Methods for Determining the Effects of Pollution on Fishes

_Project 5115 Final Report_

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Louis Tremblay
Stephen Moore
Landcare Research

Mike van den Heuvel
Forest Research

David West
Waikato University

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Foreword

Monitoring and characterising pollution is a priority to ensure that the consequences of human activities on our environment are minimum. However, our activities will always have impacts on the environment so the challenge is to determine what levels are acceptable. The methods that we utilised in this project to determine the impact of pollution on fish are effects-based. In essence, this is based on the philosophy that if you want to determine if there are impacts on the environment, “go ask a fish”. These methods have many advantages as they provide indications of cumulative effects, as contaminants are seldom found isolated but rather in mixtures. Information obtained can provide guidance to environmental managers and trigger further actions to identify causes related to the induced effects. This project was the initial step in adapting methods to New Zealand, and the methods used are being refined and improved to provide more significant outputs and appropriately protect our unique environment.

Another important aspect of the approach used for this project was to work in partnership with various parties. As scientists, we tend to work in isolation, surrounded by our colleagues and peers. This work gave us a good experience on working with different organisations and it was one of the most rewarding aspects of this project. We were fortunate to conduct work at sites from across the country and interact with highly motivated and dedicated people. Everyone was in agreement that it must priority to keep a clean environment for everyone to enjoy, and to ensure the protection of our unique fauna. It is the responsibility of each individual to protect our environment and its ecosystem.

Enjoy the reading.

_I te ora te whenua, ka ora te iwi._
_Manaki ki te whenua, manaaki ki te iwi._

_In the health of the land, lies the health of the people_
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Executive Summary

The New Zealand economy is highly based on the “clean and green” image that attracts tourism and ensures a reputation of sustainability for agricultural products. New Zealand is also a developed country with growing industry (particularly agricultural), and increasing urbanisation. These activities put pressures on the aquatic environment that may potentially lead to adverse effects on fauna and flora. Following discussions with interested stakeholder groups, it was concluded that there was a lack of methods in New Zealand to properly assess the effects of pollution on the native fishes living in impacted catchments. Consistent with international trends, this research took an “effect-based” approach to assess the effects of pollution on fish. These methods examine the responses of organisms, or populations of organisms, in the environment to multiple stresses and seek to diagnose the causative agents.

The two main methods used were the monitoring of population-level variables in wild fishes and examining biochemical responses in eels that were caged directly in areas of concern. Following consultation with tangata whenua, regional councils and industry, five study sites were selected: the Waikato and Tarawera rivers and the Waiwhetu Stream in the North Island; the Cam and Styx rivers in the South Island. These sites were selected based on strong stakeholder partnerships and on the types of stressors they received including industrial effluent, municipal wastewater and mixed agricultural runoff. Both methods employed were generally successful, though on occasion, the nature of the receiving environment or consultation with end-users precluded using one or the other method. The fish caged downstream from the pulp & paper mill and sewage effluent discharges indicated exposure to a number of organic compounds, but elevated mortality or other serious health problems were not observed. Most-dramatic differences were found in the gonad size of common bully sampled upstream and downstream of the effluent discharges on the Tarawera River. Gonads of the fish sampled downstream were significantly smaller and suggested that the fish had already spawned. Analysis of stable isotope signatures suggested that the populations sampled were not migrating between the sites, and subsequent sampling indicates that common bully in the Rangitiki River have similar responses to those in the lower Tarawera. The precise reasons for the differences in these populations is not yet known and reflects our lack of knowledge of the basic biology of many New Zealand native species.

Both caged eel and wild common bully at the South Island locations showed evidence of exposure to organic compounds. Eels showed the highest response at the sewage effluent site on the Cam River and bully showed the highest response in the lower Styx River, though it was unclear what the cause might be in the case of the latter. Wild common bully did show some indication of nutrient enrichment in the Cam River in particular. However, it was difficult to find suitable small lowland reference locations in Canterbury due to the intensity of land use.

The Waikato River is the biggest in New Zealand and receives contaminants from many different sources, and catfish and wild eel were sampled at five sites. A variety of parameters were measured in the fish upstream and downstream from major discharges...
along a large part of the river’s length. Brown bullhead catfish, a pest species, seemed to proliferate in the river, particularly where temperature and nutrients were elevated due to various discharges. Though there were numerous indicators of elevated exposure to organic compounds and metals throughout the river, serious fish health problems were not identified. The only exception was Lake Aratiatia where catfish show a complete inability to recruit young individuals into the population; this effect could not be explained. Bullheads also responded strongly to habitat variability, as the species prefers warm temperatures and slow-moving water.

Wild shortfin eel also revealed a number of indicators showing exposure to pollution. Eel grow well in both lake and riverine environments with the exception of the lower reaches of the river. It is hypothesised that strong fishing pressure reduces the number of large eels, which subsequently increases densities of smaller eels, resulting in slow growth. The Waiwhetu Stream was the most degraded site studied. The requirements for the caging studies could not be met and no common bully were found. Fish community and macroinvertebrate community surveys were conducted at this site. Recent reports had indicated abundant common bully in the stream but none could be found in our study. Their apparent absence was a major finding of the fish community survey and is of significant concern, as bully were found at all other sites. Data from the macroinvertebrate survey showed that diversity was generally poor as compared to a dataset of urban streams. Overall the Waiwhetu was heavily influenced by elevated temperature, loss of habitat due to flood control, exotic weeds, and a large number of pollution sources. In addition, two significant pollution events were witnessed during the short period of sampling.

Overall this research identified that a wide range of impacts are occurring on fishes in New Zealand. However, the impact of pollution on New Zealand rivers is not extreme when compared with the level of adverse effects seen in other more populous and industrial countries. Probably one of the most significant observations for New Zealand was that some sources of pollution, particularly where temperature is elevated, might be benefiting pest fish populations and thus directly threatening native biodiversity. It is concluded that our environment must continue to be monitored using effects-based methodologies in order to ensure the protection of our unique ecosystem and to encourage sustainable activities.

We should also be looking beyond monitoring to assess the degree of adverse effects that may or may not have occurred. In the future we must be monitoring to demonstrate the positive effects of restoration from new low-impact development designs, created to enrich rather than degrade the environment.
1 Introduction

New Zealand is a country with many natural wonders and is regarded by many as one of the best places to live. The economy is based on a “clean and green” image that attracts tourism and ensures quality values of our agricultural products. However, as a result of agricultural activities, urbanisation, and industrialisation there are significant pressures placed upon the New Zealand environment, which might alter its natural state. One such pressure on the environment is the release of chemical contaminants.

Because freshwater systems often act as a sink for contaminants, receiving a variety of anthropogenic compounds from both point and non-point sources, we chose to study fishes, as they represent the species most likely to be exposed to contaminants. As environmental toxicologists we identified a lack of appropriate methods by which to evaluate potential adverse effects of pollution on fishes in their natural environment.

Furthermore, the spiritual and life-giving values of water are paramount to tangata whenua. Consultation has clearly indicated that Maori wish to assert their tino rangatiratanga rights and responsibilities as stated under Article II of the Treaty of Waitangi, regarding management of environmental resources.

All human activities will impact on the environment and it is a management decision to set thresholds acceptable to society. This is a complex job that involves incorporating, economic consequences and environmental impacts as well as social views and cultural values. Environmental impacts usually lie within the realm of science to determine and the discipline that determines the probability of negative impacts to the environment is called ecological risk assessment. Ecological risk assessment has three basic components: chemical criteria, laboratory toxicity bioassays, and environmental effects monitoring. Chemical criteria involve setting “safe” limits for chemicals released into the environment, based on laboratory studies to determine what concentrations of individual chemicals may be detrimental to receptor organisms, i.e. the species exposed to those chemicals. Used alone, chemical criteria suffer a number of deficiencies. They are limited by the number of chemicals being analysed by any monitoring programme. Also, they have limited ability to deal with the complex mixtures of compounds typically found in pollution sources. Therefore, chemical criteria do not address the potentially toxic effects of unidentified compounds in effluents.

Biological toxicity tests provide complementary information in that they use several different species to assess the toxicity of complex pollution sources. Though toxicity tests do integrate the effects of complex mixtures, they examine a select group of impacts, such as mortality, over a relatively short term with a limited number of species. These assays do not examine the effects of long-term chronic exposure, ignore subtle sub-lethal toxicity endpoints, do not examine ecological interactions between species, and are divorced from the complex physical and chemical factors in the real world. Thus, these tools serve only to illustrate an immediate hazard to biota, and negative toxicity results cannot be reliably extrapolated to protecting the environment as they so often are in New Zealand.
Ultimately, the most environmentally relevant way to characterise risk to the environment is to assess the biological integrity of those environments directly. Environmental effects monitoring lags behind these other tools due to the difficulty in understanding the complex variables that govern the natural environment. Internationally, and in New Zealand, these tools are well developed for assessing the status of benthic invertebrate communities (Quinn and Hickey, 1990; Cairns and Pratt, 1993; Hickey and Clements, 1998) but are less developed nationally for evaluating fish population health. Due to the inherent complexity of ecosystems, effects-based monitoring often lacks sufficient certainty to assign causality when impacts are observed. Effects-based studies also are time and resource intensive and observations can take place after the environmental damage has occurred. It has recently been asserted that in almost all cases, environmental damage has already occurred after years of adverse human activity. Thus, rather than attempting to predict the risk of such activities from laboratory studies, we should be going out and measuring the level of damage (Tannenbaum, 2005).

Together, the three components of ecological risk assessment represent the best available science to appropriately monitor and protect the environment. The use of any one component alone to assess effects or environmental “compliance” represents a misuse of these tools. The Resource Management Act (1991) of New Zealand is a unique effects-based legislation. Determination of the presence or absence of an adverse impact in aquatic systems requires a diverse set of tools or methods. Chemical criteria and whole-effluent toxicity testing are frequently used in resource consents in New Zealand. However, these tools alone are not adequate to assess the risk or damage to the environment. Environmental effects monitoring methods must continue to develop, particularly where effects on fishes and other species are of concern. The purpose of this work was to adapt current international methods of environmental effects monitoring to New Zealand and to communicate the nature of the methods and results of the environmental assessments to stakeholders.

### 1.1 Background on Environmental Effects Assessment

Assessment of environmental effects can be performed at different levels of biological organisation (Fig. 1). As a general rule, as biological organisation increases in complexity, the environmental relevance increases – this results in a higher level of uncertainty linked to the complexity of the systems. As the level of biological complexity decreases it is easier to attribute the cause of the observations that are being made. This has led to two schools of thought in environmental risk assessment. The first emerged from traditional toxicology (originating with human health studies) and is based on the premise that observations at lower levels of biological organisation can be used to predict changes at higher levels. This is often termed a bottom-up approach as studies originate from lower levels of biological organisation and seek to establish the predictive validity of the endpoints measured. The second school of thought originates from ecology and maintains that it is not possible to predict changes at higher levels of biological organisation from observations made lower in the hierarchy due to “emergent” or unpredictable properties of complex systems. This top-down approach seeks to identify change at higher levels of organisation and find the cause...
This study was developed in order to combine top-down and bottom-up approaches (Fig. 1). This was accomplished by monitoring population-level variables in wild fishes and also examining molecular, and biochemical responses in eels caged at the study sites. As with ecological risk assessment, the combination of approaches is more powerful than the individual approaches used (Fig. 2). The wild-fish-monitoring approach provides the highest environmental relevance. Specifically, this method allows examination of the three factors critical for the continuance of a fish population: survival, growth, and reproduction. Though the measurement of population-level variables can be partially diagnostic of cause, it is often limited in its ability to determine cause and effect. The eel-caging methodology is a relatively short-term exposure, but the duration and nature of exposure is exactly known. Furthermore, the sub-organismal endpoints measured are diagnostic of exposure and effects of particular classes of compounds. These endpoints can be varied dependent on the suspected toxicants present in the particular system.

