Improved Monitoring by Using Genotyping to Correct Biases in the RTC Index of Possum Numbers

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Landcare Research Contract Report: LC0203/060

PREPARED FOR:
Animal Health Board
PO Box 3412, Wellington

DATE: February 2003
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Summary

Project and Client
The Animal Health Board (AHB) commissioned Landcare Research, Lincoln, to determine whether it was feasible to uniquely identify individual possums from DNA recovered from their faecal pellets, and, if so, to conduct a field trial to show how the technology could be used to assess the trappability of possums, using the leg-hold traps widely used to monitor possum density. Development was completed in 2000/2001, and the field trial in 2001/2002.

Objectives
To provide a new tool for assessing absolute animal densities in order to test and validate monitoring methods, by:
- developing a method for extracting DNA from possum faeces
- field-testing an approach combining DNA profiling with trapping to estimate possum numbers on traplines.

Methods
We developed a protocol for recovery of DNA from faecal samples using faecal pellets collected from captive possums. We then conducted weathering trials to assess the effect of pellet ‘age’ (time since deposition) on the recoverability of faecal DNA.

Genomic DNA was extracted from tail tips using the BioRad AquaPure Genomic DNA Isolation Kit. Isolation of DNA from faecal pellets was carried out using the QiaAmp DNA Stool Mini Kit. Eight variable microsatellite loci that have been identified previously were amplified and analysed from trapped possums and from pellet samples. Genotyping was carried out using the Genotyper 3.1 (Applied Biosystems) software.

In a demonstration field trial (in the Hokonui Hills and Catlins Forest) possums were trapped over 9 nights, and faecal pellets were collected immediately before and after trapping. By determining the extent to which DNA profiles obtained from faeces were represented amongst the trapped possums, we estimated the total number of possums recently present along the trpline.

Results
Our protocol for extraction of DNA from possum faeces involves purification of faecal homogenate through an ion exchange column with additional magnesium chloride to facilitate carrying out PCR amplification of fragments. Most possum DNA was concentrated in the outer layer of faecal pellets.

Recoverability of useable DNA was high for fresh samples, and was also high for field samples up to 27 days old if they had not been exposed to rain. Recoverability fell sharply with increasing exposure to actual or simulated rainfall.

In the field trial 4–10 possums were caught on each trpline over the 9 nights, with an overall mean 3-fine-night RTCl of 4%. High levels of variation in the genetic markers used (average of 8.3 alleles/locus) meant that each of the 41 trapped animals had a unique marker profile. A
total of 62 faecal extractions were attempted from pellets collected on four of the traplines; 17 failed completely, 3 produced insufficient DNA, and 42 were successful. For these four traplines, 56 separate profiles were identified from the total of 71 identifications from tail tips and faeces. Only seven (33%) of the 21 unique profiles from faecal samples collected prior to trapping were also identified amongst the profiles for trapped possums.

The number of possums using each trapline (as estimated from pre-trapping and trapping samples) ranged between 13 and 21, compared with a threefold variation in RTCI over the first three fine nights. The number actually trapped on each trapline over the first three fine nights ranged between 5% and 45% of the number of possums estimated to have been present immediately before trapping began.

Conclusions

Faecal DNA can be successfully recovered in sufficient quantity to permit the genotyping of possums. Obtaining possum genotypes from faecal DNA with the current methodology is likely to cost about $100 per sample, but larger-scale studies and/or more frequent use of the methodology are likely to reduce that cost.

For the Southland possums surveyed there was sufficient variation in the microsatellite markers used to permit complete and unequivocal separation of all 41 possums trapped.

Only one-third of possum genotypes identified as having been present recently on the trap lines were represented amongst trapped possums. Possible explanations are (i) that some alleles actually present were not identified (so called ‘allelic drop out’) creating apparently different combinations of markers for different pellets that originated from a single individual; (ii) trappability of possums was low because a high proportion of them spent little time at ground level at the time of the trapping; (iii) trappability was low at these two sites because some of the possums were trap shy; and (iv) trappability was low because possum use on a small part of their total range in any one night.

There are no major impediments to the immediate ‘operational’ use of this technology to address research and management questions about possum density. In particular the technology offers, for the first time, a practical way of assessing whether some proportion of a surviving possum population is untrappable. The main problem at very low possum densities is likely to be finding sufficient pellets.

Recommendations

• Further investment is needed to streamline the faecal DNA recovery process, to investigate the magnitude of any bias caused by allelic drop out, and to gather more data to determine the factors influencing the probability of identity P(ID) using faecal DNA (i.e. number of markers vs DNA quantity/sample).

• Two-dimensional (e.g. grid-based) sampling designs for collecting faecal pellets should be developed.

• As an extension of this proof-of-concept trial, the technology could also be used (in conjunction with trapping) to help monitor possum survival (if any) during the aerial poison operation currently being planned for the Hokonui Ranges.
1. Introduction

The Animal Health Board (AHB) commissioned Landcare Research, Lincoln, to determine whether it was feasible to uniquely identify individual possums from DNA recovered from their faecal pellets, and, if so, to conduct a field trial to show how the technology could be used to assess the trappability of possums, using the leg-hold traps widely used to monitor possum density. Development and testing of laboratory procedures for recovery of faecal DNA was completed in 2000/2001, and the field trial in 2001/2002.

2. Background

The AHB relies on leg-hold trapping as its primary tool for objectively monitoring the effectiveness of its possum control programmes (AHB 2000). As a result it is investing heavily in research to improve the reliability of the residual trap catch index (RTCI) as an index of possum numbers, both as a tool to assist research, and for setting targets and triggers for possum reduction and Tb management. This project forms part of that investment, and aims to develop a method of measuring absolute possum numbers in an unbiased way. This will enable the influences of biases in RTCI estimates to be identified quantitatively and, together with information from other AHB-funded RTCI projects, will ultimately provide the information to modify RTCI protocols accordingly to minimise such biases.

Estimates of absolute animal densities can usually only be obtained at great expense and are often heavily biased by variable trappability. However, faecal pellets are far more abundant than possums, and the probabilities of ‘capturing’ faeces from different individuals should not vary greatly, or, perhaps more importantly, should not be closely linked to trappability. An ability to identify individual animals from DNA in their faeces therefore has the potential to remove one of the greatest impediments to ecological research in general – an inability to confidently provide unbiased estimates of the absolute densities of animals (Kohn & Wayne 1997). The flow-on benefits that potentially derive from this will be better and more-efficient monitoring of possums, and other pests, and therefore better and more cost-effective management of vector populations.

