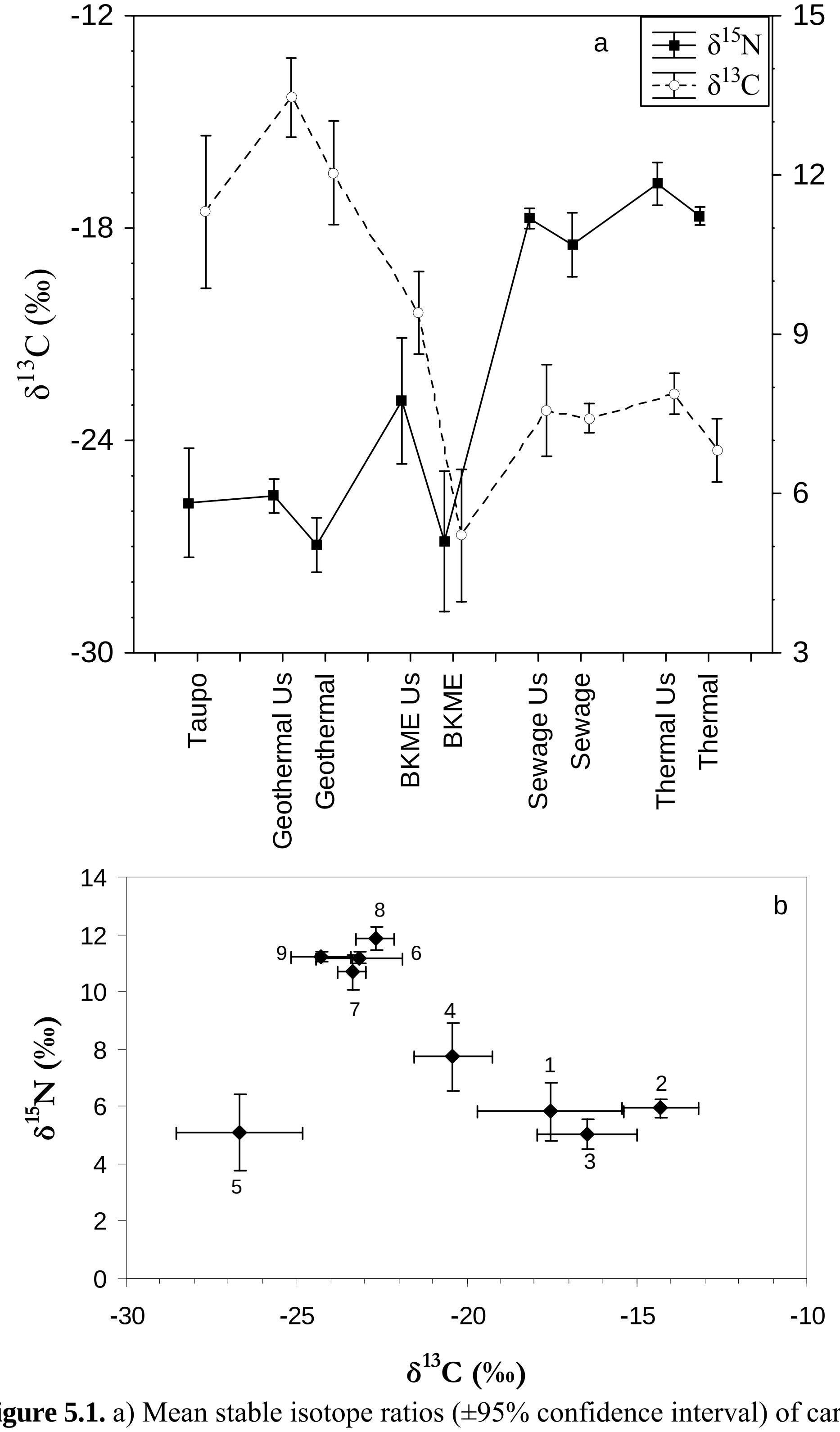

Figure 5.1. a) Mean stable isotope ratios (±95% confidence interval) of carbon and nitrogen in adult female common bully from nine sites in the Waikato River, New Zealand. b) Dual isotope plot of mean (±95% confidence interval) in $\delta^{13}C$ and $\delta^{15}N$. Sites are numbered from the most upstream site (1) Lake Taupo, (2) Geothermal upstream, (3) Geothermal, (4) Pulp and paper upstream, (5) Pulp and paper, (6) Sewage upstream, (7) Sewage, (8) Thermal upstream, to (9) Thermal, the most downstream site. b) Longitudinal patterns in $\delta^{13}C$ and $\delta^{15}N$ from upstream Lake Taupo to downstream Huntly (thermal) sites.

Table 5.2. Mean blood parameters, (Haematocrit (Hct), red blood cell count (RBC), mean cell volume (MCV), haemoglobin (Hb), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC)) in common bully from the Waikato River, New Zealand. Us = Upstream reference. Differences between paired sites ($p < 0.05$, ANOVA) are denoted by superscript letters.

Site	No.		Hct (%)		RBC (cells/L x 10^{13})		MCV (L x 10^{-12})		Hb (g/L)		MCH (g/cell x 10^{-10})		MCHC (g/L)		Lct (%)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Taupo	1	2	47	21	-	-	-	-	-	-	-	-	-	-	1.43	1.93
Geothermal Us	10	22	34	27	0.15	0.15	0.22	0.17^{A}	44.0	38.8	0.29	0.25	137	157^{B}	1.60	0.97
Geothermal	9	16	42	33	0.18	0.15	0.22	0.21^{A}	48.0	37.9	0.26	0.25	136	123^{B}	2.01	0.65
BKME Us	3	14	30	29	0.17	0.20	0.17	0.15	52.3	50.5	0.31	0.26	189	180	2.77	1.14
BKME	6	15	29	29	0.18	0.20	0.16	0.15	50.5	52.4	0.29	0.27	180	190	1.99	1.04
Sewage Us	7	18	26	29	0.16	0.19	0.16^{C}	0.15	42.8	49.3	0.27^{D}	0.27	176	175	0.60^{E}	0.82
Sewage	5	14	38	30	0.17	0.16	0.22^{C}	0.19	58.4	45.2	0.35^{D}	0.29	168	162	1.95^{E}	1.19
Thermal Us	12	12	33	29^{F}	0.18	0.18	0.18	0.16^{G}	50.2	48.1	0.28	0.27^{H}	160	172	1.06	0.59
Thermal	11	13	35	42^{F}	0.18	0.20	0.20	0.21^{G}	54.3	59.9	0.30	0.30^{H}	168	147	0.86	1.11