To develop and test the proposed monitoring methods, a number of sites were chosen throughout New Zealand. Site selection was based on consultation with regulators and stakeholders in order to determine aquatic systems where pollution is of concern. This resulted in a number of locations with wide variety of pollution sources. In some systems, it was possible to examine the hypothesis that environmental effects could be cumulative (or additive) moving from upstream to downstream. The study needs of each system were assessed in a site-specific manner, and research priorities based on the needs of regulators and tangata whenua. Alternative methods of environmental effects monitoring had to be used where the original study plan was not appropriate.
Figure 2: Strategy for environmental effects monitoring with fishes

**Eel caging**

Allows characterization of dose and exposure time while physiological changes are monitored

**Wild fish monitoring**

Allows determination of long term, population level effects
2 Methodology

2.1 Eel-Caging Methodology

The caging of fish, or *in situ* exposure, has been used throughout the world to maintain a controlled level of exposure while providing an added level of environmental relevance as compared to laboratory studies. In contrast to laboratory studies, caging studies accurately reflect the variability in chemical concentrations with time that occur in receiving environments. Caging exposures also mimic physical variables such as daylight and temperature that wild organisms are subject to and provide exposure to contaminated sediments. Thus, *in situ* exposures represent a level of biological complexity between that of laboratory studies and wild fish monitoring. A number of species have been used for caging studies including goldfish, trout, koaro, inanga, white sucker, fathead minnows and carp. In New Zealand, the shortfin eel has been used, with mixed success in studies prior to this one.

In our experience, the shortfin eel (*Anguilla australis*) is an appropriate choice for the following reasons:

1) It is an indigenous species that can be found throughout New Zealand.
2) Shortfin eel are not considered threatened and can be obtained in sufficient numbers.
3) It is a taonga to tangata whenua.
4) It has a commercially important fishery.
5) It is a robust fish able to tolerate the stresses of capture, transport, and handling.
6) It is relatively large compared to other potential species; this allows for tissue samples of sufficient size for biological and chemical measurements.
7) Due to the late age-to-maturity (>15 years), shortfin eel populations are vulnerable to anthropogenic stressors and would not recover quickly from such adverse impacts.
8) Considerable cultural and scientific expertise and experience is available in the use of this species.
9) Due to the previous use of this technique, historical data are available from some of the sites in this study.

Caging of eel allows an accurate determination of the level and duration of exposure to stressors. The level of exposure is in many cases an uncertainty for wild-fish sampling. Therefore, this gives this approach an advantage as to the certainty with which we can attribute effect to particular causes. However, the ability to observe and gather population-level variables is beyond the scope of caging studies and thus such studies can only incorporate the direct effects of toxicants on the caged fish. An important characteristic of studies with juvenile fishes, such as the eel method used here, is the inability to examine reproduction and growth, two of the most critical endpoints to the integrity of fish populations.
2.2 Caging Methodology

The eel-caging protocol involved caging fish at selected impacted and relatively pristine sites and comparing physiological responses between both groups. This is known as a “compare and contrast” method and statistics must be employed to determine if there are statistical differences between the impacted and unimpacted sites. Using these comparisons, caging biota in particular locations allows the quantification of environmental exposure and effects on the organism. Removal of test organisms from a large, unaffected population allows testing to be performed in smaller streams without extensive sampling of, or risk to, the resident populations of fishes.

The cage design and methods chosen must minimise stress on biota due to the caging itself and minimise the use of live animals while maintaining adequate sample sizes to make statistical comparisons. Considerable development of these methods had been undertaken prior to these studies. A cage design was employed that incorporates a nylon mesh inner cage, the shape of which is supported by metal hoops (similar to a fyke net), with an outer cage of coarse stainless steel mesh (Fig. 3). This outer cage was used to provide security, as it can be locked closed, and further physical support for the inner cage while allowing protection from large debris that may come downstream. The mesh design allowed for the best flow of water through the cage and exposure to the bottom sediments, both to obtain representative levels of exposure and to provide the best environment for the welfare of the animals. The permeability of the cage design also reduced the flow-resistance of the structure in fast-flowing streams. Cages were secured to steel stakes driven into the bank, with chains and padlocks to provide some security from tampering and prevent cages from washing away. Eels can also display aggressive behaviour to each other. To minimise aggression between fish and provide refuge, PVC pipes of a diameter 80 mm or more were put into the cages.

Figure 3: Eel cage design
Eels were captured from a number of relatively uncontaminated sites in the North and South islands and, after a holding period of at least 14 days to allow proper acclimation, were placed in the cages at the selected locations. Cages were examined regularly to ensure that eels had not escaped and the cages were secure and submerged. After 21 days of exposure, eels were removed from their cages, the level of mortality recorded, and eels were transported to the laboratory to collect tissues for the various endpoints measured. To minimise stress during the transport, eels were sedated by a combination of anaesthetic and submersion in an ice bath until being killed for the sampling. The procedure for sampling involved the removal of blood from the vein that runs along the bottom of the spine behind the anus and storage of the blood on ice with an anticoagulant. At some locations, further subsamples of blood were stored in order to measure blood parameters (further details in section 2.3.6). The remainder of the blood was spun in a centrifuge, the liquid or plasma removed and the plasma frozen for other analyses such as circulating sex steroids. The length and weight of the eel was recorded and an external examination for any lesions followed. Internal organs were removed and weighed and certain organs such as the liver and the gall bladder were frozen in liquid nitrogen for further analysis. The carcass of the eel was frozen and archived for future tissue analysis. The otoliths, or ear bones used for aging, were removed from the carcass at a later date.

2.3 Sub-organismal Endpoints

This section will describe and explain the variety of endpoints measured at the lower levels of biological organisation, usually termed biomarkers (Adams et al., 2001). These include biochemical, cellular and chemical endpoints. Many of these endpoints were employed both in the eel-caging and the wild-fish components of this study. However, these endpoints are the primary variables examined in eel caging and thus are addressed in this section.

2.3.1 Liver detoxification enzymes

Measuring certain liver enzymes is a method of analysing for exposure to some classes of chemicals that are flat (planar) or can twist to become flat. These compounds also consist of two or more six-carbon rings with a series of double bonds. The most well known classes of these compounds are dioxins, furans and polychlorinated biphenyls (PCBs; Fig. 4). However, a fourth class, the polycyclic aromatic hydrocarbons (or PAHs), are far more common in the environment as they are products of burning, found in fossil fuels and can also be produced by bacteria. A number of natural plant compounds, such as flavones, can also cause elevations of these enzymes. The chlorinated dioxins, furans, PCBs and PAHs are not easily broken down in the liver of exposed organisms (or by bacteria) and have low solubility in water. Thus, they are well known for their persistence in the environment and their ability to accumulate in fatty tissue. Non-chlorinated PAHs are a bit different in that they are more easily metabolised and excreted by animals. The mechanism of clearance of these types of chemical occurs primarily in the liver and is designed to make the compounds more water soluble so they can be passed on to the gall bladder through the bile and evacuated through the organism’s intestine. The first step in this metabolism is usually to add oxygen to the molecule, or oxidation. This is usually followed by the addition of a
sugar molecule, or other water-soluble structure, to that oxygen – a process called conjugation. These chemical reactions occur with the assistance of enzymes.

**Figure 4: Examples of compounds that induce the production of detoxification enzymes**

![Examples of compounds](image)

Enzymes are the proteins within living organisms that process all chemical reactions. The enzyme measured by this method and which causes the oxidation described above is cytochrome P450 1A1. This enzyme is found in the liver (an organ used to reduce the toxicity of many compounds in the body) of many animals. When chemicals are present in certain cells, they turn on or up-regulate the cytochrome P450 1A1 gene responsible for producing this enzyme. Exposure to these compounds increases the activity of cytochrome P450 1A1 by binding to a protein (the aryl hydrocarbon receptor) within a cell, which in turn activates the gene encoding for this cytochrome P450 protein.

Up-regulation of this enzyme is an indication that the animal has been exposed to the classes of compounds mentioned above. This effect cannot necessarily be deemed as an adverse or toxic effect as it is just a natural mechanism to clear chemicals. However, substantial enzyme induction of this type is an indication of significant exposures to these chemicals. Furthermore, there are situations where compounds such as PAHs are metabolised to chemicals that are much more toxic than the starting material. The classic example of this is the well-known ability of some PAHs to become cancer causing upon oxidation.

There are different methods to measure cytochrome P450 1A1, the most common being to measure it indirectly through its activity, or ability to perform chemical reactions. This is measured via the conversion of one compound, 7-ethoxyresorufin, into another compound, resorufin, that can be selectively and easily measured. From this the name 7-ethoxyresorufin-O-deethylase or EROD is coined as the name for the endpoint. The amount of product measured more or less directly relates to the amount of cytochrome P450 1A1 activity present. This method is rapid and easy to perform but there are a few limitations. Certain compounds can actually interfere with the chemical reaction and the response between the amount of compound and activity is not a linear one.
2.3.2 Bile chemistry

The process by which chemicals are broken down for excretion into the bile is described in the previous section. As many compounds that do not accumulate in tissue are actively excreted by this means, it provides an excellent tool to measure exposure to chemicals in the short term, as those chemicals usually cannot be found in tissue. Such chemicals can become quite concentrated in the bile and easily be measured. Bile analysis can be performed for just about any type of compound that concentrates in the bile. For this study, analyses concentrated on PAHs and on wood-derived compounds in waste effluent from pulp & paper mills.

Softwoods, and particularly pine species such as radiata pine, have high levels of natural compounds called resin acids (Fig. 5). These compounds are relatively toxic and serve to defend the tree against damage by insects. In a pulp mill, resin acids and other undesired compounds are removed from the wood fibre by chemical or physical processes. Though they are mostly (>90%) removed from effluents by well-operated treatment systems, they still represent one of the most significant organic contaminants released into the environment. Resin acids are conjugated in the liver and in order to measure them these sugar groups must be removed by treatment of the bile with a strong caustic. Individual resin acids can then be extracted and measured with instrumental chemistry measurement techniques such as gas chromatography, which separates compounds on a long, very narrow bore column followed by detection at the end according to their molecular size. There are many different resin acids and, to simplify reporting, results are expressed by adding them up as total resin acids.

**Figure 5: Abietic acid, a resin acid**

Polycyclic aromatic hydrocarbons are also easily measured in bile as they all tend to have the ability to fluoresce. Fluorescence is a reaction where light strikes a molecule and, after a short delay, light of a different colour (wavelength) is released from the molecule. The colour of the light coming in, or excitation, and of the light released, or emission, is very selective to the molecule. This is most useful since PAHs are metabolised in the liver, they form any number of other chemicals, and usually the parent compound is no longer present. However, the fluorescence of those metabolites is relatively similar to the parent molecules, thus it is not necessary to identify the potentially 100’s of metabolites of a mixture of PAHs.
Determine effects of pollution on fishes
to which an organism may be exposed. These bile fluorescence measurements are usually expressed as a PAH-equivalent concentration as compared to the parent PAH for which excitation and emission colours are chosen. A number of parent PAHs are chosen as they represent different sources (Fig. 6). Retene is very characteristic of pulp & paper mill effluent and wood combustion. Pyrene is very high where combustion occurs. Naphthalene and benzo[a]pyrene are found in petroleum. Though the different wavelengths used can be indicative of the type of structure, the method cannot conclusively determine the nature of the material to which an organism is exposed, as it does not identify individual compounds.

**Figure 6: Polycyclic aromatic hydrocarbons and fluorescence wavelengths**

<table>
<thead>
<tr>
<th>Name</th>
<th>Wavelengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]pyrene</td>
<td>380nm/430nm</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>290nm/355nm</td>
</tr>
<tr>
<td>Pyrene</td>
<td>341nm/383nm</td>
</tr>
<tr>
<td>Retene</td>
<td>302nm/372nm</td>
</tr>
</tbody>
</table>

### 2.3.3 Impairment to nervous function

Certain compounds released into the environment have the potential to impair the integrity of the nervous system in exposed organisms. This is particularly true for two classes of pesticides: organophosphates and carbamates. These chemicals work by acting directly on the nervous systems of insects, but have a similar effect on non-target organisms. Nerve cells communicate and transmit signals by sending chemical signals between them. This chemical signal also needs to be turned off very rapidly or uncontrolled nerve impulses would result, in a similar manner to what happens in a seizure. The enzyme that turns off this signal is called acetylcholinesterase.

When compounds block and turn off this enzyme, nervous damage and death can result. This indicator of exposure and effect has been well documented in birds exposed to insecticides from spraying operations, but is less explored in fishes. The enzyme can be easily measured by the chemical reactions that it performs, similar to the measurement of the EROD enzyme.