The ability to identify individual animals from their faeces is made possible by recent advances in DNA genotyping technology (and most notably by the development of a suite of microsatellite markers for possums; Taylor & Cooper 1998) and the now almost routine recovery of useable DNA from herbivore and carnivore hair and faeces (Hoss et al. 1992; Litvaitis & Litvaitis 1996; Kohn et al. 1999; Mowat & Strobeck, 2000). In this project we aimed to further develop and apply these technologies to effectively ‘mark’ (i.e. uniquely distinguish) a sample of the possums present near a trpline, and then use trapping to catch a second sample of possums, the DNA from which would be used to determine how many of the trapped possums were also present in the ‘faecal’ sample. From this we used a simple mark–recapture analysis to estimate the absolute number of possums using the trpline during the survey period. This parallels similar use overseas of DNA technology to estimate the abundance of rare animals such as bears (Kohn et al. 1995; Woods et al. 1996,1999).
3. Objectives

To provide a new tool for assessing absolute animal densities in order to test and validate monitoring methods, by:

- developing a method of extracting DNA from possum faeces
- field-testing an approach combining DNA profiling with trapping to estimate possum numbers on trap lines.

4. Methods

We developed a protocol for recovery of DNA from faecal samples using faecal pellets collected from captive possums (Section 4.1). Once the requisite protocols and skills had been developed we conducted a weathering trial to assess the effect of pellet ‘age’ (time since deposition) on the recoverability of faecal DNA (Section 4.2). Finally, we conducted a ‘proof-of-concept’ field trial to show how faecal DNA could be used to investigate possum trappability (Section 4.3).

4.1 DNA methods

**DNA extraction**
Genomic DNA was extracted from 13.3–51.2 mg of tissue derived from possum tail tips using the BioRad AquaPure Genomic DNA Isolation Kit (Cat#732-6340) followed by resuspension in 100 μl of hydration buffer. Isolation of DNA from faecal pellets was carried out from between 71 and 125 mg of material, which comprised the outer layer peeled off with a sterile scalpel. Genomic DNA was then extracted from this peeled layer using the QiaAmp DNA Stool Mini Kit (Qiagen Cat# 51504) followed by resuspension in 100 μl of hydration buffer.

**Mitochondrial DNA amplification**
We used mitochondrial DNA (mtDNA) as a control in the initial trials to determine whether amplifiable DNA could be recovered from possum faecal pellets. This was due to the general stability of mtDNA and its presence in high copy number in genomic DNA extractions. PCR amplifications were performed in 25-μl reactions containing 1 μl of DNA extract from faeces, 1× PCR buffer with MgCl₂ (50 mM Tris/HCl, 10 mM KCl, 5 mM [NH₄]₂SO₄, 2 mM MgCl₂, pH 8.3), 200 uM each dNTP, 0.5 μl of 10 μM each primer, 0.2 mg/ml BSA and 1.5 U of FastStart Taq DNA Polymerase (Roche Diagnostics).

Primers used to amplify a 430 bp of the central conserved domain and right variable domain of the mtDNA control region were:

(i) **Tv3F**  5'-CACTAGCATATCATCACCAT-3'
(ii) **Tv3'R** and 5' TGTATCCATATTACCTT-3'.

Amplification conditions on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) were: Initial denaturation at 95°C for 4 min; 40 cycles of 40 s at 94°C, 40 s at 55°C, 40 s at 72°C, and a final extension of 10 min at 72°C.
Microsatellite amplification and analysis

Eight variable microsatellite loci that have been previously identified for possums (Taylor & Cooper 1998) were amplified and analysed from trapped possums and from pellet samples.

From the DNA extract derived from tail tissue, multiple × PCR amplifications were performed in 10-μl reactions containing 0.5 μl of DNA extract (25–75 ng/μl), 1× PCR buffer with MgCl₂ (50 mM Tris/HCl, 10 mM KCl, 5 mM [NH₄]₂SO₄, 2 mM MgCl₂, pH 8.3), 200 μM each dNTP, 0.4–0.6 μl of 10-μM each primer and 0.8 U of FastStart Taq DNA Polymerase (Roche Diagnostics). Amplification conditions on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) consisted of initial denaturation at 95°C for 4 min; 10 touchdown cycles of 30 s at 94°C, 45 s at 65°C–55°C, 45 s at 72°C; 25 cycles of 30 s at 94°C, 45 s at 55°C, 45 s at 72°C, and a final extension of 40 min at 72°C.

The PCR protocol was slightly modified for DNA recovered from faecal pellets. Multiplex PCR amplifications were performed in 10-μl reactions containing 3 μl of DNA extract, 1× PCR buffer with MgCl₂ (50 mM Tris/HCl, 10 mM KCl, 5 mM [NH₄]₂SO₄, 2 mM MgCl₂, pH 8.3), 200 μM each dNTP, 0.4–0.6 μl of 10 μM each primer, 0.1 mg/ml BSA and 0.8 U of FastStart Taq DNA Polymerase (Roche Diagnostics). Initial denaturation at 95°C for 4 min; 10 touchdown cycles of 30 s at 94°C, 45 s at 65°C–55°C, 45 s at 72°C; 30–35 cycles (depending on DNA quantity) of 30 s at 94°C, 45 s at 55°C, 45 s at 72°C, and a final extension of 40 min at 72°C. Depending on the amount of DNA recovered from faecal pellets, microsatellite primers were either multiplexed or amplified individually.

In both tissue- and faecal-derived DNA, the following sets of primers were combined to enable multiplexing within one PCR reaction:

Multiplex Set 1: TV16, TV53 (0.6 μl primer) TV54, TV64 (0.4 μl primer)
Multiplex Set 2: TV19, TV12 (0.6 μl primer) TV27, TV58 (0.4 μl primer).

The 5'-end of the forward primer of each pair was fluorescently labelled with either 6FAM, NED or VIC dyes (Applied Biosystems) and amplification products were separated using capillary electrophoresis (ABI PRISM 310). Alleles were sized relative to an internal size standard (GS-350 ROX) using GENESCAN 3.1 (Applied Biosystems).

