



# Methods for marking New Zealand wildlife

Amphibians, reptiles and marine mammals

Ngaio J. Beausoleil, David J. Mellor, Kevin J. Stafford



Department of Conservation  
*Te Papa Atawhai*

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Cover: Archey's frog (*Leiopelma archeyi*) Multi-imaging device records four different image angles of each frog in a single digital photograph, allowing rapid documentation of natural markings which can be used to identify individual frogs. Photo: Avi Holzapfel, DOC.

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Corresponding author: D.J. Mellor, Animal Welfare Science and Bioethics Centre, Massey University, Private Bag 11-222, Palmerston North, New Zealand. Email: [D.J.Mellor@massey.ac.nz](mailto:D.J.Mellor@massey.ac.nz)

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# Introduction

Marking individuals or groups of animals is an integral part of wildlife research and management, and usually involves the application of an artificial mark. However, marking can affect the animals by altering: their behaviours or interactions with their own or other species; their health and welfare; their capacity to survive or reproduce; population dynamics; ecological balance and other factors. Such disturbances should be minimised for ethical, scientific and practical reasons. This can be done by recognising the advantages and disadvantages of different marks and marking procedures, and by employing the most effective and humane ways of applying the chosen marks (Mellor et al. 2004).

The purpose of this book is to describe key features of marking methods that have particular relevance to New Zealand species. Therefore, the information presented refers mainly to methods that have been or may be used to mark New Zealand wildlife. Information on other species is included when there are no reports of marking similar New Zealand species. Likewise, information on methods used in dissimilar species is included if their use seems plausible in New Zealand species, or if such information is deemed to be of value in some other way. It must be noted that this book does not explicitly address the effects of capture and handling on wild animals, but concentrates on the effects of the application, wearing and observation of identifying marks.

It is recommended that the whole of the *Methods* section be read before the sections on the different animal groups because information presented in that section is applicable and important to all groups. In addition, reference should also be made to the general safeguards, practical and animal welfare considerations and information about public perceptions associated with each marking method. These issues are discussed in the companion document entitled *Marking amphibians, reptiles and marine mammals: animal welfare, practicalities and public perceptions in New Zealand* (Mellor et al. 2004).

## WHY MARK WILDLIFE?

Often it is necessary in wildlife research to mark individuals or groups of animals. Reasons for marking animals include:

- To identify individuals or groups of animals to study demographics, behaviour, ecology and other aspects of the lives of wild animals.
- To estimate population size and to determine rates of survival, reproduction and recruitment within specific populations.
- To identify particular stocks and rates of stock mixing (this type of information is used extensively to monitor populations undergoing conservation management).
- To identify individual animals in behavioural studies.
- To develop and verify aging techniques and to ascertain growth rates in individual animals.

## GENERAL SAFEGUARDS FOR MARKING WILDLIFE

Regardless of the method chosen for marking a population of animals, there are general safeguards that researchers have an ethical duty to apply (Mellor et al. 2004).

- It must be demonstrated that marking is necessary to achieve the proposed research objectives.
- The purposes and benefits of the method chosen must be sufficient to justify its adverse effects.
- Devices and methods must be selected carefully (see, *Selecting an appropriate marking method*, pp. 3-10).
- Personnel should assess marking procedures that are new, or new to the particular population, or modifications of existing methods, on captive individuals or allied species before attempting to mark wild populations.
- Mark-related effects on parameters such as survival, reproductive success, behaviour and intra- and inter-specific interactions must be quantitatively and objectively assessed, and measures devised to minimise them. Both short- and long-term effects should be evaluated, and effects should be assessed separately in all age groups and sexes to be marked (see, *Direct evaluation of the effects of marking*, pp. 11-13).
- Data analysis must take account of mark-related effects.
- Only experienced and/or well-trained personnel who are proficient in the method should carry out marking.
- Since handling may cause short-term stress, use gentle and minimal handling, and for the shortest time possible.
- Accidental injury during marking should be treated, and if sufficiently serious, the animal should be humanely euthanased.
- Personnel must minimise the transmission of infectious diseases and parasites between animals during the marking procedure.
- Whenever possible, personnel should monitor the health and welfare of marked animals.
- Whenever appropriate, personnel should remove devices at the end of the study.
- Devices applied to juvenile or growing animals should be designed to expand or drop off.
- Marking should not compromise conservation strategies for endangered or threatened species (e.g. kill methods or those that adversely affect reproduction should not be used), nor should it adversely affect the ecological balance or the environment.