### 2.3.4 Biochemical measures of reproductive function

Reproduction is critical to the maintenance of life and is primarily governed by the endocrine system. The endocrine system is central to the control of the various processes that maintain life, through complex feedback mechanisms modulated by chemical messengers called hormones. Those hormones travel through the body via the blood where they act on target tissues by turning on or off certain functions. There is a complex system of feedback within the organism in order to maintain levels of hormones (Fig. 7) that is not yet fully understood.
The initial signals or cues to initiate reproductive development are external, and may include such things as temperature or changes to the length of daylight hours. These stimuli cause the hypothalamus, a part of the brain, to release hormones such as gonadotropin-releasing hormones. This in turn causes the pituitary, also associated with the brain, to release proteins called gonadotropins. Gonadotropins act on the reproductive tissue, the ovaries or testes that are collectively called gonads, to start the process of adult reproductive development.

In response to these hormones, the gonads release sex steroid hormones. Sex steroid hormones act on other organs as well as on the gonads themselves to build reproductive tissue from energy stores from within the fish. One of these hormones, oestradiol, is the fish oestrogen and causes a protein called vitellogenin to be made in the liver. Vitellogenin is common to all egg-laying organisms and is transported through the blood to the eggs to become the primary energy source for developing eggs. In male fish, male hormones or androgens fulfil the function of controlling the development of reproductive tissue. These hormones include 11-ketotestosterone and testosterone. Testosterone is also important in males and females for another reason; it feeds back on the brain in a negative way – too much testosterone slows the process down. By this means, reproductive development is closely controlled.

A number of chemicals can interfere with the delicate balance of this reproductive process and of other endocrine functions at various places throughout the axis. These types of compounds are collectively referred to as “endocrine disrupters”. For example, oestrogens in the environment can cause male or juvenile fishes to synthesise vitellogenin. This is known to happen due to exposure of certain compounds found in cleaning products, but
also due to residues of natural hormones and oral contraceptives found in sewage effluent. The measurement of vitellogenin then becomes a useful biomarker to indicate exposure to these compounds. Steroid hormones are also critical to the development process, so if they are there in insufficient quantities, reproduction will not proceed normally. A number of types of effluent have been observed to cause the gonads to produce lower levels of steroids. The mechanisms of this are generally not known, and this endpoint is not as specific as vitellogenin synthesis. Even high levels of stress are known to decrease steroid production in some species of fishes. The complex structures of the major steroid hormones are shown in Fig. 8. All steroids come from cholesterol, including steroids that are important to other components of the endocrine system such as the corticosteroid stress hormones. Generally, it is the final steroid products testosterone, oestradiol and 11-ketotestosterone that are measured as indicators of possible reproductive dysfunction. These can be easily and sensitively measured in blood using techniques such as radioimmunoassay (RIA).

![Figure 8: Major sex steroid hormones in fishes](image)

**Testosterone  Estradiol  11-Ketotestosterone**

### 2.3.6 Blood parameters (haematology)

As with human health diagnostics, blood parameters in fishes react and adapt to any number of environmental changes including low dissolved oxygen, changes in temperature, general stress and chemical stress. Hormones can be measured in the plasma. The primary target is the red blood cell, which is critical for carrying oxygen to the tissue. Red blood cells can become reduced in number, can change in size, or levels of the oxygen-carrying pigment haemoglobin can be altered. For example, stress or adrenalin can cause red blood cells to swell very quickly. Higher temperatures will result in an increase in the number of red blood cells because it is more difficult to transport oxygen. Red blood cell volume is measured by spinning blood in a fine tube to separate the red blood cells from the liquid phase. The length of this packed red blood cell column is measured, producing the haematocrit, or percentage by volume of red blood cells. The number of red blood cells can also be directly evaluated by counting them under a microscope on a calibrated grid or haemocytometer. Haemoglobin is also measured directly from its colour in blood.

Another important function of blood is immune function. A variety of types of white blood cells perform different functions to protect organisms from disease. An increase in white blood cells can indicate that a disease is present whereas a decrease can signal an increased susceptibility to infection. White blood cells can be counted directly under a microscope or
they can be measured in a similar fashion to the haematocrit.

2.3.7 Measures of immune function – lysozyme activity

Immune function is highly complex and generally divided into two categories: specific and non-specific. Specific defences are those such as antibodies that respond only to a specific disease organism. As fishes are continually exposed to water, more general or non-specific defence mechanisms likely play a far more important role than they would in humans. One such defence mechanism is lysozyme, an enzyme that specifically targets part of the cell wall unique to bacteria. In fishes, lysozyme is an important general defence, both on skin and in blood. Lysozyme is measured by incubating blood plasma with heat-killed bacteria (*Micrococcus lysodeikticus*) and measuring amount of destruction of the bacterial cell wall.

2.4 Wild-Fish Monitoring Framework

2.4.1 Framework description

The wild-fish monitoring framework employed in this research is designed to directly address the performance of fish populations in response to multiple environmental stressors. The original concept was developed by Colby and Nepsy (1981) and Colby (1984) who suggested that responses of fish populations to particular types of stressors are unique and predictable. These responses were observed to primarily be a response to environmental conditions, not genetic differences. These methods have their origin in the study of over-fishing or exploitation as well as in habitat modification. It has been long recognised that over-fishing leads to a population made up of smaller, younger individuals. The decrease in the number of adults can lead to increases in growth rate as more food becomes available for younger fishes. From these earlier observations of exploitation, it became apparent that other impacts may be detected by population characteristics, including those that may be caused by chemical stress (Munkittrick and Dixon, 1989; Munkittrick, 1992).

Based on the scientific literature it was possible to characterise a wide variety of response patterns in fish populations due to eutrophication, acidification, impoundment, predation and pollution sources. Subsequent identification of additional patterns and improvements to the methodology have been suggested by Gibbons and Munkittrick (1994). A notable addition was the simplification of the fisheries data to three basic groups: age structure changes, alteration in energy expenditure, and changes in energy storage. These categories are described in more detail within following sections pertaining to biological endpoints.

As with the eel studies, the use of this monitoring framework is on a compare-and-contrast basis – that is comparison of impacted sites with relatively unimpacted or reference sites. Valid statistical comparisons must be made between sites to determine differences. In complex systems, with a large number of sites, traditional statistics fail to have sufficient
power to show differences. Thus alternative techniques for the examination of data are a major challenge of this science area. Probably the greatest value of these techniques is the examination of changes to fish populations over time, as no population is static. However, for this study and the sites chosen, no useable background data exist.

Monitoring frameworks cannot identify all possible environmental effects. For example, this method cannot incorporate some behavioural responses (e.g. avoidance). The framework is designed for initial assessments of fish populations from impacted environments, and where effects are observed confirmatory studies are required to understand the causes in cases where it is not apparent. However, the method provides a cost-effective and insightful first look at fish populations that requires no specialised techniques or equipment.

2.4.2 Application of monitoring framework to New Zealand

The freshwater aquatic environment of New Zealand is primarily composed of small to medium-sized streams and the native fish fauna is unique in the world. Many of the species present are highly migratory during certain times in their life history. For the successful implementation of the above monitoring framework, a suitable monitoring species must be chosen. Ideally, the chosen species must have the following characteristics:

1) Be present in sufficient numbers to allow efficient capture of required sample sizes (without impacting on the population) of reproductively active (adult) fish.
2) Adults must be relatively territorial during the period when capture is occurring to ensure exposure has occurred. In the absence of migration barriers, it is difficult to determine the severity and duration of exposure to environmental stressors if using a highly migratory species.
3) The species must have a wide geographical range to facilitate comparisons between regions.
4) Populations must not be subject to commercial exploitation, as this will minimise the ability to detect the impacts of pollution.

In New Zealand, freshwater fish diversity is poor and there are not many species of fish that fit the above characteristics. Large-bodied fish, such as eel, may be difficult to capture in sufficient numbers as adults. Furthermore, much of the eel reproductive development takes place during sea migration and the two common species are heavily exploited in certain regions. In similar stream situations this monitoring technique has been effectively applied to small-bodied fishes (Gibbons et al. 1998a; 1998b). As such, bully species (Gobiomorphus sp.) may provide the most ideal monitoring species. However, the best monitoring species for each location must be assessed on a site-by-site basis during preliminary investigations of site characteristics. At some sites in this study, the appropriate species were not present and alternative methods of effects assessment were used, such as fish and invertebrate communities.
2.4.3 Wild-fish collection and sampling

Wild fish were collected by a variety of capture techniques. For the common bully (*Gobiomorus cotidianus*), minnow traps, electrofishing, spotlight dip-netting, and seine netting were all used depending on the nature of the stream and the density of the populations. Wild shortfin eel and brown bullhead catfish were collected using fyke nets. Sampling was conducted as per descriptions for sampling of caged shortfin eel. The parameters for age, energy allocation, and energy utilisation are dealt with specifically below.

2.4.4 Measures of age structure

Age structure is a critical variable in assessing the status of fish populations. Age structures were estimated for wild fish by determining age using various methods. Shifts upward in age structure can indicate a failure of recruitment while shifts downward can indicate such things as exploitation or predation of adults. Observations such as decreased age at maturity can indicate toxicants that act on reproductive systems or can reflect more rapid growth of younger individuals such as can occur with exploitation patterns in some species of fishes.

Fish age is usually determined by examining the rings or annuli on hard or bony structures. With eels, otoliths (ear bones) are usually used. This procedure involves removing the otolith from the scull, cracking the bone, followed by burning and counting of the annuli. For brown bullhead catfish, counting of the annuli on the fourth vertebrae has been used previously in New Zealand (Patchell, 1977; Barnes, 1996) and was found to be an acceptable method.

The only published method for the common bully was the use of scales for ageing (Stephens, 1982). This was attempted and found to be inadequate. Bully otoliths are quite large but very opaque. Otoliths were mounted on slides and polished with very fine sandpaper on either side to allow better reading (Fig. 9). Otoliths were read under a dissecting microscope against a black background with a drop of oil on the otolith. Efforts to stain or burn otoliths did not improve annuli resolution.

Figure 9: Common bully otolith
2.4.5 Measures of energy storage

In fishes, energy is usually stored in the form of lipid or fat in tissue, particularly muscle, liver, and intestinal fat bodies. In fishes with higher accumulated energy stores, the ratio of body weight to body length, or condition factor, will be increased. In other words, they are heavier per unit length. The ratio of liver weight to body weight, or liver-somatic index (LSI), will also be higher when liver energy stores are increased (although other factors can influence this as well). Both condition factor and LSI were measured at completion of the eel-caging experiment and in the species of wild fish chosen at particular sites.

2.4.6 Measures of energy utilisation

There are two main ways in which fishes can use energy: growth and reproduction. In wild fishes, adult-specific aspects of reproduction can be assessed directly through measurement of gonad size and fecundity. The gonad size, as a function of body weight (gonado somatic index or GSI), is a direct reflection of the energy put into reproduction. The number of eggs per unit body weight, or fecundity, also reflects the reproductive potential of adult fishes. Both gonad size and fecundity can vary with age, thus this is also a variable that must be considered.

Reproduction encompasses the whole process of gonad growth, egg laying, fertilisation and hatching. So the ultimate measure of reproductive success is the recruitment of new adults into the population, and this is best determined by age structure of the population, for example a high mean age means few individuals being recruited into the adult population.

Growth is a straightforward measure of energy allocation. However, most species of fishes continue growing for their entire life span. Thus the relationship between size and age is the best measure of growth.
2.4.7 Recognition of population patterns

Patterns were categorised according to changes in age, energy allocation, and energy utilisation. Responses were characterised as being increased, no change, or decreased as compared with reference populations, or as compared with the average values where larger and comparable datasets existed. Some examples of hypothesised patterns are illustrated in Table 1.
### Table 1: Patterns of population response observed in wild fish populations

<table>
<thead>
<tr>
<th>Pattern (age/energy storage/energy expenditure)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+/+</td>
<td>Increased food supply</td>
</tr>
<tr>
<td>+/0/0</td>
<td>Recruitment failure</td>
</tr>
<tr>
<td>+/-/-</td>
<td>Multiple stressors</td>
</tr>
<tr>
<td>0/-/-</td>
<td>Food limitation</td>
</tr>
<tr>
<td>0/-/0</td>
<td>Niche shift</td>
</tr>
<tr>
<td>+/-/+</td>
<td>Metabolic redistribution</td>
</tr>
<tr>
<td>+/-/+</td>
<td>Chronic recruitment failure</td>
</tr>
<tr>
<td>0/0/0</td>
<td>No response</td>
</tr>
</tbody>
</table>

+ indicates an increase, - a decrease, 0 no change

Many unknowns exist with regards to the use of population parameters. For example, will all species react to stressors in a similar way? Where multiple stressors or influences are present, can one environmental variable mask another? For instance, could an increased food supply obscure the detection of an endocrine disrupter? Fishes are the largest group of vertebrates on earth with over 20,000 known species. Knowledge of many of those species is limited. Efforts to understand how populations response to stress, such as has been undertaken herein, will also serve to enhance our basic knowledge of fish population biology.
3 Site Descriptions

Following the consultation process, five study sites were selected from different regions in New Zealand (Fig. 10). The selection criteria included not only strong partnerships but also reflected the types of stressors occurring in the New Zealand environment, including industrial effluent, municipal wastewater and mixed agricultural runoff. Table 2 summarises the groups involved in the process leading to this research project and the anthropogenic pressures placed on the rivers. The rivers are located across the country and all have paramount economical, social and cultural values.