Genotyping was carried out using the Genotyper 3.1 (Applied Biosystems) software. The poor quality and limited quantity of DNA available from some faecal pellets resulted in nondetection of some alleles (so-called “allelic drop out”). It was not always possible to distinguish between homozygosity and non-detection, so we took a conservative approach to assigning genotypes by classifying genotypes as unique only when the profile differed from all others at at least two loci and if the differences made sense in terms of expected heterozygosity levels and Mendelian inheritance.

4.2 Weathering effects on faecal DNA recoverability

Fresh possum pellets (1–2 days old) were collected from 11 different possums housed in the animal facility at Landcare Research. The pellets were placed on the ground under a grove of kowhai trees with a numbered flag marking the site for each possum’s pellets. A sample of 2–5 pellets from each possum was taken at weekly intervals, placed in a plastic bag, frozen, and analysed for the presence of DNA. No rain fell for the first 27 days but there was constant drizzle or rain after this period up until the final sampling at 44 days.
A test using simulated rainfall was also conducted using a further sample of fresh possum pellets taken from 10 possums in the animal facility. These were placed on a lawn and exposed to increasing amounts of simulated rain from a garden sprinkler. A rain gauge was used to measure the amount of water falling on the pellets and a sample of 4–5 pellets from each possum was taken after 0, 8, 20, 40, 60, 100, 140, 180, 220, 400, and 640 mm of simulated rainfall. Samples were placed in separate plastic bags and frozen until they could be analysed for the presence of DNA.

To help define field criteria that would maximise the probability of recovering faecal DNA, faecal pellets were collected from a forest site on Banks Peninsula. Pellets were subjectively classified into five nominal age classes: fresh and glossy (thought to have been deposited no more than a few days previously), dull ‘semi-glossy’ (0.5–2 weeks old), matt (>2 weeks old), rough (some surface loss due to rain, but less than 2 weeks old), and disintegrated (significant material and shape lost, age not estimated). Up to 20 pellets from each group were collected and the proportion of each group yielding usable DNA estimated. If the first 10 pellets from each group all yielded usable DNA, no further pellets were tested.

4.3 Field trials

A field trial was first attempted at Maungatautari, Waikato, in spring 2001, in conjunction with another AHB-funded project ‘Effect of habitat, season, trap shyness and timing on RTC estimates’ (Contract R10506), but was eventually abandoned because very few fresh faecal pellets could be found (despite mean RTCIs of about 5% being recorded; D. Forsyth, pers. comm.). In an attempt to identify why pellets were scarce we assessed short-term pellet disappearance rates on two occasions. First, 30 groups of three faecal pellets (taken from killed possums) were placed 20 m apart along a transect, and checked 1 day later. Later, seven lines of 20 plots spaced 20 m apart were established with single pellets placed on the ground beside a wire spoke at each site. The pellets were checked for any disturbance or presence of any rat faeces each day for 2 days. Video cameras were set up at three sites to monitor piles of about 20 possum pellets (two groups of 10 pellets, about 50 cm apart). Rat abundance was also monitored with tracking tunnels placed at every third trap along the pellet lines.

A second field trial was subsequently conducted during February–March 2002 in two native forest areas (Hokonui Hills and Catlins Forest) where there were low densities of possums (about 5% RTCI). Three lines of 20 leg-hold traps placed 20 m apart along 400-m tranlines were set for 9 nights in each of the two areas, in an attempt to capture all trappable possums using those tranlines during that period. Possums caught were killed, the age, sex and site noted, a jaw taken for future ageing, and the tail tip collected for DNA analysis.

Faecal pellets were removed from the rectum of possums caught on the first day of trapping and placed at readily re-locatable positions near trap sites. This was to provide a sample of known-age pellets that we could use to help visually standardise and validate our classification of ‘wild’ pellets of unknown age.

Immediately before (on the day traps were set), and immediately after (on the days traps were lifted) trapping, about 10–20 faecal pellets subjectively classed as ‘fresh’ (nominally less than 9 days old) were collected from along belt transects 20 m wide centred on each of the tranlines. Samples were placed in separate plastic bags and labelled with the nearest trap
location. The search for pellets involved looking in the most open and best sites under favoured food trees, or along obvious trails. We tried to ensure that two pellets were collected from different locations for every 40-m section of trapline but this was not always achievable. Where more than two pellets were found in a 40-m section of trapline, only the two freshest pellets were kept, with older pellets being discarded as fresher pellets were found. All pellets and animal samples were frozen until analysed. DNA was extracted from the tail tips from possums from all six traplines, but we could only analyse faecal pellets from four of the traplines (two from each area) with the resources available.

The number of unique genotypes represented in the faecal samples before and after trapping and amongst the trapped possums was determined. We then estimated the number of possums using the trapline. For this we used a modified Lincoln Index, using the formulas originally given by Chapman (1951; as cited in Pollock et al. 1990):

\[ N = \left[ \frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)} \right] - 1 \]

\[ 95\%CI = 1.96 \times \sqrt{\left( \frac{(n_1 + 1)(n_2 + 1)(n_1 - m_2)(n_2 - m_2)}{(m_2 + 1)^2} \right)} \]

where \( N \) = total number of possums likely to have been present within 10 m of the trapline during the trapping period or about 9 days prior to that, \( n_1 \) is the number of distinct genotypes in the pre-trapping faecal sample, \( n_2 \) is the number of distinct genotypes amongst the animals trapped (which in all instances was the same as the number trapped), and \( m_2 \) is the number of genotypes from trapped possums that were represented in the pre-trapping faecal sample. Separate estimates of the same parameter were calculated using the pre- and post-trapping samples, or the trapping and post-trapping samples, as the ‘mark’ and ‘recapture’ samples. There are likely to be some biases in these latter estimates (see Results section), and more sophisticated mark-recapture analyses could be used to account for these, but, in the context of this trial, the use of Lincoln Indices is adequate for demonstrating proof of concept.

5. Results

5.1 Recovery of faecal DNA

A protocol for extraction of DNA from possum faeces was successfully developed. The process requires purification of faecal homogenate through an ion exchange column with additional magnesium chloride to facilitate PCR amplification of fragments. Experience showed that most possum DNA was concentrated in the outer layer of faecal pellets. Typically about 60 mg of the outer layer of faecal pellets were required to successfully amplify a region of the mitochondrial DNA. By using only the outermost layer of material from each pellet we were able to consistently obtain usable quantities of DNA from all completely fresh pellets obtained from captive animals.