5.2.5 Length frequency, age and growth

Beach seining and minnow trapping caught a wide range of common bully from 20-30 mm young of the year to >100 mm large adults (Figure 5.2). A trend for discharge site fish to be larger than upstream paired sites was apparent (Figure 5.2) and lengths of sub-sampled adult fish from paired sites (Table 5.3) also showed this trend, although only BKME males and thermal females were significantly longer and heavier than upstream paired sites (p = 0.015, 0.006, 0.001, and 0.001 respectively, Mann-Whitney *U* Test) (Table 5.3). Fish from the geothermal sites were larger and Lake Taupo bullies smaller than overall river mean length and weights. Sub-sampled bully were younger than bully from respective upstream paired sites at BKME and sewage sites ($p < 0.01$, Mann-Whitney *U* Test, Table 5.3). Overall sub-sampled bully from the riverine sites were younger than bully from the upstream lake sites. Length at age 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. As suggested by length frequency distributions and mean length at age (Figure 5.2), growth as assessed by the length at age model was significantly different at all four discharge sites (Table 5.3). All discharge sites except the sewage site had higher ultimate growth (L_{∞}) than the paired reference site. The reverse trend was apparent at the sewage site. Cumulative effects were not apparent over longitudinal range of sites we sampled.

5.2.6 Condition factor, liver somatic index (LSI) and spleen somatic index (SSI)

Female bully from the BKME site were in better condition than those from the upstream site (Figure 5.3). Significant interaction terms in male bully $\log_{10}$ weight-$\log_{10}$ length ANCOVAs at geothermal and sewage suggest that larger fish from the impact site were in poorer condition than large fish upstream (Figure 5.3). Male and female bully from the sewage site and female bully from the BKME site had better than mean condition compared to the overall river mean (Figure 5.3).

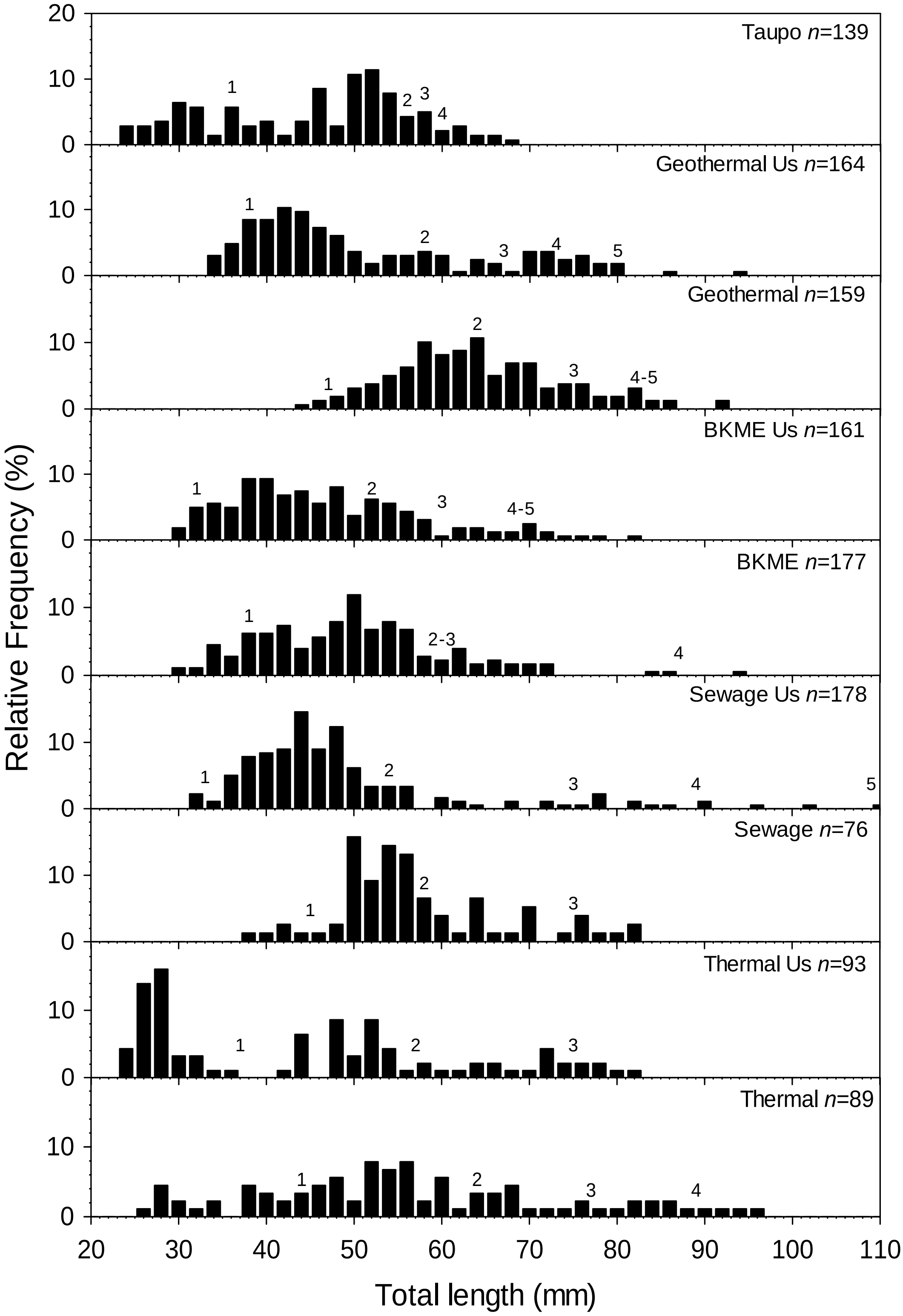

Figure 5.2. Lengths of common bully at nine sites in the Waikato River, New Zealand. Numbers above bars are aligned with approximate mean lengths at age shown. At the BKME Us site difficulties in finding bully large enough to sample led to selection of larger fish from large numbers of smaller fish, therefore small fish are under-represented in length frequency measurements.

Table 5.3. Characteristics of sub-sampled common bully from discharge and upstream reference (Us) sites. Maximum length (L_∞) and growth constant (k) calculated using combined ages of males and females and a modified von Bertalanffy equation for aged fish. Values are given as mean ± SE. Differences between paired sites ($p < 0.01$, $^{A\text{-}D}$ residual sum of squares procedure (Chen et al., 1992) , ^{E} Mann-Whitney U Test) are denoted by different superscript letters.