## THE IDEAL MARK

Ideally, an identifying mark should meet all of the following criteria (Lewke & Stroud 1974; Ferner 1979; Neitfeld et al. 1994; Reaser 1995).

An ideal mark should:

- Allow the animal to be as free of pain and/or stress<sup>1</sup> as possible.
- Identify the animal as an individual, if desired.
- Be easy to apply in both the laboratory and the field.
- Be easily and unambiguously read or observed.
- Be reliable over the duration of the study.
- Be cost-effective.
- Be adaptable to animals of different sizes.
- Utilise materials that are easy to obtain.

An ideal mark should not:

- Cause death.
- Have sub-lethal effects on fitness, e.g. reduced growth or reproductive rates.
- Influence the behaviour of marked individuals.
- Influence the behaviour of other animals towards the marked individual.
- Affect the future probability of capturing marked individuals relative to unmarked individuals.

Of course, no one marking method is able to satisfy all of these criteria; there is no ideal method of marking wild animals. There will always be a trade-off between the acquisition of knowledge and the disturbance caused by acquiring it. In order to study natural systems, we must necessarily disturb them; the undisturbed system is unknown. Some might argue that data collected from systems disturbed by marking do not reflect that system as it functions normally. However, if studies are biologically and ethically sound, their benefits should outweigh the detriment caused by such investigations. Selection of a marking method depends primarily on the ability of the method to fulfil the objectives of the study, while causing the least overall impact on the animals involved (Ehmann 2000).

## SELECTING AN APPROPRIATE MARKING METHOD

The most appropriate method may differ with species, population, sub-population, season, research group and many other factors. Therefore, researchers must be rigorous and consistent in their assessment of the most biologically and ethically appropriate marking method for the animals under study. Acceptable methods should be determined by individual researchers, research institutes (e.g. through Animal Ethics Committees) or governing bodies, and should reflect the goals and limitations of the particular study. Selection of the appropriate marking method should involve careful

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<sup>1</sup> Stress represents physiological responses to significant challenges, which can be emotional and/or physical. They elicit well-documented 'fight-or-flight' responses and changes that help to deal with possible injuries. Externally observable signs of stress include aggression, struggling or freezing behaviours, abnormal postures, vocalisation or its absence, impaired grooming, altered activity patterns, shivering, altered breathing, change in skin colour and body temperature change. The associated physiological responses may be measured.

consideration of the following issues (Ferner 1979; Heyer et al. 1994; Reaser 1995; Baker & Johanos 2002):

- Specific objectives of the study and the nature of the data required.
- Duration of the study.
- Level of recognition required.
- Life history and physical attributes of the species involved.
- Welfare of the animals involved.
- Size and conservation status of the population.
- Amount of time and resources available to researchers.
- Level of training and experience of researchers.
- Extent of public access to the study site.

### **Specific objectives of the study and the nature of the data required**

A marking method should be able to provide the level and nature of information required to meet the specific objectives of the study. For example, there would be little point in using a marking method that allowed only recognition of marked versus unmarked animals in a study of social behaviour, when a method that identified individual animals would provide more valuable information. Similarly, if information on the undisturbed behaviour of the animals is required, then marks should allow remote identification of individuals or groups.

The marking method selected should not influence the variables under study. Marks and marking procedures can alter the behaviour, reproductive success, intra- and inter-specific interactions and survivability of the bearer. For example, a mark used to estimate the annual survivorship of individuals in a population should not alter an animal's conspicuousness to potential predators. Any data collected using such a mark would misrepresent the natural survival rate of that population. Similarly, if the objective of a study is to determine the annual number of reproductive events in a population, a mark that affects the capacity of an animal to engage in mating behaviour or that influences the bearer's attractiveness to conspecifics, will have a significant effect on the data collected. It is not only negative changes in such variables, but also positive changes, that can adversely affect the data collected, and subsequent outcomes.

### **Duration of the study**

A mark must be effective for the duration of the study. A mark that is lost before the conclusion of a study can seriously bias the results. For example, tag loss in demographic studies of Antarctic seal species can cause overestimation of population size, which might affect the design of conservation strategies. In addition, if a mark is lost prematurely, the marked animal may have suffered because of marking without any redeeming benefit in terms of knowledge gained. This type of imbalance cannot be ethically justified. Researchers have an obligation to select a method that has been proven to last for the appropriate amount of time when applied to the species of interest. If the durability of a mark is unknown, the researcher has an obligation to conduct a preliminary study to determine how long the mark will endure and allow accurate identification (e.g. be legible).