5.2 Weathering effects on faecal DNA recoverability

Usable DNA could be recovered from all five samples analysed from those that had been placed out in the field for a 27-day period with no rain. No usable DNA could be recovered
from any of the seven pellets analysed from that that had been exposed for 44 days in the same trial (rain fell between day 27 and day 44).

DNA was also extracted and microsatellite markers amplified from all 10 pellets exposed to up to 180 mm of simulated rain. At 400 mm, all 10 samples yielded DNA but microsatellite markers were successfully amplified from only three of them. Nine out of 10 pellets receiving 600 mm of simulated rainfall samples also yielded some DNA, but no amplification of microsatellites was achieved from any of them.

For pellets collected from native forest, DNA was recovered from all 10 pellets classified as fresh and ‘glossy’ (fully intact pellets thought to have been deposited no more than a few days previously) and also from all 10 classified as ‘semi-glossy’ (fully intact but with a dull surface, perhaps 0.5–2 weeks old) but from only about half the pellets in the three other categories of more disintegrated or (nominally) older pellets.

The results suggest that DNA will be generally recoverable from most intact pellets that are less than 2–4 weeks old provided there has been no heavy rainfall (i.e. <200 mm).

5.3 Field trials

Maungatautari

As already noted, it proved extremely difficult to find fresh faecal pellets at Maungatautari, apparently due to their rapid disappearance. In the first disappearance trial, 19 of 30 groups of three pellets had one or more pellets missing completely after 1 night, and four groups of three were completely gone. In the second disappearance trial, 49 of the 140 pellets placed near spokes appeared to have been disturbed or covered after just 2 nights; 4 pellets could not be found, 18 had been partly or wholly covered by litter, and the remainder (27) had apparently been moved away from the spoke. The first of the two nights was fine, but the second was not, and the trial was abandoned. The mean rodent tracking rate (recorded only on the second night) was 5%, far lower than expected (possibly because of the heavy rain on that night). Rats, possums, and cats were recorded on the videos, but no interactions with the pellets were observed. However, one pellet disappeared from one of the monitored sites (agent not detected) and four pellets were moved 3–30 cm from the pellet pile at another (agent again not detected).

Hokonui/Catlins

Mean 3-fine-night RTCI for the three Hokonui traplines was 3.9% (± 3.8% 95% CI) with 4, 5 and 10 possums caught, respectively, on each line over the 9 nights. Mean 3-fine-night RTCI for the three Catlins traplines was 4.5% (± 6.1%) with 5, 5, and 10 possums caught, respectively, on each line. A high catch rate was recorded on the first night, but on all subsequent nights the catch rate was low but more or less constant, indicating that further trapping would likely have continued to catch additional possums (Fig. 1)

Extraction of DNA from the tail tips was straightforward and reliable. High levels of variation in the genetic markers used (average of 8.3 alleles/locus) made individual identification easy, with each of the 41 possums trapped able to be assigned a distinct genotype.

It was more difficult to reliably extract sufficient DNA to carry out the required PCR reactions from the faecal pellets from along traplines. A total of 62 extractions were
attempted; of these 17 failed completely, 3 produced insufficient DNA to assign an identity, and 42 were successful. Later extractions were less time-consuming than initial ones, but this was at the cost of slightly reduced success, i.e. we analysed more samples rather than wasting effort attempting to overcome low DNA yields in initial extractions. Experience showed that repeating the extractions in such cases seldom provided enough DNA to obtain a usable profile.

![Graph showing trend in RTCl over nine nights.](image)

**Fig. 1** Trend in RTCI over nine nights, showing the RTCI for each individual night, averaged across the four traplines used for faecal DNA analyses, and the cumulative total RTCI for that night and all previous nights (square symbols).

For the four traplines for which both ‘trapped’ and ‘faecal’ DNA profiles are available, a total of 56 unique profiles was identified from a total of 71 profiles (i.e.; 15 faecal profiles matched other faecal profiles). Of the 56 unique profiles, 21 were recorded in pre-trapping faecal samples, 29 in trapped possums, and 18 in post-trapping faecal samples (Table 1, Fig. 2). Overall, 26 profiles identified from faeces were not recorded amongst trapped possums, while 17 of the trapped possums were not identified among the faecal profiles, indicating a strong likelihood that some additional possums were not detected by either trapping or faecal DNA. An estimate of that number is provided by a Lincoln Index comparing pre-trapping faecal profiles with trapping profiles: seven (33%) of the ‘pre-trap’ possums were trapped (Fig. 2), implying that 9 nights of trapping caught only one-third of the possums that had been present on the trapline at some time during the undefined period before and including trapping, i.e. the total ‘population’ using the four traplines during that time was about 82 ± 37 possums (Table 1), suggesting that 26 possums had not been detected. The 95% CIs around the estimates for individual traplines ranged between 30 and 82% of the estimate, and were highest for the traplines with few or no recaptures.

Of the trapped possums, only five were recorded amongst the 18 profiles identified from post-trapping faecal samples (Fig. 2), implying that trapping had accounted for 28% of the population. It is possible that this estimate is biased low by the rainfall that fell during the 9 nights, as that rainfall would have made it more difficult to find, and then recover DNA from, faecal pellets from possums that were trapped (and removed) early in the study. Consistent with this, only 17% of the post-trapping faecal profiles matched pre-trapping faecal profiles. As a consequence the Lincoln Indices generated by comparing pre- and post-trapping profiles and by comparing trapping and post-trapping profiles were generally higher than those from the comparison of pre-trapping and trapping profiles. Note that each index estimates possum usage of the trapline over slightly different periods, so are not necessarily estimating exactly the same parameter.
Table 1 Numbers of individuals identified from faeces and trapping (for four traplines, and in total) and of 'recaptures' between pairs of samples. Lincoln indices for each of the sample pairs are compared with trapping data from each trapline and overall. For each trapline, the first column represents the faecal pellet sample collected before trapping, the second column represents the trapped possums, and the third column the faecal pellet sample collected after control.