Site	No. sub-sampled		Length (mm)		Weight (g)		Age (year)		Growth	
	Male	Female	Male	Female	Male	Female	Male	Female	L_∞ (mm)	k
River average	175	246	61.7 ± 0.9	65.0 ± 0.8	2.96 ± 0.16	3.58 ± 0.13	2.5 ± 0.06	2.6 ± 0.05		
Taupo	12	15	57.3 ± 1.2	58.6 ± 1.2	1.96 ± 0.12	2.39 ± 0.16	2.9 ± 0.19	3.0 ± 0.20	59.2	1.10
Geothermal Us	20	31	66.2 ± 1.8	69.4 ± 1.8	3.51 ± 0.35	4.13 ± 0.38	3.1 ± 0.24	3.3 ± 0.19	78.2^{A}	0.56
Geothermal	21	28	71.3 ± 2.0	74.3 ± 1.5	4.29 ± 0.37	4.71 ± 0.32	2.7 ± 0.16	3.1 ± 0.13	81.0^{A}	0.70
BKME Us	21	28	56.4 ± 1.8^{E}	61.2 ± 1.7	2.02 ± 0.27^{E}	2.79 ± 0.24	2.8 ± 0.15^{E}	2.9 ± 0.15^{E}	66.6^{B}	0.66
BKME	23	27	59.7 ± 0.9^{E}	63.9 ± 2.1	2.45 ± 0.11^{E}	3.72 ± 0.44	2.0 ± 0.06^{E}	2.3 ± 0.14^{E}	83.9^{B}	0.53
Sewage Us	20	31	58.7 ± 3.3	66.7 ± 2.9	3.06 ± 0.83	4.23 ± 0.56	2.2 ± 0.17	2.7 ± 0.13^{E}	130.3^{C}	0.21
Sewage	21	31	56.4 ± 1.4	61.4 ± 2.3	2.22 ± 0.16	3.24 ± 0.34	2.1 ± 0.07	2.1 ± 0.12^{E}	82.0^{C}	0.55
Thermal Us	21	23	62.1 ± 2.4	58.3 ± 2.3^{E}	2.75 ± 0.29	2.50 ± 0.33^{E}	2.1 ± 0.07	2.2 ± 0.08	79.8^{D}	0.56
Thermal	16	32	67.4 ± 5.0	66.3 ± 2.0^{E}	4.35 ± 0.83	3.66 ± 0.33^{E}	2.3 ± 0.27	2.4 ± 0.12	85.8^{D}	0.62

Female bully from the BKME site had larger livers than those from the upstream site (Figure 5.3). Geothermal site females had smaller livers than upstream females (Figure 5.3). No consistent longitudinal trend in condition (condition factor or LSI) in lake or river sites was apparent (Figure 5.3). Spleen size was quite variable although males and females showed same trends (Figure 5.3). Slopes of $\log_{10}$ spleen weight- $\log_{10}$ fish weight were significantly different from upstream paired site (significant interaction in ANCOVA) for male bully at the BKME site and female bully at the sewage site.

5.2.7 Gonadosomatic index (GSI), fecundity and follicle size

Male bully GSI were all in the 0.12 to 4.7% of body weight range, mean site male GSI was more variable than female GSI (Figure 5.4a and b) and the only significant difference was found at the geothermal site where male gonads were smaller than upstream reference site. During the course of examining dissected male gonads it was noted that seminal or ‘testicular glands” excluded from GSI varied in size and could be quite large being up to 2.5 times the weight of testes. Adult female common bully GSI ranged from as low as 0.4 to 22% of body weight, although mean gonad size (GSI) of sub-sampled females at most sites was consistent at about 8% (Figure 5.4b). Lake Taupo reference females had lower GSI than the river mean (Figure 5.4b). No significant differences in female gonad size were found in any of the paired sites.

Fecundity estimates for females with a GSI greater than 5% (maturing females) ranged from 205 to 2123 follicles/g of female body weight. Mean site fecundity/g did not vary to this extent and were in the 600 to 1200 range (Figure 5.4c). There were no significant differences between paired sites although non-homogeneous variances prevented valid comparisons between the BKME sites. Ultimate mean follicle diameters ranged from 0.43 to 0.72 mm (Figure 5.4d). For the entire dataset of females where estimates of fecundity were possible, there was a positive relationship between fecundity and somatic weight (r =0.77, $p < 0.001$). Although a negative relationship (r =-0.45, $p < 0.001$) between fecundity/g and somatic weight indicates that larger females had relatively fewer follicles. This could be explained by larger females having larger follicles but no significant relationship was found, there was however a positive relationship between GSI and mean follicle diameter (r =0.67, $p < 0.001$) which may have confounded relationship between female size and follicle diameter.