Figure 10: Study site locations
Table 2: Study sites investigated including point and non-point sources of contaminants and partners involved in the research

<table>
<thead>
<tr>
<th>Study site</th>
<th>Type of stressors</th>
<th>Partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarawera River, Bay of Plenty</td>
<td>Two pulp &amp; paper mills, geothermal power, Kawerau municipal sewage, Edgcumbe sewage and agricultural inputs</td>
<td>Environment Bay of Plenty, Carter Holt Harvey Tissue, Norske-Skog, Tuwharetoa ki Kawerau</td>
</tr>
<tr>
<td>Waikato River</td>
<td>One pulp &amp; paper mill, City of Hamilton and mixed geothermal, municipal and agricultural inputs</td>
<td>Environment Waikato, Carter Holt Harvey Pulp and Paper, Waikato Tainui</td>
</tr>
<tr>
<td>Cam River, Canterbury</td>
<td>Municipal sewage, general industry and agricultural inputs</td>
<td>Waimakariri District Council, Environment Canterbury, Ngai Tahu, Cam River Working Party</td>
</tr>
<tr>
<td>Styx River, Christchurch</td>
<td>Wood treatment plant, urban inputs, general small industry</td>
<td>Christchurch City Council, Ngai Tahu, Guardians of the Styx</td>
</tr>
<tr>
<td>Waiwhetu Stream, Hutt Valley</td>
<td>Multiple industrial and urban inputs</td>
<td>Wellington Regional Council, Waiwhetu Stream Action Plan, Atiawa</td>
</tr>
</tbody>
</table>

3.1 Tarawera River

One of the greatest areas of concern in the Bay of Plenty remains the Tarawera River. The Tarawera receives effluent from two pulp & paper mills and treated municipal sewage effluent from Kawerau and Edgcumbe. The lower river is also influenced by agriculture and channelisation for flood protection. In recent years, improvements have been seen in the condition of the river following upgrades of the treatment technologies in the pulp & paper mills. However, it is still yet to be determined whether these improvements have reached the point where biological effects in fishes have been eliminated.

Three sites for shortfin eel caging and wild fish sampling were selected on the river. A reference site was located upstream from the paper mill effluent outfalls. Two additional sites were located downstream of Kawerau and reflected the influence of mixed municipal sewage, geothermal power water, and pulp & paper mill effluent. Site three will be downstream of SH30 and contains all of the influences present at site two, plus effluent from a second pulp & paper mill.

3.2 Waikato River

The Waikato River is the longest river in New Zealand and is of great spiritual and cultural value to the tangata whenua. Waterways in the Waikato catchment are heavily influenced by human activity with the major impacts thought to be due to industrial wastewater, hydroelectric dams, agricultural runoff, and municipal wastewater. It was decided that eel-caging studies in the Waikato River would be replaced by wild-eel sampling. Also the exotic species bullhead was selected for the wild fish survey.
3.3 Canterbury Region

The Cam River is located in North Canterbury. Its catchment is undergoing pressures from urban development with the populations of Rangiora and Kaiapoi growing rapidly. The catchment receives multiple stressors from a variety of agricultural, industrial and municipal sources. There have been a number of complaints over the years of poor water quality, referring to general “unhealthy” appearance, an apparent lack of fish and invertebrates, and algal slime growths (Main, 1998). The Rangiora oxidation ponds are discharged into South Brook, which increases the coloration and contributes nutrients. An Environment Canterbury report in the late 1990s stated that the Cam River contains high levels of faecal coliforms and that the effluent discharge had little effect on overall indicator bacteria concentrations (Main, 1998).

The Cam River Working Party is a community-based group with a mission statement to “stop and restore the ecosystem and natural values of the Cam and Kaiapoi river systems for the safety and enjoyment of the users”. One of the major concerns is discharges from the Rangiora wastewater treatment plant.

The other site in the Canterbury Region is the Styx River. There are indications that when Europeans first settled in the area, the Styx River and its tributaries were surrounded by extensive wetlands (raupo and flax) and sand dunes. Today the Styx River originates in the Harewood area and is intermittently filled with stormflow. It has been extensively modified through farming and drainage schemes and is flowing through intensive urban development projects. The Styx River is highly valued by those people closely associated with it and much effort is aimed at enhancing and restoring the catchment. These efforts include the Guardians of the Styx, a community group that promotes the protection, restoration, and raises awareness of the values of the Styx River. Another important activity coordinated by the Christchurch City Council is the Styx Living Laboratory, which aims to facilitate partnerships between the groups interested in enhancement of the river. A centre is planned that will help centralise research efforts and facilitate interpretation and education of the local people. The Styx River is a catchment influenced by increasing urbanisation, a wood treatment plant, and general small industry.

3.4 Waiwhetu Stream

The site of interest in the Wellington area is the Waiwhetu Steam. The Waiwhetu Stream is located in the Hutt Valley and is a generally degraded urban stream with input from small industry. Contamination by both organics and metals has been suggested and the levels of metal contamination in the lower stream are exceedingly high and organic contaminants such as DDT are also known to be present (Sheppard, 2001; Sheppard and Goff, 2001; 2002).
4 Descriptions of the Studies

4.1 Tarawera River

4.1.1 Methods employed

Wild fish monitoring and shortfin eel caging were the methods employed on the Tarawera River. Shortfin eel were caged at three sites in the river (Fig. 11). The reference site was in Kawerau (Tarawera 1) as there is little settlement or anthropogenic input upstream of this site. The first downstream site was below the chemi-thermo-mechanical pulp mill and Kawerau municipal sewage inputs (Tarawera 2), but upstream of geothermal power generation inputs. The furthest downstream site was downstream of all of the above-mentioned inputs and also of the thermo-mechanical/bleached kraft mill wastewater input (Tarawera 3).

Figure 11: Caging locations on the Tarawera River

Eel caging was conducted from 10 to 31 March 2003. Eels were sourced from Lake Taikehu, a small lake on the eastern edge of the Rangitaiki Plains with abundant eel populations. This lake received virtually no agricultural input and the catchment is dominated by exotic forest plantations. Two eel cages with 10 eels in each were used at the study sites to ensure a successful outcome, and to provide the required sample sizes. After 21 days of exposure, eels were examined externally, and the length, weight and organ weights recorded. The biochemical parameters measured were liver detoxification enzymes.
and blood plasma steroids. An assessment of red and white blood cell parameters was also conducted. Due to the presence of pulp mill effluent in the river eel bile was analysed for the presence of pulp-mill-related organic compounds. As certain aromatic compounds can be produced in treatment systems and in accumulated sediment, fluorescence analysis of bile was also conducted.

Common bully were sampled in the river at an upstream location, and below the combined pulp mill inputs. The reference population of common bully was sampled from the Tarawera River within the vicinity of Kawerau (Tarawera 1). Minnow traps were ineffective in this region and bully were captured by dip-netting with a spotlight at night. Downstream populations were sampled from below where SH30 crosses the Tarawera River. Capture was conducted at suitable locations over approximately a 5-km reach of the river. Common bully in the downstream areas were captured effectively in minnow traps. This area receives the combined input from Kawerau sewage, two pulp mills and geothermal inputs. Parameters measured in common bully include length, weight, organ weights, age and liver detoxification enzymes. Subsequent to the initial examination of common bully in summer, bully were examined from the lower Tarawera in winter and at both sites again during the following summer. During the second summer sampling, common bully were also sampled from the lower Rangitaiki River. Tissue from common bully from the initial period of sampling was measured for heavy isotopes of carbon ($^{13}$C) and nitrogen ($^{15}$N). These analyses were conducted to determine if there was significant migration of common bully between the sampling sites.

4.1.2 Results and discussion

Eel caging has been employed on the Tarawera River previously and this current study was one of the more successful efforts. The cage design employed proved successful for maintaining eels in excellent condition. Sites were chosen that had some level of shelter from the high water velocity encountered in the Tarawera River. A total of 20 eels were deployed in cages at each location. All eels were accounted for at the furthest downstream site. The middle location had four eels either missing or dead, and the reference site had two eels missing. It is difficult to determine if missing eels died or escaped given the propensity for eels to escape from cages. Dead eels within a cage may also have been consumed by the other eels. Two eel carcasses were found at the second cage site indicating at least 10% mortality. This mortality can be caused by a number of factors including aggression from other eels, stress due to capture and handling, local oxygen/temperature conditions, or toxic compounds released from sediment. Certainly the cages at the intermediate site were placed in a backwater with significant amounts of organic sediment and this could have contributed to the observed mortality. By comparison, the reference cage location had the highest flows as there were no similar backwaters and this area generally has no organic-rich sediment. The furthest downstream site was also in a location with accumulated sediment, presumably from the pulp & paper operations, but not to the same extent as the middle set of cages.

Eels from the furthest downstream site were significantly lighter per unit length compared with the eels from the upstream reference location (Fig. 12). A similar, though not
statistically significant, trend was also observed with liver size. Eel condition would be expected to decrease if they were not actively consuming food. Eels can and do consume food as it enters the cage, so presumably less food was available in the furthest downstream cages. The downstream set of cages also had no missing eels; consumption of other eels in the two sets of upstream cages may have also provided nutrition for these eels.

**Figure 12: Condition factor and liver size in eels caged in the Tarawera River**

There were no differences found in spleen size (not shown) or in the proportion of white blood cells as measured by leucocrit (Table 3). These results indicate no perturbation to components of the immune system as could be caused by either chemicals or disease. A number of red blood cell parameters were examined including measures of cell number and haemoglobin, the oxygen-carrying compound in blood cells. There were no significant differences in any of the parameters examined (Table 3). Blood endpoints are non-specific and alterations in blood cell characteristics may indicate both stress and adaptation. For example, blood cell parameters would be expected to change with temperature or dissolved oxygen in order to allow the eels to transport the amounts of oxygen they require. Many chemicals could also cause alterations to blood profiles and so these would be expected to change in almost any case where acute toxicity was likely.

**Table 3: Blood parameters in shortfin eel caged in the Tarawera River**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Reference (n=12)</th>
<th>Downstream 1 (n=12)</th>
<th>Downstream 2 (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/L)</td>
<td>97.5</td>
<td>99.7</td>
<td>94.9</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>29.3</td>
<td>28.7</td>
<td>29.0</td>
</tr>
<tr>
<td>Leucocrit (%)</td>
<td>0.89</td>
<td>1.17</td>
<td>1.11</td>
</tr>
<tr>
<td>Mean cell haemoglobin (g/cell x 10^{-12})</td>
<td>0.68</td>
<td>0.72</td>
<td>0.65</td>
</tr>
<tr>
<td>Haemoglobin per Cell Volume (g/L)</td>
<td>335</td>
<td>350</td>
<td>326</td>
</tr>
<tr>
<td>Mean cell volume (L x 10^{-12})</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Red blood cells (cells/L x 10^{-12})</td>
<td>0.15</td>
<td>0.14</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Sex steroid hormones were also measured in the blood of caged eels. Elevated levels of these hormones are not expected as none of the eels used demonstrated any evidence of reproductive development. However, steroids in immature fishes have been previously used to measure an effect on the reproductive system in other species (Tremblay and Van Der Kraak 1998; 1999). In this examination, testosterone and oestradiol were found at measurable levels in all of the eels tested. There was a significant increase in plasma testosterone and oestradiol at the site furthest downstream (Fig. 13). This is contrary to any published results, which show that in many cases exposure to pulp & paper effluents...
causes a reduction in circulating steroid levels, potentially leading to effects on reproduction (Munkittrick et al., 1992). Our observation cannot be easily explained; however, the effect is a modest one and certainly not adverse. It is possible that minor changes in exposure conditions, such as temperature, could have caused this observation. Alternatively, there may have been factors associated with the upstream cage that caused a reduction in steroid hormone levels.