<table>
<thead>
<tr>
<th></th>
<th>Hokonui 1</th>
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<th>Hokonui 3</th>
<th></th>
<th>Catlins 1</th>
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<th>Catlins 2</th>
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<td>21</td>
<td>82</td>
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<td>RTCI: First 3 fine nights</td>
<td>1.7%</td>
<td>8.3%</td>
<td>3.3%</td>
<td>10.0%</td>
<td>5.8%</td>
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<td>RTCI: 9 nights (%) trapped (First 3 fine nights)</td>
<td>2.2%</td>
<td>5.6%</td>
<td>2.8%</td>
<td>5.6%</td>
<td>4.0%</td>
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\(^1\) Pre recaptures = number of possums identified from the pre-trapping faecal samples that were subsequently identified amongst trapped possums or, separately, in the post-trapping faecal sample

\(^2\) Trapped recaptures = number of profiles from trapped possums that were also found in post-trapping faecal samples.
Fig. 2 Distribution of genotypes. The location of faecal pellets or possums for which a DNA profile was obtained is shown for each of the 400-m-long transects, using the number of the nearest trap site – traps were numbered sequentially 1–20. For each trapline, the first column represents the faecal pellet sample collected before trapping, the second column represents the trapped possums, and the third column the faecal pellet sample collected after control. Each unique genotype has been assigned a different letter, with genotypes ‘captured’ more than once connected by the dashed lines.

The number of possums using each trapline (as estimated from the proportion of matches between pre-trapping faecal profiles and trapping profiles) ranged between 13 and 21, compared with a threefold variation between lines in the RTCI for the first three fine nights (Table 1). There was no clear relationship between the estimated number and RTCI although the line with highest RTCI had the highest estimated number of possums present (which is the expected relationship). The number of possums actually trapped over the first three fine nights ranged between 5% and 46% of the number estimated to have been present immediately before and during trapping (Table 1).

There were no identifications that did not make sense – none of the profiles from one area or trapline matched any of those from the other area or trapline. Further, all the faecal profiles that matched trapped profiles were collected within 180 m of where the possum was trapped. This strengthens our belief that there was sufficient variation at the eight loci assessed to uniquely identify all possums. The scant data also suggests that few possums in these areas range over areas larger than 200 m in diameter.
6. Discussion and Conclusions

6.1 Faecal DNA extraction

Faecal DNA was successfully recovered in sufficient quantity to permit the genotyping of possums. The faecal extraction process was relatively time-consuming in this study, partly because we initially spent a lot of time and effort trying to recover DNA from every sample. With increasing experience, however, laboratory staff became increasingly adept at deciding which extractions were likely to be successful.

Using the above process, we estimate that 50 samples would take 40 hours of technician time to process and a material cost of $28/sample. We believe that obtaining possum genotypes from faecal DNA with the current methodology will be achievable at a cost of less than $100 per sample. Larger-scale studies and/or more frequent use of the methodology should create substantial economies of scale and reduce this cost.

For the Southland possums surveyed there was sufficient variation at the eight loci for which we currently have microsatellite markers to permit complete and unequivocal separation of all 41 possums trapped. This indicates that there will be only a small or zero downward bias in estimating the numbers of possums from the number of distinct genotypes (as a result of different possums having the same set of alleles for this set of eight markers). Increasing the number of markers will further reduce that small potential for bias.

One potential limitation to the use of faecal DNA is the difficulty we found in locating faecal pellets at the Maungatautari site. On two separate occasions we observed interference or relatively rapid disappearance of fresh pellets placed at marked sites, suggesting strongly that this was the underlying cause of the lack of pellets. Because rat densities at Maungatautari are thought to be high, we suspect rats were the cause of the interference, but were unable to confirm that. If rats do affect the disappearance rate of possum faecal pellets, estimates of possum abundance based on faecal pellet counts may be seriously and unpredictably biased, depending on rat abundance and behaviour.

6.2 Possum trappability and density assessment

We expected that by trapping for 9 successive nights we would have caught most of the possums using the trapline, even though some rain (mostly light) fell on 4 or 5 of the nights, depending on the area. Our results suggest, instead, that most possums that had recently been present on, or within 10 m of, the traplines were not trapped, in both the Hokonui and the Catlins sites. That is puzzling.

Possible explanations are:

(i) The number of genotypes identified from faecal pellets may have been substantially overestimated because the comparatively poor-quality DNA from faecal samples sometimes may have resulted in some alleles actually present not being identified (allelic drop out). This could potentially create apparently different combinations of markers for different pellets that originated from a single individual. Kohn et al. (1998) assessed the importance of this bias by independently genotyping two samples from each of 59 coyote faecal samples. They found a genotyping error of about 0.05
per locus, but concluded that that did not substantially bias their overall estimates of population size.

(ii) Trappability of possums was low at these two sites because many of them spend little time at ground level at the time of the trapping. Trapping or any other ground-based control is likely to selectively remove the possums that spend the most time at ground level. Ground-based trap-catch monitoring would then overestimate the proportion of the population removed. Further, if survivor behaviour subsequently changed so that more of the survivors spent more time at ground level, then RTCIs are likely to increase through time even if there was no change in possum numbers.

(iii) Trappability was low at these two sites because some of the possums were trap shy.

(iv) Trappability was low at these two sites because possum home ranges are large and possums used only small parts of their range on any one night but much larger portions over a nine-night period.

We have no way of distinguishing between these explanations (and all four could apply simultaneously), but speculate that the second may be the most important, primarily because it fits with the increasingly frequent observation that RTCI increases far more rapidly after control than can be explained by breeding or immigration (Nugent et al. 2001). Such increases would appear unlikely if trap shyness were the cause, as toxin shyness, at least, is long lasting (Morgan & Hickling 2000).

6.3 Feasibility of the technology

This study confirms that it is technically feasible to recover faecal DNA and individually identify possums from it. Although the investigation of trappability was, effectively, an ancillary ‘demonstration’ objective, the outcome highlights the potential power of faecal DNA recovery in helping overcome the otherwise intractable problem of attempting to monitor the abundance of animals that are not readily trapped.

There are no major impediments to the immediate ‘operational’ use of this technology to address research and management questions about possum density, particularly questions that relate to whether or not some possums are not being detected during, or are surviving, control operations. In particular, the method may provide a simple way of assessing the magnitude of post-control bias (if any) in RTCI, by comparing the ratio of possums trapped to the number present (as identified from faeces) immediately following, and then 6 and 12 months after control.