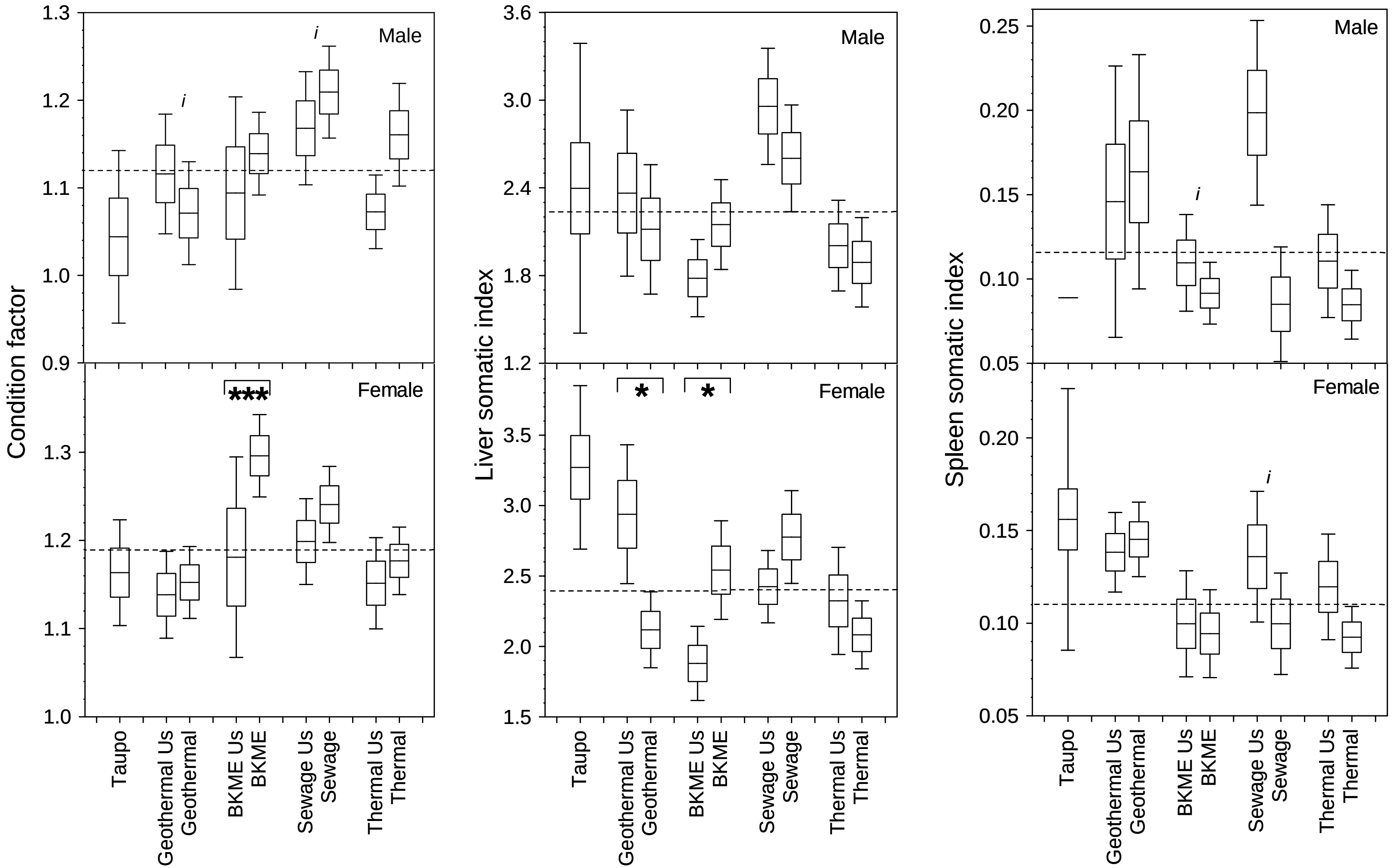

Figure 5.3. Somatic indices for mature male (top) and female (bottom) common bully. Boxes are mean ± SE and whiskers are 95% confidence intervals. Asterisks indicate pairs of values significantly different at * $p < 0.05$ and *** $p < 0.001$. An *i* indicates different slope of organ vs. body weight relationships between paired sites. An analysis of covariance could not be carried out on male common bully from the paired sewage sites due to non-homogeneity of variances. Dashed horizontal line is river mean.

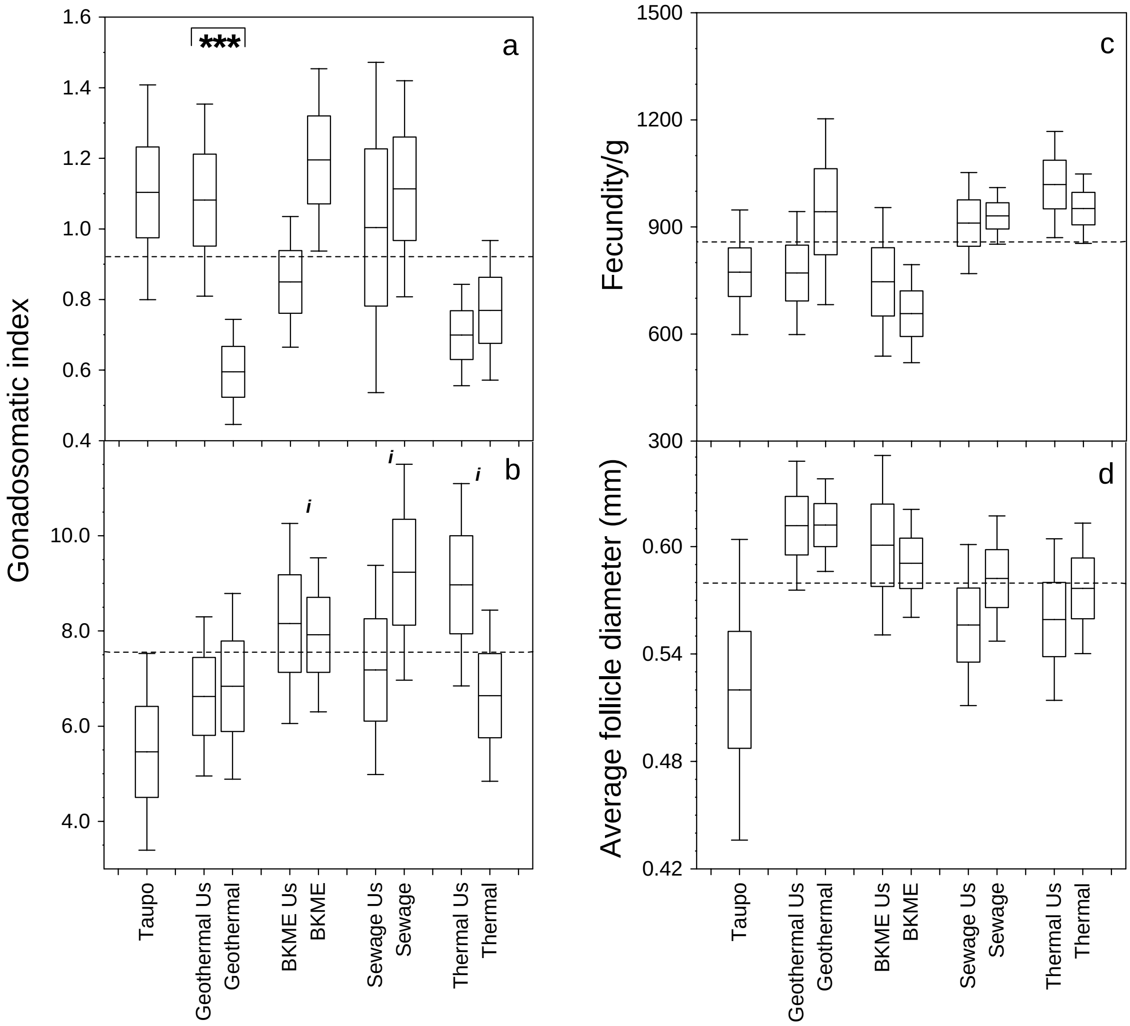

Figure 5.4. Gonadosomatic Index (GSI), for mature male (a) and female (b) common bully, and fecundity per gram (c), and mean follicle diameter for mature females (d). Asterisks indicate pairs of values significantly different at * $p < 0.05$, an *i* indicates different slope of gonad vs. body weight relationships between paired sites. Boxes are mean ± SE and whiskers are 95% confidence intervals. Dashed horizontal line is river mean.