If mark loss is inevitable, as is the case with tags used in seal species, the rate of mark loss must be calculated, and this rate must be incorporated into the results of the study and its outcomes (e.g. modelled population estimates). It is important that estimated rates of mark loss be specific to the marking equipment, species and habitat in question, as loss rates vary greatly with these factors. Other factors that may affect the rate of mark loss include operator technique and experience, which are inherently difficult both to standardise and quantify.

If a less harmful, temporary mark is sufficient then this type of method should be used in preference to a more invasive, longer-lasting mark. The use of a temporary mark will mean that the effects of marking are also likely to be short term. Conversely, if a temporary mark is lost before the end of a study, any harm associated with that mark would be unjustifiable, and a more permanent method should have been used.

## **Level of recognition required**

### ***Proximity***

Researchers must decide the distance at which they require recognition of the mark. Some marks allow identification of marked animals from a considerable distance. Others, especially those on small animals, require recapture and handling of the marked animals to identify them. If minimising disturbance is of primary importance to the study objectives or to the welfare of the animals, then the marking method should allow recognition at a distance, without recapture and handling. However, researchers must be aware that marks allowing remote identification or recognition are also likely to increase the conspicuousness of the bearer to predators or prey, or can alter the appearance enough to affect intraspecific interactions.

### ***Specificity***

Some studies require that marked animals be distinguished only from unmarked animals. For example, estimates of population size do not require identification of individual animals. Mathematical models are used to convert sample numbers (ratio of marked to unmarked animals) into estimates of the total population size. In contrast, most ethological studies require the identification of individuals to assess facets of animal behaviour such as predator-prey interactions, home ranges, habitat use, foraging and reproductive behaviours and social interactions. A mark that does not identify the animal as an individual is of little use in such studies.

## **Life history and physical attributes of the species involved**

The physical and anatomical attributes, habits and ecology of a species will play a major role in deciding which marking method is appropriate. A method appropriate for use in whales is unlikely to be useful for studying frogs. Physically, the animal's size, the presence or absence of appendages, and tissue characteristics are some of the features that will influence mark choice. Small size limits the use of certain marking methods, primarily because of the need for low mark-to-body weight ratios. This ratio should not exceed 10%, but devices should be kept as small as possible (American Society of Mammalogists 1987; Heyer et al. 1994).

The particular requirements of each species must be considered. For example, it may not be appropriate to remove those structures with special functions, such as the toes of a climbing or digging animal. In addition, the size or conspicuousness of certain anatomical structures should not be accentuated (e.g. structures involved in social signalling) as this can artificially change intraspecific relationships or social rank. Seasonal differences in size, shape, behaviour, habitat or sensitivity to human presence may also influence the selection of an appropriate mark. Adverse effects may occur only in conjunction with certain behaviours or environmental conditions, or only in certain age groups or sexes. Therefore, in order to avoid mark-related influences on physiology, behaviour or survival, researchers must be thoroughly acquainted with the physiology, ecology and ethology of their study populations before marking occurs.

In addition to the physical attributes, researchers must be aware of the species' responses to capture, handling and restraint. While some species appear to tolerate human presence, capture and handling well, others are extremely sensitive and may die if placed under severe or repeated stress during procedures associated with marking. Responses to capture and handling might also vary throughout the year or at different life stages. For example, it is generally considered prudent to leave animals undisturbed during mating, parturition and egg laying.

### **Welfare of the animals involved**

Scientists need to weigh the benefits of the research against the adverse consequences for the individual animal, population and ecosystem. Each marking method involves a compromise between the effect on the subject animal and the quality of data collected. However, it is likely that a method that has a significant effect on the animal will be associated with less reliable data, especially for behavioural studies.

Marking can affect animals in three ways:

- The act of marking.
- Presence of the mark.
- Observation of the mark.

#### ***The act of marking***

The application of a mark can cause tissue damage, pain and stress, may temporarily restrict movement or feeding and can increase the risk of infection to the animal. More permanent methods such as branding, tattooing, tagging or surgical implantation are generally more stressful and cause more pain than temporary superficial methods such as painting and hair clipping. Researchers must take great care to minimise pain and stress, as well as the risk of infection when invasive methods are used.