It also provides a completely non-invasive way of assessing possum density from one sample taken on a single day, because with any sample of possum pellets, some individuals will be ‘captured’ once, others twice and others more than twice, and the frequencies of capture can be used to calculate actual densities (Kohn et al. 1998). This will require two-dimensional sampling regimes (such as sampling grids), rather than the one-dimensional trapline used in this method. When possums are abundant the high density of animals present means that large numbers of pellets would need to be identified to have useful recapture rates, but for extremely low density populations, the paucity of animals becomes something of an advantage. For example, at 1% RTCI (thought to equate to 0.1 possums/ha) a 100-ha area would contain only 10 possums, and a random sample of 20-30 pellets from throughout the area would provide a reasonably precise estimate of possum density. The main problem is likely to be finding that number of pellets, and we have no experience yet to judge how difficult that might be.
7. Recommendations

Further investment is needed to streamline the faecal DNA recovery process, to investigate the magnitude of any bias caused by allelic drop out, and to gather more data to determine the factors influencing the probability of identity $P(ID)$ using faecal DNA (i.e. number of markers vs DNA quantity/sample). We propose that this be done as part of a larger project aimed at applying the technology to particular research or management problems.

Two-dimensional (e.g. grid-based) sampling designs for collecting faecal pellets should be developed, using either existing models developed to simulate possum trapping, or new designs for estimating local possum density. These designs should then be field-tested, ideally to address high priority questions about the reliability of RTCI.

As an extension of this proof-of-concept trial, the technology could also be used (in conjunction with trapping) to help monitor possum survival (if any) during the aerial poison operation being planned for the Hokonui Ranges.

8. Acknowledgements

We thank Chris Brausch (Landcare Research) and Mark Hunter, Lyndon Dynes and Randal Beal (Environment Southland) for helping with the organisation of the main field trial and for running the trapping lines. We also thank Jackie Whitford, Neil Fitzgerald and Steve Hough (Landcare Research) for attempting the initial trial at Maungatautari. Andrea Byrom and Stephen Ball provided useful comments on early drafts.

9. References


Hi Tamsin,

Please find attached the files for sections 1 to 5.

A few points to note:

1. I am sending the CVs in another email.

2. I am having problems seeing some of the documents to 100%. There appears to some type of glitch in the way my email is reading them. Could you please re-set them from 150% to 100%.

3. I would very much appreciate it if you (or someone who knows what they are doing) briefly checks these documents for any basic errors/omissions. I realise the "deal" isn't that your edit/proof-read them - I am not expecting that - just basic administrative stuff.

Thanks for all your help. Please don't hesitate to contact me if you have any queries etc.

Regrades,

Murray Patterson
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**Funding Requested**

|----------|---------|---------|---------|---------|---------|---------|
1.1 Executive Summary
This research will enable cites and regions to plan for Sustainable Development, by providing them with authoritative information on alternative future development scenarios. These scenarios will seek to illuminate and link the economic, social and environmental dimensions of Sustainable Development. The scenarios will project changes in time frames ranging up to 30 years, using user-friendly dynamic models. The scenario development process will be evidence based, relying on detailed and well researched empirical data, which is often spatially referenced. This process will also be inclusive and collaborative, building a common understanding of the sustainability options amongst stakeholders.

Three linked objectives are designed to aid the decoupling of economic growth and social progress from environmental harm: (1) Urban Metabolism: The aim of this objective is to understand the biophysical functioning of cities in New Zealand. This should directly lead to a number of insights into the eco-efficiency and sustainability of urban systems. Two case studies are planned (Auckland and Christchurch), emphasizing issues such as water use and solid wastes; (2) Scenario Development: The aim of this objective is to build on the first objective by providing a wider socio-economic framework (extended metabolism model) for developing city and regional scenarios. Special emphasis will be placed on understanding the socio-economic dimensions of change and how these affect the metabolism of cities and environmental impacts. A spatial-dynamic modelling capacity will be developed particularly to assist in detailed planning of Sustainable Development pathways; (3) Indicators of Progress to Sustainable Development. This objective focuses on developing indicator and reporting systems that can guide and monitor progress to Sustainable Development. Overall measures of sustainability performance will be derived and advocated. This objective will be undertaken in close collaboration with SNZ and MFE programmes.

The benefits that stem from this research are primarily environmental but there are also significant interconnected economic and social benefits. The urban metabolism approach will identify ways of reducing the mass throughput of urban and regional systems in New Zealand. It is calculated that if our research leads to only a 1% yr reduction in mass throughput in Auckland and Christchurch, then this will capture $49 million yr of environmental benefits (less global warming, less eutrophication, less resource depletion and so forth). Our research will also identify ways of optimising the environmental benefits gained from better use of ecosystem services, particularly as they relate to land use change. The triple-bottom line approach of the research guarantees that economic and social benefits will also ensue. For example, in our case studies of Nelson-Tasman and with Ngati Raukawa, regional development benefits (income, jobs) will be a focus. Commercial marketing benefits also arise from enhancing New Zealand's clean green image and from business opportunities that will be identified in our urban metabolism analysis.

To date, research efforts in New Zealand in the above areas has been fragmented and the level of capability is limited. A major aim of this research (and two linked proposals) is therefore to build up capacity and develop a critical mass of expertise in this general area of ecological economics. The research will be undertaken by a team drawn from Landcare Research Ltd, Resource and Environmental Planning Programme (Massey University) and Market Economics Ltd. All senior members of the team are very experienced in interdisciplinary policy related research and have excellent track records in interacting with end-users and communicating the results of their research to a variety of audiences. Strong overseas research links will also be utilised. We will particularly work closely with the Resource Futures Group (CSIRO).
2. Proposed Research Programme

2.1 Strategic Intent

Outcome
The outcome that this proposal seeks to contribute to is the "decoupling of economic growth and societal progress from environmental harm". This will be achieved by developing information, tools of analysis and insights concerning the "interconnection" of economic activity and environmental impact. Only when these interconnections are fully understood can policy-makers and planners, understand how policies and actions can be put in place to promote decoupling.

Research Vision
The thinking that drives this Programme is that cities and regions need to be able to visualise future (sustainable) development pathways in an integrative fashion. This will lead to an awareness of future sustainability issues which will "connect" the economic, environmental and social dimensions of change. No single tool can achieve this integration of all of these factors – instead a collection of tools and approaches are required. "What if" scenario modelling will be one of the main approaches used in this Programme, which has particular strengths in linking economic and social activity to environmental impacts, as well as having the ability to track changes over a 20-30 year time-frame. Urban metabolism, ecological footprinting, ecosystem services and integrative indicator systems are other approaches that will also be used in this research. Cities and regions have a variety of growth and development issues, a few of which have been selected to develop this "futuring" capability. These issues include: water, solid wastes, energy, tourism, transport and water quality.