5.2.8 Summary of population parameters

Population and physiological variables were categorized as being age structure, energy storage or energy allocation variables (Table 5.4). Age structure was based on the mean age and the relative distribution of the youngest year class versus adult common bully. Energy storage was indicated by condition factor and liver size, while estimates of energy expenditure included: gonad size; fecundity; and growth. The response pattern at the geothermal site suggests common bully were unable to efficiently convert energy into reproductive tissue. The pattern at the BKME site suggests that despite abundant food, recruitment is not occurring at a comparable rate to the upstream reference site. Changes in age structure, energy storage and energy allocation at the sewage site are not clear, and were biased by 2 to 3 large fish caught at the upstream reference site making conclusions difficult. The response pattern at the thermal site is also hard to associate with a generalized response, but again increase in growth at the thermal site dominated energy allocation. The majority of parameters increased at discharge sites. Energy appears to be allocated to growth rather than reproduction, and despite increased allocation of energy to growth, bully appear able to maintain energy stores.

Table 5.4. Comparisons of: age structure (mean age, age distribution); energy storage (condition factor, LSI); and energy allocation (growth, gonad weight, fecundity) at impact and paired reference sites. A + indicates a significant increase, a – a significant decrease and a 0 no change in at least one variable at the impact site.

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

5.3 Discussion

Common bully captured at sites throughout the Waikato River reflected the physiochemical nature and especially the thermal regime of water at those sites. Point-source discharges had mostly positive effects on common bully individuals. However, comparatively few juvenile bully were caught at discharge sites and the reduction in gonad size of males at the geothermal site may be related to this. Recruitment limitation and a similar lack of younger age classes was seen in brown bullhead *Ameiurus nebulosus* (Lesueur, 1819) at geothermal sites (West et al., 2006). The pulp mill effluent caused increased energy allocation (growth) and storage. Common bully individuals caught from the sewage site were not visibly impacted by the discharge and were growing faster than upstream paired site. Thermal power generation resulted in larger length at age and decreased energy allocation (growth) but no differences in energy storage. Physicochemical and habitat characteristics of sites influenced common bully populations over the geographic range of sites I sampled, but there was little evidence of additive or cumulative changes along the river. A lack of recruitment and allocation of energy to reproduction was apparent at discharge sites.

The consistency of stable isotopic signatures of common bully showed they were resident at sites and even ratios at the closest sites (geothermal) were significantly different, this supports residence of small-bodied fish species as seen in a previous study (Gray et al., 2004). It also suggests that fish reflect the ecology of the site they were caught from as seen in a previous study of Waikato River tributaries (Hicks, 1997). The fish from the BKME site had very different stable isotope signatures supporting their assimilation of terrestrial pulp and paper sourced carbon and nitrogen, an observation supported by a study of Canadian Rivers receiving pulp mill effluents (Wayland and Hobson, 2001). The diet of common bully from the arm of Lake Maraetai that pulp mill effluent is discharged into is dominated by snails (Boubée et al., 1995) which graze on the likely source of pulp and paper carbon and nitrogen. The similarity of $\delta^{13}C$ and $\delta^{15}N$ at the most spatially separated paired (sewage) sites is surprising but could be explained by the majority of fish at the sewage upstream site being caught just downstream of the confluence of a major agricultural catchment which might be expected to have isotope ratios more similar to the lower Waikato River. Strong longitudinal trends in $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are consistent with increasing inputs from tributaries (lower $^{13}C/^{12}C$ runoff) diluting the high ^{13}C (equilibrated with atmospheric CO_2) Lake Taupo water and increased inputs of ^{15}N rich nitrogen (for example agricultural fertilizers and treated sewage) in a downstream direction. The same longitudinal trend was seen in $\delta^{13}C$ values of dissolved inorganic carbon in Waikato River water samples taken from Lake Taupo to Huntly (Fitzgerald, 1996). Enrichment in ^{15}N in fish flesh has been correlated to increased amounts of agricultural land in the catchments that fish were caught from, as seen in studies overseas (Lake et al., 2001, Harrington et al., 1998), and in Waikato River tributaries (Hicks, 1997). High fish muscle $\delta^{15}N$ have also been correlated with increasing amounts of sewage waste in Australian estuaries (Schlacher et al., 2005). While $^{15}N/^{14}N$ ratios can also be affected by a range of processes such as nitrification or denitrification, ammonia volatilisation and/or leaching of depleted NO_3^-, all of which may interact (Bedard-Haughn et al., 2003), the fact that the trend in sites spread over such a large distance is consistent and large (approximately 6‰) suggests it is driven by a common factor such as enrichment from agricultural and treated sewage sources.

The total haemoglobin of common bully blood showed changes consistent with physiological responses to water temperature at all sites. Unlike brown bullhead at this site (West et al., 2006) and five of the six pulp and paper effluent studies reviewed in (Folmar, 1993), no decrease in Hct was seen in common bully at the BKME site. The fact that the expected increase in Hct or total haemoglobin did not occur may also indicate an inability by the bully to adapt to warmer and lower dissolved oxygen conditions in the BKME discharge site. Previous comparisons (Richardson et al., 1996, Richardson and Boubée, 1999) of blood parameters in bully sampled from BKME discharge and upstream Lake Maraetai sites have also shown reductions in Hct, but combination of sexes and different site pairings make direct comparison difficult. The only significant increase in white blood cells (Lct) occurred at the sewage site and could indicate an impact. However, the literature reviewed in (Folmar, 1993) showed inconsistent patterns of response for Lct. It is also difficult to distinguish if difference in Lct between sewage paired sites isn't actually due to Lct at the sewage upstream site being significantly depressed. Previous studies of Lct in common bully blood (Richardson et al., 1996, Richardson and Boubée, 1999) have also found highly variable levels and although Lct was lower at the BKME discharge site in the 1996 study (Richardson et al., 1996) comparisons using fish health profiles (FHP) (Richardson, 1998) showed it was not outside normal ranges. Difficulties in obtaining fish especially males large enough to give good blood samples contributed to within site variability which ultimately limited the linkage of fish physiological responses to components of discharges.