Figure 13: Testosterone and estradiol in eels caged in the Tarawera River

![Testosterone and estradiol levels in eels from different sites](image)

Measures of exposure used in this study include liver detoxification enzymes, bile resin acids, and bile fluorescence. These endpoints were chosen as they represent measures of exposure to chemicals associated with pulp & paper effluents. Resin acids, compounds found specifically in pine trees, were non-detectable in most samples of bile from the eels caged at the reference site in Kawerau. The mean total resin acids were 1, 2513, and 827 μg/g for the reference, middle, and furthest downstream site respectively. This was surprising as the concentration of pulp & paper effluent at the furthest downstream site is more than 10-fold higher than at the locations just upstream of the thermo-mechanical/kraft mill. It is possible that these results indicate exposure to a contaminated area of sediment at the site between the two pulp mill outfalls.

Measurement of the liver detoxification enzyme EROD and bile fluorescence showed similar trends (Fig. 14). EROD and bile fluorescence were only significantly different at the site furthest downstream.

Figure 14: Liver detoxification enzymes and bile fluorescence in eels caged in the Tarawera River

![Liver detoxification enzymes and bile fluorescence levels in eels from different sites](image)
Bile fluorescence indicates exposure to polycyclic aromatic hydrocarbons or PAHs that are known to be produced by the bacterial transformation of resin acids. PAHs are well known to cause increases in liver detoxification enzymes, so a causal relationship between EROD and PAHs is indicated. Elevated liver detoxification enzymes have been observed at many pulp mills around the world including in mesocosm studies with rainbow trout using the Tasman effluent (van den Heuvel et al., 2002; van den Heuvel and Ellis, 2002). However, these results are curious because a number of recent studies with rainbow trout indicate that the Tasman mill effluent no longer causes elevated EROD activity (van den Heuvel et al., 2004; Ellis et al., 2004). The primary PAH formed, retene, is known to be formed in the absence of oxygen, such as may occur in sediments. These results again suggest the observed effects may be sediment-related.

Examinations of wild common bully in the Tarawera River revealed some dramatic differences in physiology between the upstream and downstream sites. The most glaring difference was a very substantial difference in gonad size in both male and female bully. Those captured downstream of inputs into the river had no observable reproductive development as can be observed using the ratio of gonad size to body weight (Fig. 15). The gonads of those female bully captured in the downstream reaches had the appearance of those stages of reproductive development following spawning where no gonad growth is occurring.

This unusual pattern of reproductive development was accompanied by elevated liver size at the upstream location, though condition factor was not different (data not shown). Given that the downstream and upstream fish were at a completely different reproductive development period, comparison of these data is not meaningful. Common bully in the two reaches of the river were similar in age/length structure (Fig. 16). Tarawera River bully are quite large and appear to be fast growing in both the upstream and downstream reaches of the river. The size with age was very similar for upstream and downstream sites despite the fact that the relative density of the upstream and downstream populations was very different. Downstream, common bully were very abundant and easy to capture. Minnow traps were not effective upstream and much effort had to be directed at spotlighting in order to capture significant numbers of bullies.
Given the substantial differences in the reproductive development of the two populations, considerable effort has been directed at determining the possible reasons for this observation. There were several possible reasons and the first line of examination was to determine if large, reproductively developing common bully could potentially be migrating to the upstream site. Since inputs of carbon and nitrogen in the lower river are substantially different from those in the upper river, we reasoned that if bully were not migratory, we could observe a significantly different elemental fingerprint as measured using the heavier stable isotopes of carbon and nitrogen. These measurements revealed very distinct isotopic patterns indicating that the two populations were indeed separate (Fig. 17). The carbon values in the tissue of bully sampled downstream from the mills in particular strongly reflect the input of carbon into the river from the pulp & paper wastewaters.
Given that populations of bully did not appear to be migratory, it was suspected that downstream bully may have been spawning at a different time of year. Sampling was conducted at the downstream location in the July following the January sampling presented above. In July, the downstream common bully showed normal reproductive development indicating that this population is developing normally, but is spawning in advance of the upstream population. In our experience with the common bully in numerous sites throughout New Zealand we had only encountered summer spawners. However, the exact date of spawning of the downstream bullies is not yet known and could potentially be anywhere from 1 to 6 months in advance of the upstream bullies.

It was hypothesised that conditions in the lower river over the past decades of pulp & paper mill operations could have influenced the spawning patterns of common bully. In January 2004 we again sampled common bully in the Tarawera River and added the Rangitaiki River to this examination. Common bully in the Tarawera River showed similar reproductive patterns to the previous year. However, the Rangitaiki River results also indicated that common bully were earlier spawners. At this point, we suspect that bully in the lower Tarawera and Rangitaiki could be seagoing during their early juvenile stages, whereas upstream summer-spawning populations may be resident in fresh water only. Subsequent investigations are examining the migratory and genetic patterns of these populations of common bully.

Only a limited number of biochemical and chemical tools are available for the common bully. This is in part due to the smaller nature of this species with subsequent difficulties in obtaining sufficient blood or other tissues for analysis. The only endpoint of this category examined was the liver detoxification enzymes (Fig. 18). Similar to the eel results, the EROD endpoint indicated a significant increase in this enzyme. However, this endpoint is known to vary with the state of reproductive development in females, though not in males. So these data may not be comparable given the reproductive differences observed.

**Figure 18: Detoxification enzymes in common bully liver from the Tarawera River**
4.1.3 Conclusions

Both caged shortfin eels and wild common bully demonstrated exposure to chemicals contained in or derived from discharges into the Tarawera River. The responses were more substantial than expected given previous results of experiments that utilised mesocosm and laboratory studies to examine impacts of one of the two effluents from pulp & paper mills. These previous studies demonstrated that though subtle reproductive and biochemical effects were previously observed, these effects disappeared a number of years ago, including liver detoxification enzyme induction. There is a difference in exposure between effluent and actual exposure within the river itself. It is strongly suspected that these discrepancies between effluent and river studies are due to the presence of sediments in the river that may be contaminated with historical pulp & paper-related compounds.

Due to the unexpected difference in the reproductive timing of the upstream and downstream Tarawera River common bully, no comparison of reproductive success has yet been performed. Given the knowledge that the Rangitaiki River is similar to the Lower Tarawera in regards to spawning time, this comparison can now be conducted with the Rangitaiki River.

This study has certainly served to indicate one example of the limited understanding of the basic biology of New Zealand native fishes. The common bully has proved to be an excellent monitoring species. However, a more comprehensive understanding of the basic biology is required in order to use this native species to understand the life-sustaining capability of the New Zealand environment. Similarly, biochemical tools to assist in demonstrating causality are non-existent from most native species and development of such tools is required.

Despite many years of study, the ecology of the lower Tarawera River is still poorly characterised. Ongoing studies are required to document changes in the river with ongoing improvement, but also to provide a benchmark against which to assess the impacts of subsequent potential developments, such as further geothermal power generation, on the river.

4.1.4 Recommendations

• Long-term studies on population and community changes to fishes, as well as nutrient and colour fate should be initiated on the river.
• This should include a comparison of Tarawera and Rangitaiki common bullies.
• Genetic and morphological studies should be conducted to understand the unique patterns of reproduction observed in the common bully so that this species can be better utilised as a monitoring species.
4.2 Waikato River

4.2.1 Methods employed

Resident brown bullhead catfish (*Ameiurus nebulosus*) and shortfin eel populations were sampled at nine and seven sites, respectively, in the Waikato River. The catfish was the original wild species chosen, as it is one of only two species present in virtually the entire length of the river. There is a significant amount of data on catfish ecology and physiology, no significant catfish fishery, and as it is a pest species it can be sampled in unlimited numbers. Wild eels were sampled in lieu of eel caging studies primarily due to the interest expressed from Waikato Tainui in examining the wild eel populations. Owing to the sheer magnitude of the Waikato study, it would have been logistically very difficult to conduct caging at all sites simultaneously.

Paired upstream and downstream sites were used in lacustrine (slow moving or lake) and riverine habitats, with Lake Taupo and the Mangaotama Stream (Waipa River) sampled as reference lake and stream populations of bullhead and eel respectively. Bullhead responses to geothermal, pulp & paper, municipal sewage, and thermal discharges and shortfin eel responses to the same discharges (except geothermal) were investigated (Fig. 19). Sites were spread from the upper to lower reaches of the river and evidence of cumulative effects was also assessed.

Sampling for bullhead and eel was carried out within three weeks in October–November 2002 and March 2003 respectively. Times were chosen to coincide with maturation peaks where fecundity and reproductive endpoints can be measured (bullhead), and peak activity or catch rates (eel). Between 7 and 20 Fyke nets per site were set overnight along the margins of lake, river or stream sites to capture bullhead and eels. Counts of fish captured were used to calculate Catch Per Unit Effort (CPUE, fish per net per night). Habitats fished were matched as closely as possible between upstream and downstream sites, with backwaters being targeted for bullhead. Subsampled bullhead and eel were examined externally, and the length, weight and organ weights recorded. Full sampling of blood and other variables was carried out on 12 larger mature male and female bullhead and 10–12 eels and an effort was made to keep size range of fish similar between paired sites. The fifth vertebrae and otoliths were used to age bullhead and eel, respectively. The biochemical parameters measured were liver detoxification enzymes and blood plasma steroids. An assessment of red and white blood cell parameters was also conducted. Bile was analysed for the presence of pulp-mill-related organic compounds. As certain aromatic compounds can be produced in treatment systems and in accumulated sediment, fluorescence analysis of bile was also conducted.
Figure 19: Waikato River catfish ( ) and shortfin eel ( ) sampling sites with point source discharges: 1, Wairakei Geothermal Power Station; 2, Kinleith pulp & paper mill; 3, Hamilton City municipal sewage; 4, Huntly Thermal Power Station
4.2.2 Results and discussion

Temperature measurements were made at all sites over the year during which sampling was completed, using temperature loggers. Temperature values over an entire year are expressed as degree days above 10°C as this has been observed as the threshold for growth of brown bullhead catfish (Keast, 1985). Temperature data (Fig. 20) showed significant temperature increases within the effluent plumes of geothermal, pulp & paper, and thermal power sites. This temperature effect generally did not persist at downstream sites after mixing.

![Figure 20: Cumulative temperature (degree days above 10°C) at all sampling locations during the year of sampling](image)

Sufficient bullhead and eel to carry out subsampling for biochemical endpoints were caught at all nine sites, although only four male and female bullhead were captured at the Huntly upstream site. No significant differences were found between CPUE at paired bullhead sites upstream and downstream of individual discharges (Fig. 21). Overall, Lake Taupo and Maraetai sites had higher CPUE than other sites and Lake Maraetai sites were significantly higher than all sites except Lake Taupo and Huntly downstream (Fig. 21). The highest numbers of eel were caught at the two most downstream Waikato River sites (Huntly upstream and downstream) and CPUE at the Huntly downstream site was significantly higher than at any of the other sites (Fig. 21).
Large numbers of small bullhead were caught in the Maraetai and Huntly downstream sites and average lengths were significantly different from upstream reference sites. Average lengths of bullhead caught at other paired sites were similar. In comparison to all sites Lake Aratiatia and Huntly upstream bullhead were larger than the overall river average. Lake Taupo and to a lesser extent Maraetai upstream bullhead appear to be slow growing compared with those at other sites. Aratiatia downstream bullhead were some of the largest we captured and also appear to be some of the fastest growing (Figs 22, 23). Growth was further assessed by fitting of a growth curve (Von Bertalanffy function) to the size–age data. This produces the variable Lmax, the maximum length, or length at infinite age. Examination of the Lmax (Fig. 23) revealed a large range of maximum length among the sites. Lake Aratiatia sites have the largest catfish while Cambridge and Taupo sites had among the smallest. Presumably, this range of sizes is due to low densities at some sites reducing competition for food and elevated water temperatures, which bullhead prefer. However, other sites such as Cambridge have a relatively low density (as indicated by CPUE) and small bullhead. As this site is highly riverine, it is likely that it presents very poor habitat for bullhead and subsequently has poor food availability.
Eel catches from the lower Waikato River sites were dominated by small eels (Fig. 21). While eel caught from the two Lake Maraetai sites were larger, this may be due to limited recruitment at these sites, although eel at the Hamilton upstream site were also larger than lower Waikato River eel and it is doubtful that recruitment would be limiting at this site. A more likely explanation is that eel growth is faster at sites where we caught larger eels and this is supported by greater length at age of these eels (Fig. 24).
Bullhead of both sexes from the Maraetai downstream site had higher condition scores than those at the upstream Lake Maraetai site (Fig. 25).