Research Context
There has been a fragmented and disjointed approach to research in this area in New Zealand and certainly no concerted funding by FRST. Through discretionary funding by Landcare Research and Sustainable Management Fund support, our team has constructed integrated Economic-Environmental Accounts for the Northland, Auckland and Waikato regions. An earlier version of these Accounts is available on the EcoLink database. These Accounts provide a starting point for our research. However, the modelling and scenario development capability required for this research is poorly developed. The Parliamentary Commissioner for the Environment (2000) accordingly states "like the development of national scenarios, the development of urban visions and scenarios that recognise and address sustainable development have been limited". The PCE (2000) adds "there is virtually no capacity to develop sustainability models that incorporate social, economic and cultural elements". There are however a number of examples of this type of policy modelling – Robbert Associates (Canada), RIVM (Netherlands) and CSIRO (Australia) which provide useful templates for future developments.

Indicator development in New Zealand although quite extensive, hitherto has not really focussed on integrating the economic, environmental and social dimensions of Sustainable Development (refer to Patterson, 2002 and SNZ, 2002).

Alignment with Strategies
Strategic Portfolio Outlines This research will make a strong contribution to the outcomes outlined in the Sustainable Cities and Settlements SPO. At a strategic level the research will specifically "develop mechanisms that will simultaneously
address both social/economic imperatives and environmental imperatives of sustainable human settlements” (SPO, p.3); and will investigate “resource use efficiency, decreased overall demand on resources, and the decoupling of economic growth from increased resource consumption” (SPO, p.4). In terms of the strategic objectives outlined in the SPO, this research will have a strong focus on the “Improved Eco-Efficiency of Settlements” directly addressing many of the points outlined in this SPO objective. A significant contribution will also be made to the “Development of Ecosystem Integrity within Settlements” Objective, particularly through our linked “Ecosystem Services” Proposal. The systems based and interdisciplinary research being put forward in our proposal, very much follows the “holistic, integrated, systems oriented” approach, by not only connecting the economic, social and environmental factors but also by seeking to understand the interconnections of urban behaviour in the broadest sense. This is exemplified by our “whole-of-system” approach to energy and material flows in urban systems, that attempts to move beyond the “end-of-pipe”/“effects based” approach.

**Government Strategies**

Our research will contribute to achieving the goals of the Government’s Sustainable Development Strategy. As stated in *The Government Approach to Sustainable Development* one of the main priorities is “decoupling of economic growth from environmental harm” – ascertaining how this can be achieved is one of our main research goals. The government’s *Programme of Action for Sustainable Development* nominates four priority areas for immediate attention – three of these (water quality and allocation, energy and sustainable cities particularly Auckland) are direct foci of our research programme. Our proposed research will also: (1) assist the *New Zealand Waste Strategy*, particularly in pinpointing how materials can be used more efficiently and in providing a systematic information base on levels of waste and progress to waste management goals; (2) address two of the challenges outlined in the *Ministry for the Environment’s draft Research Strategy* – viz, “decouple environmental processes from economic growth” and “improving information for measuring progress (to Sustainable Development)”; (3) support the *National Energy Efficiency and Conservation Strategy*, specifically with respect to Goal 1 (reduce CO₂ emissions) and the high level target of “energy efficiency”.

**Local Government Act 2002**

Local Government’s role following the new Local Government Act will be a key driver for Sustainable Development, through developing community outcomes and long term community plans. This will require the formulation of alternative future pathways (scenarios) to achieving sustainable development in their area.

**Linked Proposals**

This proposal has strong links (in both directions) with other proposals being put forward by the Institute of Ecological Economics and Landcare Research Ltd. Some of these links are critical. Please refer to the linking document for further information.

## 2.2 Potential Benefit to New Zealand

### 2.2.1 Expected Benefits for New Zealand

**Environmental Benefits**

*Overall Environmental Benefit Gained by Reducing Material Throughput* There is now mounting international pressure to move away from “end-of-pipeline” environmental management, to a more holistic approach. The central argument put forward by organisations such as the Wuppertal Institute (Germany) is that society must reduce its *material throughput* if its environmental impact is to be reduced. Reducing material throughput in urban and regional systems is important as it
simultaneously reduces resource depletion ("front of pipeline effects") and pollution impacts ("end of pipeline effects"). This leads to a range of environmental, economic and social benefits, which are crucial ingredients for improving the sustainability performance of cities and regions.

The environmental benefits to be gained by reducing mass throughput are large and difficult to estimate with any certainty. They are also highly interconnected which leads to double-counting problems in estimating total benefits. A summary of the indicative savings to be made by reducing material throughput is however put forward by the following table:

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<th>Savings from Reduced Throughput</th>
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<tr>
<td>Description</td>
<td>Flow Rate (tonnes/yr)</td>
<td>Description of Main Impacts</td>
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<tr>
<td>Landfill Wastes</td>
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<td>water &amp; soil contamination, global warming, odours, amenity loss, numerous lifecycle impacts before discarded product reaches the landfill</td>
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<td>acidification, human health, ozone depletion</td>
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<tr>
<td>Water Use (Not Hydro)</td>
<td>1,490,000,000</td>
<td>minimum flows, habitat loss, in-stream values affected, resource depletion (e.g. aquifers)</td>
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<tr>
<td>Total</td>
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Based on this tabulated data, if it is conservatively assumed that our research leads to only a 1% reduction in mass throughput in Auckland and Christchurch, there will be $49 million/yr worth of environmental benefits.

Specific Environmental Benefits: The main material flows that are expected to be reduced (and associated reduction in negative environmental impacts) are:

- **Landfill Waste**: It is estimated that there is 3,400,000 tonnes/yr of landfill wastes in New Zealand, most of which occurs in urban areas. The main environmental impacts are: water and soil contamination (from leachates), amenity loss, odours and global warming (from fugitive CH₄). One reputable American study found that $US100 of environmental benefit results from each tonne of solid wastes eliminated from the production system. On this basis, if our research resulted in solid waste flows in Auckland and Christchurch being reduced by just 1%/yr, then this would deliver an environmental benefit valued at $3.8 million/yr.