Contrary to the small size of common bully, sagittal otoliths were found to be comparatively large. A consistent oval shape of otoliths aided preparation and aging. Results of the captive rearing of young of year and adult common bully and fluorophor marking of otoliths validated the use of otoliths to age this species. An otolith clearing agent such as propylene glycol was not used but may aid counting of annuli and reduce the need for double grinding of larger otoliths. Captured common bully were dominated by one to three year old fish with the occasional four and five year old fish. Variable and sometimes substantial growth of fish in their first year required care in assignment of first annuli. The occasional presence of a strong check presumably caused by spawning in the second year of otolith growth was also noted. The only other published study on common bully ages (Stephens, 1982) reported few fish surviving into their third year, whereas 40% of fish I aged were older than 2 years. While we were sub-sampling adult fish this still suggests that survival at most of the sites we sampled, was better than Lake Waahi, the shallow eutrophic lower Waikato River lake sampled in (Stephens, 1982). An increase in mean lengths at age, and significant increases in modelled L_∞ appeared to be strongly influenced by point-source discharges. This may have been influenced by the warmer temperatures, and in the case of BKME, increased food supply due to nutrient inputs. This was somewhat unexpected, but next to eels the preferred temperature of common bully of 20.2°C is amongst the highest of the New Zealand native fish that have been tested (Richardson et al., 1994), therefore would benefit from the warmer autumn, spring and winter temperatures at the warmer discharge sites. Greater length at age of other small fish species downstream of pulp mills has been noted in the very similar slimy sculpin (*Cottus* *cognatus*) (Galloway et al., 2003) and spoonhead sculpin (*Cottus ricei*) (Gibbons et al., 1998b).

Increased energy storage at the BKME site matches observations of responses of other fish species to pulp and paper discharges (McMaster et al., 1991, Munkittrick et al., 1994, Karels et al., 1998), and brown bullhead at this site to the BKME discharge (West et al., 2006). It is likely that bully were exposed to pulp and paper contaminants at the BKME sites as brown bullhead (West et al., 2006) and shortfin eel (*Anguilla australis* Richardson 1848) caught from the same site had pulp and paper contaminants in bile and elevated 7-ethoxyresorufin-*O*-deethylase EROD levels.

As greater energy allocation to growth was one of the most common responses at the discharge sites it is surprising that given sufficient energy stores at BKME, sewage and thermal sites, energy allocation to reproduction wasn't higher. The significant reduction in male gonad size at the geothermal site is of concern especially as male common bully are nesters and their contribution may determine reproductive success. The role of the 'testicular gland' is as yet undetermined but if it is similar to that described in reef dwelling fish *Scartella cristate* (Neat et al., 2003) then it has an important role in nesting males and should be included in estimation of gonadosomatic indices. The higher numbers of smaller common bully caught at the BKME Us site suggest that recruitment is lower at the BKME site. Despite this, mean site GSI, follicle diameter and fecundity/g of females were remarkably consistent. Although the range of fish sampled from Lake Waahi (Stephens, 1982) were smaller than bully we sampled, fecundities of similar sized individuals we measured appear much higher. Overall sites we sampled including discharge sites appear to be much more favourable habitat for common bully than Lake Waahi (Stephens, 1982).

Stable isotope signatures of common bully gave strong evidence of fish residence at sites, the dominant terrestrial signature at the BKME site and longitudinal trends seen also show common bully reflect changes in their habitat. Distinct changes in populations at each of the paired sites and changes in individual variables also suggest that fish were responding to those discharges. Despite the gradual deterioration in water quality downstream, particularly nutrient enrichment and increased turbidity, no concomitant cumulative impacts were observed in common bully. The possibility remains that the effects of sub-lethal or chronic stressors discharges on sensitive juvenile life-stages of common bully result in compensatory population responses that hinder detection of those individual effects (Power, 1997). In particular, population responses at the geothermal and BKME discharge sites warrant further study to investigate possible effects on recruitment. Results from this study support overseas examples of the use of small-bodied fish in biomonitoring. This study also confirms the suitability of common bully in the wild as a sentinel species, identified potential reproductive impairment at geothermal and BKME discharge sites that should be followed up with specific studies and provided life history information that can be used in future assessments of environmental health using common bully.

Chapter Six: General Discussion

Fish individuals and populations integrate anthropogenic effects on aquatic ecosystems to a greater extent than lower trophic levels of aquatic ecosystems. However, with greater complexity come the challenges of accounting for inter-relationships between individual fish variables, fish population variables, and habitat, and determining causality between contaminants and biological effects (Adams, 2003). These challenges can be met, and Collier (2003) recommends a set of criteria that would aid establishing causality. These criteria will still be limited by the increased complexity introduced by assessing responses at a population level especially where multiple stressors are involved. Our aim was to establish causality, or detect effects that can be followed up by targeted investigations of specific species and life stages.

6.0 Site physicochemical characteristics and experimental design

Despite the possibility of other upstream discharges affecting upstream paired reference site fish, fish at these sites appeared good controls against which to determine impacts of the targeted discharge. An example of this was at the geothermal sites in Lake Aratiatia where reduced brown bullhead and common bully recruitment was evident at both sites. Assuming the discharge was not affecting recruitment in the whole lake, then some other common cause such as flushing due to power generation was at fault. If the upstream reference site was in another catchment without common extraneous effects then the lack of recruitment at the geothermal discharge site would have been attributed perhaps erroneously to effects of the discharge. The placement of the sewage upstream site above other municipal discharges compromised matching of habitats. This highlights the conflict between selecting sites for low contamination and matching habitats. In hindsight this would have been solved by adding another site closer to Hamilton with habitat better matched to the sewage downstream site. Overall, site habitat and particularly water temperatures appear to have had greater influences on fish than chemicals in discharges. Assessment of cumulative impacts was hindered by responses of fish to the change from lacustarine to riverine habitat.

There is a potential that responses of shortfin eel, captured at the end of summer, to discharges were different than responses of brown bullhead and common bully caught at the end of autumn. This would be more likely if there were differences in discharge characteristics and water temperature at sites. BKME was the site that was marginally cooler than the upstream reference when shortfin eel were sampled, and was 1-4.5°C warmer when brown bullhead and common bully were sampled. As significant differences in blood variables were only normally seen when discharge site water temperatures were 3-4°C warmer, then it is likely that differences seen due to temperatures during brown bullhead sampling would not be seen during shortfin eel sampling. However, it should be noted that there was evidence that brown bullhead blood variables may have been impacted by the BKME discharge.