Boxes are mean ± SE and whiskers are 95% confidence intervals. Dashed horizontal line is mean value calculated from site means.
Subsequent examination of bullheads captured upstream of Hamilton revealed they were all sexually immature, and as it is inadvisable to compare parameters between mature and immature fish, no comparisons were made for condition factor or for any other subsequent measures. Condition factors for both sexes of bullhead from Lake Taupo fell below the overall river average whereas those catfish from Lake Aratiatia were often above the overall river average. Liver size followed the same trends seen in condition (Fig. 25).

As seen with bullhead downstream at Maraetai, eel were in significantly better condition and had larger livers than at the Maraetai upstream site, (Fig. 26). Unlike bullhead at the same site, eel at the Hamilton upstream site were in the best condition of all the sites; they also had the second largest livers. Significant differences were found in spleen size at the Maraetai downstream site although trends were reversed between bullhead and eel.

The range of blood parameters examined showed consistent trends between species in that increases in red blood cell numbers and oxygen-carrying capacity expected due to higher site temperatures were seen at all warmer sites except Maraetai downstream (Table 4). Despite higher temperatures and lower dissolved oxygen at the Maraetai downstream site, female bullhead haematocrit was significantly lower than at the upstream Maraetai reference site, male haematocrit (Hct) was also lower but not significantly so. In contrast, Maraetai downstream eel Hct values were higher but not significantly so (Table 5).

Blood sex steroid hormones were measured in subsampled bullhead. Sex steroid concentrations were found to covary with gonad size, indicating difference in maturational state, and GSI was used as a covariate in the comparisons. In general, increases in steroid hormones were seen at the geothermal site and decreases at the pulp mill site (Fig. 27). Both significant decreases and increases were seen at the Huntly downstream site. The only other significant differences in female steroid levels were seen between the Hamilton sites, as the upstream females were not maturing their ovaries. Examination of eggs from females from this site has revealed that they were not as mature as those from other site females and females would not have spawned in the same year as other mature females. Because of this they are not included in reproductive measures presented here.
Table 4: Blood parameters in bullhead from the Waikato River

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Taupo</th>
<th>Aratiatia</th>
<th>Maraetaiti</th>
<th>Hamilton</th>
<th>Huntly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>98.2</td>
<td>83.7</td>
<td>97.7</td>
<td>124.8</td>
<td>119.5</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>33.2</td>
<td>27.7</td>
<td>33.5</td>
<td>37.6</td>
<td>37.9</td>
</tr>
<tr>
<td>Lct (%)</td>
<td>0.79</td>
<td>0.87</td>
<td>0.87</td>
<td>1.03</td>
<td>1.07</td>
</tr>
<tr>
<td>MCH (g/cell x 10^-10)</td>
<td>0.88</td>
<td>0.94</td>
<td>0.65</td>
<td>1.12</td>
<td>0.82</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>294</td>
<td>311</td>
<td>291</td>
<td>332</td>
<td>312</td>
</tr>
<tr>
<td>MCV (L x 10^-12)</td>
<td>0.30</td>
<td>0.27</td>
<td>0.23</td>
<td>0.34</td>
<td>0.27</td>
</tr>
<tr>
<td>RBC (cells/L x 10^12)</td>
<td>0.16</td>
<td>0.13</td>
<td>0.19</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>99.7</td>
<td>90.0</td>
<td>113.5</td>
<td>117.6</td>
<td>115.2</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>34.0</td>
<td>28.1*</td>
<td>38.0*</td>
<td>37.4</td>
<td>32.0</td>
</tr>
<tr>
<td>Lct (%)</td>
<td>0.96</td>
<td>1.11</td>
<td>0.90</td>
<td>0.98</td>
<td>1.22</td>
</tr>
<tr>
<td>MCH (g/cell x 10^-10)</td>
<td>0.90</td>
<td>1.23</td>
<td>0.92</td>
<td>0.86</td>
<td>1.29</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>286</td>
<td>311</td>
<td>298</td>
<td>311</td>
<td>359</td>
</tr>
<tr>
<td>MCV (L x 10^-12)</td>
<td>0.31</td>
<td>0.40</td>
<td>0.31</td>
<td>0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>RBC (cells/L x 10^12)</td>
<td>0.11</td>
<td>0.08</td>
<td>0.18</td>
<td>0.14</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*Pair of bold values are significantly different at *p=0.05 or **0.005.

Table 5: Blood parameters in shortfin eel from the Waikato River

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Maraetai</th>
<th>Hamilton</th>
<th>Huntly</th>
<th>Waipa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Up (n=12)</td>
<td>Ds (n=12)</td>
<td>Up (n=11)</td>
<td>Ds (n=12)</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>110.7</td>
<td>115.9</td>
<td>96.9</td>
<td>91.6</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>30.9</td>
<td>31.2</td>
<td>28.9</td>
<td>26.7</td>
</tr>
<tr>
<td>Lct (%)</td>
<td>1.44</td>
<td>1.84</td>
<td>1.93**</td>
<td>1.28</td>
</tr>
<tr>
<td>MCH (g/cell x 10^-10)</td>
<td>0.34</td>
<td>0.32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>358</td>
<td>377</td>
<td>334</td>
<td>346</td>
</tr>
<tr>
<td>MCV (L x 10^-12)</td>
<td>0.09</td>
<td>0.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RBC (cells/L x 10^12)</td>
<td>0.34</td>
<td>0.38</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Pair of bold values are significantly different at *p=0.05 and **0.005, - analysis incomplete.

Figure 27: Sex steroid hormones in male (top) and female (bottom) bullhead from the Waikato River
Gonads (Gonado-Somatic Index, GSI) of maturing males and females were remarkably consistent throughout all sites and no significant differences were found (Fig. 28). GSI in Lake Maraetai males was generally lower, but this is likely due to immature fish in the dataset as it is much more difficult to discriminate immature males than females. Lake Taupo females were the only group of females that appeared to have a GSI significantly lower than the mean for the river. Bullhead ovaries were observed to have two distinct ovarian follicle size classes – those that were developing for the year in question, and those for the next year. Follicles from the ovaries were examined by digital analysis and those ovaries that did not have two distinct egg size classes were considered immature and were deleted from the analysis of gonad size, fecundity and sex steroid hormones. Low gonad size at the Hamilton upstream site was due to bullhead being immature, although the fish were of equivalent size to the sexually mature Hamilton downstream bullhead.

Figure 28: Gonado-somatic index of male (left) and female (right) bullhead catfish in the Waikato River

Fecundity in bullhead was measured by counting only maturing ovarian follicles and is expressed as number of eggs per kilogram body weight (Fig. 29). There were no significant differences between upstream and downstream locations although fecundity did vary significantly over the range of sites examined. It was also possible to measure egg size directly from the digital images. An analysis of egg size also revealed no significant differences between upstream and downstream reference sites.
The significant increase in liver detoxification enzymes (EROD) at the Maraetai downstream site is consistent with bullhead being exposed to chemicals associated with pulp & paper effluents (Fig. 30). The larger increase in males is typical of other fish responses to such effluents.
Despite bile results (Table 6) suggesting Hamilton upstream bullhead have been exposed to low-level resin acids, no corresponding increase in EROD was seen.

**Table 6: Total resin acids from pooled biles from Waikato River bullhead**

<table>
<thead>
<tr>
<th>Site</th>
<th>Upstream</th>
<th>Downstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taupo</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Aratiatia</td>
<td>17.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Maraetai</td>
<td>28.2</td>
<td>1052.8</td>
</tr>
<tr>
<td>Hamilton</td>
<td>301.8</td>
<td>18.7</td>
</tr>
<tr>
<td>Huntly</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Maraetai downstream eel also had a significant increase in EROD (Fig. 31). The difference in magnitude between species is normal, with different species showing varied amounts of increase to similar exposure conditions. The increase in eel EROD seen at the Maraetai downstream site was four times that seen in eel caged in the Tarawera River at the Tarawera 2 site (Fig. 14).

Levels of total resin acid found in bile show that Maraetai downstream bullhead were, as expected, exposed to chemicals associated with pulp & paper effluents. Surprisingly, Hamilton upstream bullhead also showed some signs of exposure (Table 6).

**Figure 31: Liver detoxification enzyme activity in wild shortfin eel captured in the Waikato River**

4.2.3 Conclusions

Size range, relative density, length at age, and condition of bullhead downstream of the discharges with a significant heat component (all except Hamilton) suggest that bullhead benefit from the added heat, as would be expected given their preference for temperature of
approximately 30°C (Cranshaw, 1974). Given the correlation between condition factor and liver size it could have been expected that sites with highest condition factors (Aratiatia upstream for bullhead and Hamilton upstream for eel) would have had the largest livers, but Maraetai downstream fish had the largest livers. It is likely that bullhead and eel at this site had enlarged livers as a consequence of exposure to contaminants from pulp & paper effluent as has been demonstrated in other similar fish studies (Munkittrick et al., 1994).

Bullhead populations sampled in the Waikato River were good indicators of the effects of discharges as they had chemical and biochemical markers that showed they were resident in and exposed to toxic components of the discharges investigated. While other components of their habitat (such as water velocity at riverine sites, fluctuating water levels at Aratiatia sites, and water temperature) made determining population responses to toxic components of discharge difficult, sub-lethal effects were obvious at the Maraetai downstream site. It may be that negative individual or community effects on bullhead were offset by the beneficial effects of added heat and perhaps nutrients from the discharges. If so, native species with lower preferred temperatures may still be compromised.

The native shortfin eel we sampled from many of the same sites showed similar responses to bullhead. However, there were some notable differences such as the Hamilton upstream site where eel and bullhead responded in diametrically opposite fashion, likely due to habitat responses. Differences in population structure due to factors such as limited recruitment at Maraetai sites and fishing pressure at lower river sites, lack of reproductive endpoints and preference for warmer water temperatures found at most discharge sites made eel a poorer indicator of discharge effects than catfish.

4.2.4 Recommendations

- Long-term studies on population and community changes to fishes, as well as on nutrients and temperature should be continued on the river.
- Given the severe pest fish problems in the Waikato, more emphasis should be placed on reducing the impacts of thermal pollution.
- Follow-up studies on the impacts of overfishing on eel populations should be considered.
4.3. South Island Studies on the Cam and Styx Rivers

4.3.1 Methods employed

Shortfin eel caging studies and wild common bully sampling were conducted at both sites within Canterbury. Secure sites where cages were placed on both rivers are indicated in Fig. 32 for the Cam River and Fig. 33 for the Styx River. Shortfin eel for these studies were from Lake Ellesmere (Waihora) and kindly provided by Mr C. Smith, a local fisherman. Fish were caught at Lake Ellesmere and transported to the Landcare Research animal facility on ice to minimise stress, and they were acclimatised for at least 2 weeks prior to exposure. Caging studies were conducted as previously described, with one cage holding 10 eels at each location.

For the wild fish study, no appropriate reference sites that were relatively free from pollution or agricultural land-use could be identified in the Canterbury Region. Upstream areas of the Cam and Styx rivers did not have common bully in sufficient numbers or the species was absent and thus these locations did not provide good reference locations. Following consultation with Environment Canterbury, it was decided to use the Selwyn River as a source of reference common bully. An additional site, a small tributary of the lower Heathcote River in Christchurch City, was also examined.
4.3.2 Results and discussion

Eel caging studies were conducted on both river systems, as appropriate sites were found along the catchment. As stated earlier, there are prerequisites for conducting a caging study, including sites with sufficient depth and shade that are as remote as possible from general public access.