Procedures that cause more than momentary or slight pain should be performed with appropriate sedation, analgesia or anaesthetic, except when otherwise justified for scientific reasons. For example, the relatively unpredictable and potentially delayed responses of some ectotherms to immobilants and anaesthetics may contraindicate their use in field studies (Pickering et al. 1982). Similarly, the use of anaesthetics in pinnipeds may lead to unpredictable

reactions, including death (Troy et al. 1997). However, the decision not to use pain relief must be compellingly justified. General anaesthetics must be chosen carefully to minimise risks to the animal. The anaesthetic should permit rapid recovery to a normal physiological and behavioural state, and animals must be kept under observation until recovery is complete (Laws 1993).

In addition to the act of marking itself, it is important to consider the potential effects of the associated capture, restraint and handling. Researchers must recognise that capture and handling can cause acute stress in wild animals, and may induce changes in physiology and behaviour, affecting survival and welfare (Pickering et al. 1982). In addition, the mere presence of humans in the environment may disturb the natural behaviour of the species of interest and other animals.

Before beginning any study involving the marking of wildlife, researchers must be thoroughly familiar with the biology of the target species, and its sensitivity to capture and restraint. In general, animals should be handled quietly, using the minimum personnel necessary. Researchers should use the least amount of restraint required to do the job properly. Tranquilisers and chemical immobilants may be appropriate if they prevent injury to animals or people during restraint. However, the use of some chemical immobilants may cause greater distress than restraint alone (Laws 1993).

### ***Presence of the mark***

As well as causing direct bodily harm, the physical presence of a mark may restrict movement or foraging, disrupt breeding or social interactions or alter distribution or migration patterns. Marks that have been incorrectly placed or poorly fitted may cause chaffing or constriction, leading to tissue damage, pain and loss of function. In addition, loose fitting devices may cause animals to become snagged on features of the environment, or may prevent effective escape (e.g. from predators) or foraging behaviour.

External marks, especially those allowing remote identification, often alter the appearance of the animal. Marking may increase the visibility of the study animals not only to human observers, but also to potential predators or prey. Because bright or contrasting colours are often used to mark animals, predators may detect marked animals more easily than unmarked animals (Kessler 1964). In addition, marked predatory animals may have decreased success in hunting if prey species are more easily able to detect them. Marked animals may also be treated differently by conspecifics, as many social interactions are facilitated by integument markings (Frankel & Baskett 1963). Modification of these natural markings may distort the messages sent to conspecifics, affecting social interactions such as mating, and aggression or submission. These effects may be acceptable for short-term studies, and external marks are generally necessary for remote identification in behavioural studies.

Although they do not alter the appearance of the animal, internal marks such as implanted telemetric equipment or Passive Integrated Transponders (PITs) can adversely affect internal structures.

### ***Observation of the mark***

Some marks allow identification from a distance, reducing the negative impacts of human presence, capture, restraint and handling otherwise inflicted on the study animal. Others marks are less conspicuous and require repeated capture and handling for all subsequent identifications. Temporary marks, such as painting, tend to allow remote identification, but recapture and remarking are necessary if they are used for longer-term studies. More permanent marks such as brands and tattoos tend to cause pain and stress at the time of application. Some researchers believe that causing an injury to an animal once in its lifetime is more humane than repeated capture in order to refresh temporary marks (Erickson et al. 1993). However, permanent marks are often small, and recapture is likely to be required for subsequent identification.

The adverse effects of marking may be immediately evident or appear long after the procedure has been performed (Neitfeld et al. 1994). Repeated capture and handling can lead to sub-clinical stress, which can accumulate to affect survivability. Such stress can make marked animals more vulnerable to the effects of other natural stressors that would not normally affect the animal; the accumulation of sub-clinical stresses can have subtle effects on the welfare of wildlife (Moberg 2000).

All the above considerations are important, not only for maximising the scientific value of the study, but also for ensuring that animal welfare is compromised as little as possible. In addition, investigators are obliged to monitor the condition of the marked animal and if necessary remove the mark at the end of the study.

### **Size and conservation status of population**

Wildlife research, including marking, must contribute to and be in harmony with conservation efforts. This is especially important in New Zealand, which is home to a large number of unique and threatened species of amphibians, reptiles and marine mammals. The loss or harm of even a small number of individuals due to research activity can have important repercussions for threatened populations. Members of endangered or threatened taxa should not be removed from the wild except in agreement with conservation efforts (British Columbia Environment Resources Inventory Committee 1997). Obviously, kill methods and methods that prevent reproduction are inappropriate for use in such populations. Likewise, methods that adversely affect the behaviour, survivability or reproductive success of threatened species may be just as inappropriate. It is also important to conduct field research on vulnerable populations in a manner that leaves associated habitats as undisturbed as possible.