6.1 Chemical and biochemical indicators of exposure to contaminants

The chemical measurements of fish tissues or fluids undertaken at sites were vital for determining residence of fish at sites and exposure to contaminants in the discharges. The combination of variables that reflect recent exposure to contaminants such as bile chemistry, with longer term indicators such as tissue metals or isotope ratios gave the strongest evidence of exposure and residence. Overall levels of contamination (metals, PAH) in brown bullhead and shortfin eel were low compared to comparable studies overseas (Eufemia et al., 1997, Arcand-Hoy and Metcalfe, 1999, Bordajandi et al., 2003, Pinkney et al., 2004, Schmitt, 2004, Ribeiro et al., 2005). Other studies of New Zealand freshwater fish have also found low levels of contaminants (Jones et al., 1995, Buckland et al., 1997).

6.2 Blood variables

Blood total haemoglobin in all three species largely responded in a predictable and consistent way to site water temperatures and dissolved oxygen. Significant differences between paired sites were usually found where discharge site temperatures were 2 to 4°C higher at time of sampling. The only impact detected in total haemoglobin of blood was in brown bullhead at the BKME site, where instead of an expected increase in total haemoglobin a significant decrease was found. Shortfin eel caught from the same site were not affected in the same way supporting their tolerance of hypoxia (Forster, 1981, Dean and Richardson, 1999, Landman et al., 2005). The possibility remains that effluent imposed differences in the blood of fish at discharge and reference sites were hidden by the magnitude of haematological responses to capture and transport stress. Factors that mitigate against this include the significant differences seen between discharge sites and their cooler reference sites and impacts seen in brown bullhead blood from the BKME site. However catecholamine-mediated cell swelling normally seen in salmonid fish does not occur in brown bullhead (Szebedinszky and Gilmour, 2002) and was also absent in eel (*Anguilla rostrata*) even under hypoxic conditions (Perry and Reid, 1992). To investigate effects of additional transport stress on brown bullhead blood, I compared relative spleen size, haematocrit and mean cell volume from fish bled on site with those bled in the laboratory. Changes consistent with increased stress-induced splenic release of red blood cells were seen. However, ascribing transport stress as the primary cause of these changes is hindered by field-bled fish coming from lake sites and lab-bled fish which came from river sites. To avoid transport stress the best place to bleed fish is immediately on removal from nets in the field. As this what not possible at all sites statistical comparison of sites was limited to paired sites treated in the same manner.

Confounding effects of pollutants (for example nitrite) on levels of haemoglobin at reference sites would have been elucidated by measurements of methaemoglobin. While the validity of blood variables as sole indicators of fish health remains in question (Houston, 1997), they appeared to be sensitive indicators of fish physiology at sites and taken together with other variables, they provided an insight into the responses of fish to all aspects of the discharges.

6.3 Catch rates, length frequency, growth, somatic indices and reproduction

Changes in the numbers, size and growth rates of fish captured at discharge sites were some of the most marked effects seen. While the majority of changes in brown bullhead and shortfin eel attributable to discharges seen were considered beneficial, comparatively low numbers of juveniles of brown bullhead and common bully at the geothermal site, and low numbers of common bully at the BKME site suggest limited recruitment. Further investigations into the recruitment levels at these sites are warranted. A large increase in numbers of juvenile brown bullhead at the BKME and thermal sites could indicate increased energy allocation to reproduction or early and more successful spawning.

Traditional measures of fish health (condition factor, liver size, gonad size, fecundity and egg size) were not highly variable at a site level, but responded consistently in explicable patterns and proved reliable indicators.

Trends in fecundity of brown bullhead and common bully were consistent with changes in female gonad size. This trend was even evident at the BKME site where brown bullhead had reduced sex steroid levels and there were reduced numbers of small common bully. As seen with the most contaminated sites in Lesko et al. (1996) brown bullhead maintained levels of fecundity similar to reference sites at all discharge sites. Common bully also maintained fecundity at similar levels at all sites a comparable maintenance of egg output at contaminated sites was seen in analogous Cottus species at Pulp and Paper Mill (Gibbons et al., 1998b, Galloway et al., 2004) and municipal sewage sites (Galloway et al., 2004). Robust statistical comparisons of female brown bullhead and common bully gonad and liver size were strengthened by inclusion of each other as covariates. The inclusion of GSI as a covariate in statistical comparisons of sex steroids in brown bullhead also aided realistic comparisons. Measurement of gonad size and blood sex steroids is possible in eels, particularly maturing individuals (Lokman et al. 1998 and Lokman and Young 1998). As the majority of the eels we caught were non-migratory the use of reproductive endpoints was not feasible. Sampling later in the year when greater numbers of migrant eels are present would make measurement of reproductive endpoints feasible. However, different rates of eel maturation between at sites may limit comparisons as was the case for Lake Maraetai sites in this study.

6.4 Summary of population parameters

Insights into fish responses to common aspects of the discharges were made possible by simultaneously sampling four types of discharges. The most obvious was temperature which had significant benefits for brown bullhead, shortfin eel and common bully. At a population level the age composition of brown bullhead and common bully showed opposite responses to discharges (Table 6.1).

Table 6.1. Summary of population indicators of age structure for three species sampled. A + indicates a significant increase in at least one variable, a – indicates a significant decrease and a 0 indicates no change between the impact site and paired upstream site.

Impact Site	Age structure (mean age, age distribution)		
	Brown bullhead	Shortfin	Bully
Geothermal power	0	N/A	+
BKME	–	0	+
Sewage	0	0	0
Thermal power	–	0	+

Condition factor and liver size were positively correlated for all species and sexes verifying their common role in energy storage (Table 6.3). However brown bullhead and common bully at the thermal site did not follow this general trend. For brown bullhead, a decrease in condition was accompanied by an increase in liver size, but in common bully an increase in condition was accompanied by a decrease in liver size. The different energy storage of these two species may reflect different energy allocation in response to increased temperature, with brown bullhead increasing reproduction in the warmer thermal discharge but common bully not able to do so. The common response pattern to BKME endorses the consistency of established population response patterns (Gibbons and Munkittrick, 1994) and nutrient enrichment associated with pulp and paper discharges in Canada, however only limited indications of the metabolic disruption seen at those discharge sites (Lowell et al., 2005) were seen in brown bullhead.