- **Water Pollutants**: Our research is expected to deliver efficiencies in reducing the loading of water pollutants particularly in urban areas, with our linked FRST proposal targeting water pollutants in the rural productive environment. Water pollutants in the urban environment cause a number of negative environmental

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1 A 1% reduction in mass throughput is conservative, given that studies have consistently shown that much larger improvements are possible — eg, Adriaanse et al.'s (1997) four country study.
impacts - Mosley (1992) estimates the economic cost of these impacts to be $960 million/yr for the urban environment, and a similar estimate is derived by extrapolating Australian studies by NIER (1996) and Hamilton (1997). If our research resulted in urban water pollutants being reduced by 1%/yr, this would deliver an environmental benefit valued at $9.6 million. Through our regional studies (Waikato, Nelson-Tasman) we would also expect some improvements in rural water pollutants.

- **Fossil Fuels and CO₂ Emissions**: Urban systems are highly dependent on the (unsustainable) consumption of fossil fuels and the resultant CO₂ emissions. It is critical for urban sustainability that throughput of fossil fuel be reduced and replaced by more sustainable and efficient energy inputs. Based on unit cost data from the OECD (1996), the impact of global warming resulting from CO₂ emissions in New Zealand, is costed at $2,650 million per annum. If our research could reduce fossil fuel consumption and greenhouse gases by 1% in Auckland and Christchurch, then this would deliver an environmental benefit valued at $10 million.

- **Other Air Pollutant (SO₂, NOₓ, volatile organic compounds, lead, particulates)**: The adverse effects of these air pollutants on human health, property and biota in urban New Zealand is costed at $1,000 million/yr, based on extrapolated data from Hamilton (1997).

- **Water Use**: Reducing the demand for water is one of the key focuses of this study that will result in both environmental and economic benefits. A lifecycle assessment approach is expected to uncover opportunities for reducing water usage. The environmental costs (loss or degradation of habitat for flora and fauna, decline in conservation values etc) are estimated to be $960 million for non-hydro water use.

**Sustaining and Utilising Ecosystem Services**: Complementary to the urban metabolism approach, will be the ecosystem services focus of our research. Ecosystem services are services (eg, water supply, waste treatment, habitat provision) provided by nature to humans. It is expected that a characteristic profile of the ecosystem services in each case study region, will point to opportunities to better manage ecosystems and deliver environmental benefits. The examination of rural land-use changes (in the Waikato and Tasman-Nelson case studies) will particularly benefit from such an analysis. For example, pastoral farming and forestry provide different ecosystem service profiles and deliver different levels of benefit – in terms of the delivery of ecosystem services, is it better to afforest a catchment as opposed to it remaining in pastoral farming? The ecosystem services approach may also provide insights into how to better manage urban environments. For example, natural ecosystem services are often under-utilized resources in areas such as the treatment of urban wastes and sewage. Or what will it cost in terms of lost ecosystem services if the eastern extension of the Auckland motorway goes ahead? Non-market valuation methods will enable us to put a monetary value on the cost of losing these ecosystem services and to thereby allow us to directly compare this loss with the economic benefits and costs of the motorway extension. Even if only a very small proportion of the terrestrial ecosystem services (valued at $44 billion) in New Zealand could be better utilised as a result of our research, this will deliver a very significant environmental benefit.

**Economic Benefits**

**Business Development**: The commercial case for reduced mass throughput has been strongly made by the advocates of dematerialisation and Factor 4 approaches – eg, by Weizsäcker, Lovins and Lovins (1997). The before-mentioned study chronicles 50 case study examples of successful businesses built around resource-use efficiency. In New Zealand, there are quite a number of examples of businesses that have improved their energy efficiency, resulting in cost reductions and other commercial benefits (EECA, 1998). Perhaps, the most significant example of improved resource use efficiency is Electricorp which reduced the water flows
through its hydro-dams by 10% for commercial benefit, as well as environmental benefits. Our research should play an important role in prospecting for opportunities for improved resource use efficiency in New Zealand, many of which can be realised through our strong linkages to policy developers, end-users and businesses.

**Economic Efficiency and Macro-Economic Impacts:** Interventions stemming from the RMA, have impacts on the economy second only to tax law interventions. Resource management policy decisions although intended to achieve environmental outcomes, inevitably have an economic impact. The scenario modelling and policy analysis tools developed in Objective 2, are designed to provide decision makers with information on the trade-offs between economic and environmental policy objectives, so that unnecessary economic costs can be avoided or at least minimised.

**Reduced Compliance Costs:** There is ongoing concern by business of the level of compliance costs in the administration of the Resource Management Act 1991. Provision of better quality information and a wider information base, is recognised as a key mechanism in reducing compliance costs. The authoritative databases on environmental impacts (linked to economic activity) generated in our research could have a role to play in this process of improving information.

**Marketing Benefits:** The *Clean Green* image of New Zealand has commercial value - it helps sell our produce on world markets. This commercial reality has been underscored recently by a study by Thornton, Paul and Kerr (2001), who for example show that a declining perception of our clean green image by overseas consumers may reduce annual profits and income in the Dairy and Tourism industries by up to $1 billion. By significantly reducing mass throughput and thereby improving environmental quality in New Zealand, this should help future-proof our clean green image and the commercial advantages it brings.

**Social Benefits**

The integrated scenario modelling approach will make policy analysts/communities aware of the social consequences of policy options which could be pursued - eg, if land is converted to forestry, how many jobs will be generated and what does this mean for the indicators of social well-being. In all of the scenario modelling projects, a significant emphasis will be on ensuring that social goals are optimised as well as economic and environmental goals. Depending on the context of the study, social goals, social cohesion, employment may have greater weighting than in others. At this stage it appears that in the Ngati Raukawa and the Nelson-Tasman projects, it is likely that social goals will be highlighted. We will also build on the Auckland Regional Economic Development Strategy (AREDS), which seeks key social outcomes from, and in parallel, to the achievement economic development goals.

The approach will draw from and complement local stakeholder knowledge and experience. It will provide a forum for developing a shared vision of a desired future. In the process, understanding and relationships will be deepened, contributing to the development of social capital (ie, strengthening sustainability networks amongst key players in Government, business, iwi and the community).

The purpose of the scenario and indicator development is also to motivate change in behaviours and policy action, by bringing to light the social dimension of Sustainable Development, which is often overlooked.

**We will work with Ngati Raukawa to explore development scenarios for the iwi, which whilst having a strong economic and social development focus, will also be respectful of tikanga, mauri and other Maori principles of natural resource management.**