Insights gained during research are often critical to devising sound conservation strategies (Baker & Johanos 2002). Marking methods that alter the behaviour or survival of animals may lead to the collection of inaccurate data, which could contribute to the development of ineffective conservation efforts. It is not only negative effects that could adversely affect conservation strategies. For example, a marking method that increases the breeding success of individuals could lead to inaccurate estimates of growth within a population. This could cause researchers to believe that the size of the population was

increasing at a higher rate than was actually the case, leading to underestimates of the conservation effort required to ensure the survival of that population. Therefore, it is imperative that the effects of potential marking methods are carefully assessed before they are used in such populations.

### **Amount of time and resources available to researchers**

Researchers must consider that although a marking method may provide the desired level of identification, there are also practical limitations to each method. Some marking methods are time-consuming or complicated to perform, and require a high degree of skill. Complicated methods also require longer handling times, which can increase the animal's stress.

Expensive or rare materials may not be accessible to some research groups. However, inferior materials may be more detrimental to the marked animal, may be lost prematurely or may decrease the quality of the data. Some methods require that cumbersome or delicate equipment be taken into the field for marking or for subsequent identification of marks. Other methods require researchers to comply with rigorous safety regulations, and their use may be restricted by laborious monitoring or safety procedures, as with radioisotope tagging.

Researchers must be aware of the practical limitations of each method, and ensure that they have adequate time, financial resources and knowledge to minimise the risk to the animals involved, while maximising the value of the data collected. They must have the time and resources to perform appropriate preliminary studies to quantify the effects of marking on the population under study. Experimental design and data analysis should account for such marking-induced effects. In addition, researchers must be committed to monitoring marked animals and to locating them at the end of the study, and, if necessary, removing the marks. They must also allocate resources for the treatment or euthanasia of any animals injured as a result of marking.

### **Level of training and experience of researchers**

The success of some marking methods is more dependent on operator technique than others. For example, the success of both hot and freeze branding, in terms of legibility and permanence, is apparently highly correlated with operator experience (Gales 2000). Other methods, such as toe clipping, are relatively immune to operator influence. Research leaders and institutions have a responsibility to ensure that scientifically and ethically sound protocols for handling, marking and monitoring are put in place and adhered to. One option is to develop and use Standard Operating Procedures that are formally approved by the institution. Documentation outlining these procedures should be issued to each new member of the research team and any affiliated personnel working with wildlife. In addition, regular checks should be undertaken to ensure that such standards are maintained and improved as necessary.

Every member of the research team who is involved in handling or marking animals should be knowledgeable and well trained in the ecology of the species, the marking method and the general symptoms of pain, stress or injury in that species. The optimum methods for aseptic techniques, mark application and treatment of injuries should be taught to all personnel working with wild

animals. Only experienced, proficient personnel should be permitted to mark wild populations. Trainee researchers should practice, under supervision, on captive animals, where potential injuries can be monitored and treated if necessary.

### **Extent of public access to the study site**

Public support for government-funded wildlife research is crucial. There will always be some people who object to interfering with wildlife in any way, and others who object to the infliction of pain and stress on any wild animal. However, the majority of interested people appreciate the role of marking in wildlife biology and conservation, and it is to those people that scientists must demonstrate that the chosen methods are both suitable and humane.

Methods that appear to seriously harm the animal or grossly alter its appearance are likely to be viewed negatively by the public (Gales n.d.; Mellor et al. 2004). The extent of public access to the study site should therefore be considered when selecting a method for marking wild populations. For remote study sites, other factors can be given priority over public perception, but for sites frequented by members of the public, the aesthetics of the procedure must weigh heavily in the selection of the marking method. It is important to remember that there is often a disparity between the *real* and *perceived* effects of marking on animal welfare, and public perception can sometimes be inaccurate. If sound scientific practice is abandoned to appease public perception and present an acceptable superficial appearance, animal welfare and the acquisition of scientific knowledge may be compromised.

A detailed discussion of public perceptions relevant to wildlife marking is provided in the companion volume (Mellor et al. 2004).