While only one of the changes in common bully reproductive measures was statistically significant, the fact that changes in numbers of small brown bullhead and common bully were consistent with trends in GSI and fecundity suggests trends were real. These trends were also consistent with population response patterns assigned to the respective species (Table 6.2).

Table 6.2. Summary of physiological indicators of energy allocation for three species sampled. A + indicates a significant increase in at least one variable, a – indicates a significant decrease and a 0 indicates no change between the impact site and paired upstream site.

Impact Site	Energy allocation (growth rate, gonad weight, fecundity)		
	Brown bullhead	Shortfin	Bully
Geothermal power	0	N/A	+
BKME	+	0	+
Sewage	+	–	–
Thermal power	–	0	+

Table 6.3. Summary of physiological indicators of energy storage for three species sampled. A + indicates a significant increase in at least one variable, a – indicates a significant decrease and a 0 indicates no change between the impact site and paired upstream site. NC indicates that comparisons were not possible due to lack of mature fish.

Impact Site	Energy storage (condition factor, liver weight)		
	Brown bullhead	Shortfin	Bully
Geothermal power	0	N/A	–
BKME	+	+	+
Sewage	NC	–	0
Thermal power	0	0	0

6.5 Was different ecology of sampled fish reflected in responses to discharges?

For the Waikato River, unimpacted sites may have low numbers of poorly conditioned brown bullhead. The poor performance of brown bullhead at cooler sites may be due to its high preferred temperature of 29°C (Cranshaw, 1974), compared to lower ambient temperature of much of the Waikato River. In addition to greater growth and better condition, the increased reproduction of brown bullhead at the warmer BKME and Thermals sites is of concern as brown bullhead are typical of many eurythermic pest fish species in New Zealand, therefore significant heat discharges will enhance conditions for pest species (West et al., 2005). The change from hydro-electric impoundments to riverine environments had detrimental effects on brown bullhead populations and the oligotrophic nature of Lake Taupo appeared to limit growth and condition of brown bullhead. The ability to capture reasonable numbers of most size classes of brown bullhead from sites, and the large size of mature brown bullhead offset some of the limitations inferred by its preference for degraded water conditions.

Shortfin eel also benefited from additional heat and possibly nutrients at discharge sites. In fact shortfin eel were the least impacted of the three species sampled and responded in predominantly positive ways to discharges. Three major factors limit the use of shortfin eel as a sentinel species in the Waikato River: 1) differing levels of recruitment due to barriers to migration (dams); 2) absence of a freshwater reproductive stage; and 3) commercial harvest practices. Variations in recruitment were one of the strongest responses observed in brown bullhead and common bully. The possibility of observing similar responses in shortfin eel was reduced by fluctuations in oceanic survival and subsequent recruitment into freshwater past migration barriers. Reproductive damage suffered by adult eels may not immediately manifest itself in the affected population due to temporal delays in recruitment and single panmictic populations (Smith et al., 2001) that supplement recruitment of impacted populations. The commercial harvest of large eels has profound effects on New Zealand populations of eels (Jellyman et al., 2000, Broad et al., 2002, McCleave and Jellyman, 2004, Beentjes et al., 2006) and all shortfin eel populations we sampled had some level of harvesting. Unknown harvest levels complicate interpretation of pollution effects. However, shortfin eel is one of the few large predatory native fish present in New Zealand freshwaters so offers an opportunity to investigate a native piscivorous fish at the top of the food chain. Piscivorous eels also offer an opportunity to assess biomagnification of contaminants such as mercury (Peterson et al., 2002) and some organic chemicals (Russell et al., 1999).

The comparatively poor condition and low numbers of common bully caught at the Lake Taupo site may be related to Lake Taupo having the lowest water temperatures of any of the sites and common bully having a preferred temperature of 20.2°C (Richardson et al., 1994). Health condition profiles (HCP) of common bully from Lake Taupo were also amongst the lowest recorded in an attempt to establish normal HCP ranges for this species and were not considered suitable for use as a healthy reference population (Richardson, 1998). I would recommend common bully for future use as a sentinel species due to its: great site fidelity; comparative ease with which all life stages can be captured; measurable reproductive endpoints (in vitro steroid production methods need to be developed); widespread occurrence; and importance in freshwater food webs. Recruitment of common bully also appears to be more sensitive to discharges than brown bullhead. Possible factors limiting performance of fish populations identified in our initial assessments should be followed up with studies targeting important aspects of fish populations for example recruitment of fish at Lake Aratiatia (geothermal) sites and recruitment of common bully populations at the BKME site.

Assessment of fish health downstream of the: Wairakei Geothermal Power Station (Geothermal); Kinleith Pulp and Paper Mill (BKME); Hamilton Municipal Waste (Sewage); and Huntly Thermal Power Station (Thermal) offered an opportunity to compare magnitude of effects of respective discharges. Comparisons of effects are largely subjective as receiving environments of the discharges differ markedly. Geothermal is in a small regularly flushed riverine impoundment, BKME is a poorly flushed arm of a large lake at Kinleith, Sewage is a swiftly flowing large river site, and Thermal is a larger slower flowing river site. These variations and the resident fishes' response may exert greater influence than the toxicity of the discharges themselves. Bearing this in mind I rank the magnitude of effects in the following order from largest to smallest: BKME; Geothermal; Thermal; and Sewage. The lack of consistent sublethal effects in the three species sampled even at the most affected site suggests that sublethal toxicity was not occurring at a major level. Concurrent chronic and acute toxicity testing (Hall and Golding, 1998b) would aid comparisons of the toxicity of discharges.

The documentation of fishes' integration of all aspects of conditions at sites was facilitated by our sampling from biochemical to population scales, an approach advocated by Munkittrick and Dixon (1989) and Adams et al. (2000). This approach is also being applied more widely (Porter and Janz, 2003, Mayon et al., 2006), perhaps due to greater access to sensitive biochemical assays, for example cytochrome P_{450} induction (Kloepper-Sams and Benton, 1994), and the greater inferential power conveyed by linking changes over the range of biological organisation. Our study provides further evidence that cause and effect can be inferred in wild fish populations if comparable reference fish populations are sampled, inter-relationships between variables are considered, and sensitive biochemical indicators are paired with individual fish and fish population variables.

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