To determine the energy consumption/utilisation of the fish following the 3-week exposure, condition factors were calculated before and after exposure. Figures 34 and 35 show the condition factors of fish caged in both rivers before the caging experiment and at the end when the fish were sampled. At both sites, the fish lost some condition. Similar trends were seen in a study conducted on the Christchurch Avon River where all fish, independently from the site, showed slight loss of body weight after 3 weeks (Tremblay, 2004). Drop in weight is a normal response in fish being caged, as they have to adapt to their new environment in the cage and to new feeding regimes different from that under laboratory conditions. Observations of the overall condition of the fish at the end of all caging experiments showed no sign of common external stress such as fin rot or lesions. Most fish had stomach contents at the end of the exposure indicating that there was food available for them during the exposure. The food included small crustaceans and fish like the common bully.
Determining effects of pollution on fishes

Lysozyme is an enzyme with bacteriolytic activity that acts as a non-specific component of innate immunity protecting the organism from infections. It is present in serum and within cells with immune function. In a previous study using caged eels, lower plasma lysozyme activities were found in fish located in more contaminated sites in the Christchurch Avon River (Tremblay, 2004). In the current caging studies, opposite trends were seen at the two sites. Fish caged in the Cam River showed decreased lysozomal activity (Fig. 36) while there was an increased activity with the Styx River study (Fig. 37). There was no trend with the spleen somatic index for both caging experiments (data not shown). It was decided that measuring lysozyme activity was not providing useful information to evaluate the fitness of the immune system, and consequently, this parameter was not measured in subsequent eel caging studies.
The induction of hepatic liver detoxification enzymes was evaluated by measuring the enzyme cytochrome P450 1A (CYP1A) though the EROD endpoint. The liver is the main detoxifying organ involved in eliminating contaminants from the body and will often increase in mass when the organism is challenged with heavy organic contamination. This enzyme has previously been used successfully in eel caging studies to measure the effects of pulp & paper mill effluents and urban contamination in the Avon River (Jones et al., 1995; Tremblay, 2004). The level of CYP1A was measured by the EROD assay for both caging studies (Figs 38, 39). Enzyme activities were elevated in fish caged immediately downstream from the Rangiora sewage treatment outfall in South Brook.
Determining effects of pollution on fishes

EROD was reduced in fish caged further downstream at the Revells Road site suggesting that the contaminants responsible for causing the effects were diluted when moving further away from the source (Fig. 38). Fish caged in the Styx River were induced at the Redwood site (Fig. 39). At this stage, it is difficult to establish the cause of the induction, as there is a new subdivision in the area, but stormwater output from a nearby shopping centre could also contribute to this effect.

For the wild fish survey, common bully were sampled using minnow traps and electrofishing. It was not possible to obtain reference fish within the same river system because of the species’ distribution limits which do not extend upstream from the sources of pollution. This is one of the limitations of using the common bully as an indicator. Therefore, fish were sampled at two alternative sites following discussion with Environment Canterbury. The first was in the Selwyn River and the second in a tributary of
the Heathcote River in Christchurch City. In the Cam River, fish were sampled downstream from the Rangiora sewage outfall. In the Styx River, fish were sampled around the Styx Living Laboratory, downstream from the Janet Stewart Reserve. Figures 40 (males) and 41 (females) show condition of the fish at the various sites. The Selwyn River fish of both sex showed the smallest condition, which reflects the natural conditions found at that site. The Selwyn River is fed from groundwater and sub alpine runoff and has a cobble bottom, and thus it represents water quality and habitat considerably different from all other sites. There were significant differences in condition factors for both fish sexes between the Cam and Styx rivers. Fish sampled in the Cam River were bigger, which may be attributed to the nutrient influx from the sewage outfall and agricultural inputs. Though not statistically significant, the Selwyn River bullies consistently had the lowest condition factor, reflecting the more oligotrophic (nutrient depleted) nature of this stream.

**Figure 40: Condition factor of male common bully sampled at the South Island sites**

<table>
<thead>
<tr>
<th>Site</th>
<th>Condition Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cam River</td>
<td>a</td>
</tr>
<tr>
<td>Styx River</td>
<td>bc</td>
</tr>
<tr>
<td>Selwyn River</td>
<td>ac</td>
</tr>
<tr>
<td>Heathcote</td>
<td>bc</td>
</tr>
</tbody>
</table>

**Figure 41: Condition factors of female common bully sampled at the South Island sites**

<table>
<thead>
<tr>
<th>Site</th>
<th>Condition Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cam River</td>
<td>a</td>
</tr>
<tr>
<td>Styx River</td>
<td>b</td>
</tr>
<tr>
<td>Selwyn River</td>
<td>ab</td>
</tr>
<tr>
<td>Heathcote</td>
<td>ab</td>
</tr>
</tbody>
</table>
Figures 42 and 43 show the liver somatic index for both fish sexes. The liver is an important organ and has multiple functions including energy storage and detoxification. The only significant difference was in male sampled in the Styx River, which had smaller livers. This is not the expected response as liver size usually parallels condition factor. It may be that the liver is not as critical as an energy storage organ in the common bully.

**Figure 42: Liver somatic index in male common bully sampled at the South Island sites**

![Graph showing liver somatic index in male common bully](image)

**Figure 43: Liver somatic index in female common bully sampled at the South Island sites**

![Graph showing liver somatic index in female common bully](image)

The gonado-somatic index (GSI) results for the common bully are provided in Fig. 44 (males) and Fig. 45 (females). GSI provides an indication of the size of reproductive organs. The only difference was again with males sampled in the Styx River. The GSI data indicate the despite the variety of trophic states and conditions at the South Island sites, there was no overt influence of these environmental variables on reproductive development of the female bully.
Determining effects of pollution on fishes

Figure 44: Gonado-somatic index in male common bully sampled at the South Island sites

Figure 45: Gonado-somatic index in female common bully sampled at the South Island sites

Figure 46 summarises EROD activity measured in the South Island common bully. The fish from the Styx River showed much higher levels of activity than those from the Cam River. EROD levels in fish sampled in the Cam River were similar to levels found in the Heathcote Tributary. That indicates the Heathcote tributary is not an appropriate reference site as it is located in the city and may receive some urban inputs. The Selwyn River had the lowest EROD activity of any of the sites, reflecting the higher water quality and lack of anthropogenic pollution in the system. As compared to the Selwyn River bully, EROD in males from the Styx River was induced nearly six-fold.

The way the bully responded was opposite to what was shown with the eel caging experiment where induction was higher downstream from the sewage outfall in the South Brook than in fish caged in the Styx River (Figs 38, 39). The bully in the Cam River were sampled away from the Rangiora sewage outfall and there is significant dilution as the
South Brook meets the main Cam River, which may partly explain the discrepancy between species. It is more difficult to identify the cause of EROD induction in the Styx River at the Living Laboratory site. The chemicals responsible may be of historical origin and deposited in the sediments. There has been increasing urbanisation in the area over the last few years, and it is possible that EROD activity is associated with runoff from this development. Further research is required to identify the source of contamination.

**Figure 46: Hepatic ethoxyresorufin-O-deethylase (EROD) activity in male and female common bully sampled in the South Island**

![Hepatic EROD activity graph](image)

Each bar represents the mean ± standard error.

### 4.3.3 Conclusions

The results show that the sources of pollution present in both river systems did not lead to acute toxicity, as none of the caged fish died or showed physical damage such as the presence of fin rot or skin tumour. Of all the parameters measured following the 3-week exposure, the induction of the hepatic EROD was the most significant. Both fish species showed increased level activities of that enzyme. At this stage, because of our limited knowledge of the two species, it is difficult to conclude whether these levels pose significant threat. Fish caged downstream from the sewage outfall on South Brook and in the Redwood area of the Styx River had elevated EROD activity indicating that the fish were exposed to organic pollutants such as PAHs; but that the activity was rapidly diluted as it moved away from the source. However, with the common bully, fish sampled in the Styx River showed the highest levels of hepatic EROD activity. In the Styx River, there are no point sources of contamination such as the Rangiora sewage discharge, therefore it is
difficult to determine the cause of this effect. Further investigations are warranted to identify the sources of contaminants responsible and determine the long-term effects on the health of the exposed fauna.

Finding appropriate reference sites to collect control common bully was not possible within the Cam and Styx river systems, due to the distribution of this species not extending upstream from the pollution sources. Therefore, it was necessary to collect fish from other less impacted rivers like the Selwyn and a tributary of the Heathcote. This alternative is not ideal as the general habitats of these rivers were not similar to the two rivers studied. The Selwyn River is a mountain-fed system with a fast flow and a cobble bottom quite different from both Cam and Styx rivers. Fish sampled in the Heathcote Tributary had elevated levels of hepatic EROD activity indicating that these fish were exposed to organic contaminants. This demonstrates some of the limitations of this approach and provides a further rationale for the use of in situ methods such as the caging studies.

4.3.4 Recommendations

- It is important to identify the biologically active contaminants in the Rangiora sewage effluent responsible for the EROD activity. Both water and sediment should be extracted and tested using a biological test to evaluate the activity. The chemicals in the extracts containing the activity could then be identified by chemical analysis.
- Similarly, further investigate the source of contaminants responsible for the EROD induction at the Styx Living Laboratory area of the Styx River site by using a toxicity identification and evaluation (TIE) approach. There has been intensive urbanisation in this area and also there is a refuse station nearby that may contribute to the activity.
4.4 Waiwhetu Stream

4.4.1 Methods employed

Examination of toxicological impacts on the Waiwhetu Stream using the methods proposed for this study proved difficult. The target wild fish species, the common bully, was not found in the stream, precluding this part of the study. Eel caging was not deemed feasible because of the lack of suitable secure sites at both the downstream (most heavily contaminated) and upstream regions of the stream. There was also a lack of suitable locations for reference sites upstream and these would have provided a poor basis of comparison with downstream areas, which are tidal.

Fish and macroinvertebrate community surveys were performed in lieu of the originally proposed work. Fish surveys were conducted using electric fishing where possible. In tidal regions, where conductivity was high, minnow traps and visual observations were used to assess the species present. Macroinvertebrates were sampled, using a kick-net, from a variety of reaches of the stream and identified to species level. Periphyton on substrates collected from the Waiwhetu Stream were also identified.

4.4.2 Results and discussion

The Waiwhetu Stream was sampled between 29 November and 2 December 2002. During this time temperature readings and water conductivity values were taken at high tide (Fig. 47). Salinity influences were apparent in a significant part of the lower stream at least as far as Gracefield School on Riverside Drive. A significant increase in stream temperature occurred between the headwaters near the cemetery and the outlet of the concrete flood control channel. During the short period of sampling on the Waiwhetu stream, two visually obvious pollution events were documented. The first was an oily sheen on the lower stream that was followed back to a storm drain on the lower river (Fig. 48). The second event involved the stream becoming a turbid white colour. This second event was apparent in the upper reaches of the stream below the concrete flood barrier, but the event ceased before it could be traced to a specific location.
The macroinvertebrate community was examined at eight locations throughout the Waiwhetu Stream (Fig. 49). Communities in the lower reaches, where contamination is known to be highest, were typical of marine fauna, and as a result were not easily comparable to the freshwater reaches of the stream. The community structure was expressed as an MCI, a diversity index commonly used in New Zealand. However, as MCI varies with stream type as well as with impacts on the stream, the MCI was expressed by plotting it against the number of species present (Fig. 50).
To put these data in context, they were compared with a dataset derived from Auckland urban streams. Overall, most of the Waiwhetu sites in the lower part of the stream had relatively low MCI when compared against the number of species. This indicates that all sites below the cemetery support poor invertebrate communities, with low numbers (or a complete absence of) "sensitive" invertebrates. This results in low MCI values (based on the proportion of taxa belonging to "sensitive" groups) with the exception of the site closest to the cemetery (MCI increased at that site by the presence of single individuals of three sensitive taxa). The lowermost two sites could be expected to have poor invertebrate communities due to the tidal influence, slow flow and muddy environment. These two sites are dominated by estuarine taxa that are absent/rare further upstream. The Whites Line invertebrate community is strongly affected by the weedy environment. The most unexpectedly poor communities were those further upstream near St. Ronans Drive and Lockett St because these were physically good sites with communities that were not significantly better than those of the lower reaches sites. The poor communities
immediately below the concreted upper reaches suggests two obvious causes: one is that the open, concreted environment causes warming and a lack of habitat for "sensitive" taxa – hence these taxa don't colonise the downstream reaches via downstream drift, and the other is a contamination problem entering the concrete area. The invertebrates conspicuously missing from the reaches below the concrete area are the mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) (these organisms form the EPT index) and these groups are known to be unsuited to warm water temperatures, low dissolved oxygen, slow flows, muddy beds, and severe nutrient enrichment.

It is unlikely that nutrient enrichment is the main cause of the lack of EPTs – the algae abundance did not indicate a grossly enriched site. There were no indicators of extreme organic enrichment such as rat-tail maggots. Some of the most abundant taxa at the poorer sites, e.g. oligochaetes, *Potamopyrgus* snails, orthoclad midges, *Chironomus* midges and ostracods, are all known to be well adapted to surviving in warm environments and those with low dissolved oxygen. Te MCI values from this stream are generally low, even compared with other lowland, urban streams (Auckland data), but the numbers of taxa ("taxonomic richness") are not low compared with Auckland urban sites. A really toxic environment would be expected to support low numbers of taxa.

The cemetery is a non-urban site in that the setting and physical habitat conditions are much more "natural" and riffle-like than all the downstream sites. The high MCI and taxonomic richness in the cemetery reaches is the result of lots of mayflies, stoneflies and caddisflies (over half of the taxa in the sample). These groups are usually associated with cold water, fast flow, stony beds, high dissolved oxygen, and proximity to native vegetation. The cemetery site is the most riffle-like (steeper and more stony), which makes it a better physical habitat than all of the downstream sites (more like runs than riffles). However, physical habitats of the midreaches were adequate to support at least some of the mayflies, stoneflies and caddisflies, so perhaps the open concreted reaches allow too much warming for these coldwater species or perhaps there are city stormwater quality issues. The lowermost tidal sites may not have supported mayflies, stoneflies or caddisflies even before urban development as these insects don't like salt, mud, or inconsistent flows.

The algae groups within the periphyton samples were all common, widespread types and there were no signs of unusual contamination. The main periphyton species were *Enteromorpha*, forming bank-side bands of visible green filaments at the tidal sites (the same algae thrive along Auckland and Otago estuaries). *Melosira* was abundant in the lower reaches and this alga likes a good nutrient supply and stony bed. Generally there was low abundance of other algae and the bed of the midreaches is more dominated by moss, which is unlike most other urban streams, indicating a stable bed. There were no significant abundances of filamentous bacteria, fungi or protozoa. Abundance of such groups are often seen on streambeds below gross organic nutrient sources such as sewer leaks; such growths can be seen under the microscope before "sewage fungus" growths become visible in the field. The Waiwhetu Stream also contains substantial beds of Cape pondweed in the region of Te Whiti Park. This highly invasive, exotic species of aquatic plant is known to thrive under high light conditions and is virtually absent from upstream regions with extensive shading.
Extensive fish surveys were carried out, particularly in the lower reaches of the stream (Fig. 51). Minnow traps in the saline regions of the stream captured large numbers of marine triplefins (*Grahamina nigripenne*). A few smaller eels and yellow-eyed mullet were also captured in minnow traps in this area. Larger schools of yellow-eyed mullet were also observed in this area. Substantial fishing effort was directed in the area of Te Whiti Park as this shallow, slow-moving, weedy environment was the most likely habitat for common bully. Eel species were particularly abundant in this area and inanga were also frequently observed. Neither electric shocking nor baited minnow traps were successful at capturing any bullies. Bullies are known to occur in a number of tributaries of the Hutt River. The most recent survey of fish communities in the Waiwhetu Stream was conducted in 1996 and common bully were observed as being abundant in the area of Te Whiti Park (Royds Consulting, 1996). The only other known fish community data for this stream dates back to 1947 when giant bully were observed in the lower reaches of the Waiwhetu Stream.

Common bully are one of the most ubiquitous species in lowland streams in New Zealand, are relatively insensitive to low dissolved oxygen compared with other species (Landman et al., 2004). Though few data on the temperature tolerance of bullies are available, the upper levels of temperature in the Waiwhetu Stream would not be expected to exceed the upper limit of their tolerance. The level to which common bully migrate to sea (diadromy) as juveniles is not fully characterised, but it is certainly known to occur (Closs et al., 2003), and there is no barrier to migration of bullies in this region of the stream. It is a bit of an enigma why this species, previously observed in the Waiwhetu, is now absent. Given that physical factors in the stream should not preclude this species, pollution is a potential candidate for their disappearance. Herbicide use, or other as yet uncharacterised factors, either in the stream itself or in adjacent regions, may be contributing to the absence of bully.

**Figure 51: Fish species captured in the Waiwhetu Stream**

Inanga were absent in upper regions of the stream as may be expected due to the presence of the weir upstream of the park. Inanga have poor climbing ability and would not be
expected to migrate above this weir. The two other reaches between the park and the concrete channel were examined by electric fishing. These areas contain smaller reaches with mature riparian areas. Small numbers of other galaxiids such as giant kokopu, koaro, and banded kokopu were observed to be closely associated with the more mature and shaded riparian areas. These same areas also supported the largest eels observed in the Waiwhetu, particularly in the only small, deep pool upstream of Rossiter Ave. One redfin bully and one brown trout were also captured along these reaches.

Visual observation was made of the concrete channel as there are no structures present to conceal fish, and one banded kokopu was observed swimming down the channel. The stream becomes very narrow near the headwaters that run through the cemetery and no larger fish were captured. However, significant numbers of small, unidentified, post-whitebait galaxiids were observed.

4.4.3 Conclusions

Though the lower Waiwhetu Stream is known for its very high levels of metals, deterioration of its upper regions was more substantial than expected. This includes loss of habitat and elevated temperatures due to the concrete flood control channel, a weir preventing fish migration, directly observed and anecdotal evidence of pollution pulses – likely from stormwater, invasive plants in unshaded reaches, and the conspicuous absence of a keystone lowland fish species. Unquestionably, the tidal reaches of the stream are severely contaminated and deteriorated. However, it would require an alternative design, involving comparisons with tidal areas of other streams, in order to make conclusions regarding the integrity of the ecosystem in this area. There is clearly significant fish and invertebrate life in this reach, despite the high levels of contamination. However, the tidal flushing and migratory nature of some of the species present probably ensure that biota do not reach serious levels of contaminant exposure in the lower reaches.

4.4.4 Recommendations

• Before substantial investment in clean up of the lower reaches is made, adequate pollution source identification and control must be achieved.
• The concrete channel removes significant habitat and contributes to substantial increases in temperature, thus, alternatives to this structure to restore shading, riparian zones and habitat may overall have the most significant impacts on the restoration of the Waiwhetu Stream.
• Work to establish native riparian areas, create shading, and remove exotic weeds from middle to lower reaches are well under way and should continue.
• Alternatives to the weir structure should be considered, as a means to increase upstream fish diversity.
• The possibility of examining the health of estuarine fish species, such as the triplefin, should be considered.
5 General Conclusions

The first step of this programme was to conduct a consultation exercise to identify knowledge gaps on the effects of pollution in New Zealand. Contrasting views were expressed. Certain parties consulted expressed the feeling that New Zealand fitted its “clean and green” image and they were sceptical of the need for a programme to investigate methods to study the effects of pollution. In many, but not all cases, industry was more interested than government agencies in supporting study of environmental impacts. Other parties consulted, tangata whenua in particular, expressed unreserved delight that somebody was finally interested in examining environmental issues of great cultural and spiritual relevance to them. Continuing consultation and the hui held at the study sites helped getting feedback from various interested parties. This aspect needs to be maintained and encouraged in future environmental efforts to manage resources.

Relative to other areas of the world, New Zealand certainly maintains a significant component of its natural character, largely as a benefit of its low population density. However, New Zealand is a developed country with an expanding population resulting in the intensification of urban, agricultural and industrial activities. Prior to our study, little information was available on the characterisation and monitoring of pollution effects on fish populations in New Zealand rivers receiving various anthropogenic pressures. Indeed, perceptions related to pollution issues in New Zealand have been changing even as this study progressed. Front-page headlines featuring “dying” lakes, a national publicity campaign against “dirty dairying” that culminated in a report by the Parliamentary Commissioner for the Environment, and recognition of the possibility of adverse affects of historical pesticide manufacture and usage are among the number of prominent examples of this.

This research was aimed at characterising the health status of fish using an effects-based approach whereby we interpret what the exposed organisms themselves are telling us about their environment. There are few if any endpoints more relevant to the environment than this, but there is also considerable difficulty in designing and interpreting studies for this purpose. Indeed, much of our understanding of how components of the environment respond to stress is still within the realm of scientific research. Thus, one of the primary goals of this study was to examine how international state-of-the-art effects-based fish monitoring protocols could be adapted for the unique ecosystems of New Zealand. In one study we included the MCI, which provided a good surrogate to the use of caging studies, indicating that the caging protocol needs specific requirements. Study sites from across the country were selected to represent environments receiving various pressure types occurring in New Zealand. Site selection was carried out by consulting with local groups and establishing partnerships in order to identify sites of priority concern within the various management areas. It was hoped that the sites examined would vary significantly in their nature, and in the nature of the factors impacting them, in order to provide a robust test of the methods employed. This goal was certainly realised within the sites selected.

The two methods primarily used, shortfin eel caging and wild fish survey, were to provide indications of how fish respond to the level of pollution on a short-term and long-term
basis, respectively. It is certainly apparent from this study that neither these, nor any other methods selected, will be applicable to every site and situation. A wide range of capability is required in order that effects-based methods can be adapted to each specific situation. Currently, effects-based monitoring, particularly with fishes, is largely ignored in favour of laboratory methods of determining the risk of impacts. In our opinion, such risk assessment methods are useful and anticipatory tools but their use in exclusion of real-world monitoring is not adequate to protect the environment in all cases. It is simply not possible for laboratory bioassays or chemical criteria to be extrapolated to real-world conditions in many cases. Thus, the most significant outcome of this work is to forward the understanding of effects-based monitoring using fishes in New Zealand. The approach applied for this research was to be effects-based and to provide information across the different biological scales, i.e. from the molecular (EROD) to whole individuals (common bully). Current work is looking at dynamics and genetics of populations to provide more relevant information on the sustainability of the species.

One outcome of the research was to identify the common bully as an appropriate New Zealand species with which to measure stream health. The species showed all the characteristics required to monitor the New Zealand environment and was abundant in a great number of the lowland streams examined. Further development of diagnostic biochemical tools for this species is ongoing. This study only dealt with the freshwater environment and most impacted freshwater environments are lowland systems. In areas such as Canterbury, it was not possible to find an unimpacted lowland stream nearby for comparison. Estuarine environments act as sinks for contaminants, and are also of concern. Thus, future studies will examine the utility of estuarine species such as triplefin species and flounder.

Not surprisingly, at those sites where pollution impacts are known to exist, we could observe changes in a number of biological and chemical indicators of pollution stress. Less obvious was the presence of a number of potentially serious impacts on fish populations and fish communities. Such observations included the complete absence of a keystone fish species, complete failure to recruit young fish back into the population, the local proliferation of pest fish species, and clear signs of overfishing. This research led to the hypothesis that thermal pollution, or elevations from natural temperature, could have dramatic effects on biodiversity because these changes greatly favour a number of warm water pest species.

The results presented in this report demonstrate that there are impacts on fishes in aquatic systems throughout New Zealand. However, the sites herein were chosen based on the presence of known contaminant sources, so the study results should not be extrapolated to reflect the state of the freshwater aquatic environment in New Zealand. We would not consider that the impacts of contaminants are the most serious threat to biodiversity in New Zealand. However, we would contend that, based on our consultation and research, such impacts have not been given the consideration that they are due. New Zealand should consider itself fortunate that the state of the environment is such that it is still possible to prevent further deterioration, and accomplish significant restoration, and thus to right some of the wrongs that have been perpetrated on the environment. However, there are increasing efforts to limit impacts of human activities on the receiving environment and such
programmes should be maintained and encouraged.
6 Recommendations

- Regulatory agencies need to enhance the role of effect-based monitoring in New Zealand through incorporation of ongoing environmental effects monitoring into resource consents where warranted. This monitoring can not only serve as a validation of other methods or risk assessment but can also provide a valuable baseline for measuring further improvement, or the impacts of additional development.
- Continue consultation processes with community groups, iwi, local government, and industry to ensure involvement of all groups and the inclusion of their input into management decisions.
- Further investigations should be conducted at estuaries as they act as sinks by receiving upstream cumulative pollution. Estuarine environments are under increasing pressure and resources must be allocated to protect these highly significant environments.
- Continue to improve management of effluent point-sources.
- Continue to improve the management of non-point sources such as dairy wastes, urban stormwater, road runoffs, golf courses, and horticultural growing areas. The proper management of these pollution sources is particularly challenging and requires paradigm-shifting efforts from the various stakeholders involved.
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