## **RESPONSIBILITIES OF SCIENTISTS**

Researchers working with wildlife have ethical and scientific responsibilities to minimise the adverse effects they have on the study animals, while maximising the value of the research (Halliday 1995; Powell & Proulx 2003). In addition to the *General safeguards* outlined on p. 2, wildlife researchers should strive to meet the following objectives (British Columbia Environment Resources Inventory Committee 1997; Association for the Study of Animal Behaviour 2002). Researchers should:

- Comply with all relevant national and institutional regulations pertaining to the particular species. Most guiding or governing societies now require that marking protocols minimise pain, stress and adverse effects on the study animals (e.g. Society for the Study of Amphibians and Reptiles).
- Be familiar with current literature on the species and be aware of any special considerations for the particular population under study.
- Seek the advice of experienced peers before initiating a research project.
- Receive, use and offer constructive criticism from and to colleagues regarding the efficient and ethical use of animals.
- Use the smallest number of animals possible to satisfy the goals of the study.

- Maximise the research potential of animals, e.g. by using toe-clip material for genetic analyses.
- Research and develop new techniques or ways of improving existing methods.
- Re-assess experimental methodologies whenever an injury or mortality occurs.
- Ensure that data gathered are accurate and complete.
- Publish any innovations in capture, handling, marking or analytical techniques.
- Train and supervise all research assistants to apply the same ethical and scientific standards.

## DIRECT EVALUATION OF THE EFFECTS OF MARKING

Subtle effects of capture, handling and marking can accumulate to affect behaviour and survival of the marked animals, but such effects are rarely addressed explicitly in the experimental design of research projects (Baker & Johanos 2002). If the possibility of mark-related effects is acknowledged at all, it is usually dealt with as an afterthought, by simply stating that ‘marked animals did not appear to be obviously harmed’.

In a survey of nine peer-reviewed journals, Murray & Fuller (2000) found that 90% of authors who applied artificial marks to facilitate the study of vertebrates did not address the potential effects of marking at all. Three percent of authors made an explicit assumption that the methods used did not affect the animals under study. However, such assumptions were almost always based on previous reports of no significant effects on the target, or a related, species. Seven percent of authors did present information directly relating to potential marking effects. Unfortunately, most of these studies used qualitative measures of ‘effect’ and there were no reports of the long-term effects of marking. Almost all of these studies (90%) related to the attachment of radio transmitters. This implies that other methods such as tagging and branding are automatically assumed to have no effect, perhaps because of their historical use in animal biology.

The assumption that there are no significant marking effects is critical, as it is the basis for generalising data collected from marked individuals to unmarked animals and populations (Murray & Fuller 2000). Studies with unknown effects on the animals are not only potentially detrimental to the study population, but can also generate invalid data. Quantification of the effects of a marking method is essential to allow accurate interpretation of population parameters such as mortality and emigration as well as behavioural parameters (Lemckert 1996).

There are several reasons why the effects of handling and marking are rarely evaluated systematically (Baker & Johanos 2002). These include:

- The difficulty in quantifying sub-lethal effects.
- Logistical and financial constraints.
- The lack of appropriate control animals.
- An inability to locate/track control animals.
- The duration of the study is inadequate owing to species characteristics.

Despite such limitations, some studies have explicitly evaluated the effects of marking. Schlaepfer (1998) assessed the use of pressurised fluorescent powder on small amphibians in the laboratory and field. Several authors have specifically assessed the effects of paint marking on the behaviour, survivorship and risk of predation of reptiles (Jones & Ferguson 1980; Simon & Bissinger 1983; Boone & Larue 1999; Quinn et al. 2001; Lopez et al. 2003). Wilson et al. (1986) and Baker & Johanos (2002) evaluated the effects of instrumenting marine mammals on their behaviour, energy expenditure, health and survivorship.

The effects of toe clipping on amphibians and reptiles have only occasionally been evaluated systematically (Huey et al. 1990; Dodd 1993; Luddecke & Amezcua 1999; Paulissen & Meyer 2000). This is concerning, because toe clipping is still the most common method for marking amphibians and reptiles. Most other studies using toe clipping have merely reported a subjective measure of 'effect' or stated that toe clipping had no apparent effect.

Although it is encouraging to see studies systematically evaluating the effects of marking, their paltry number in relation to the thousands of investigations that use artificial marks to identify wild animals suggests that insufficient attention has been given to this area. In addition, many of the studies intended to evaluate mark-related effects have been plagued by flaws in experimental design. Common failings include: a lack of appropriate controls, inappropriate treatment of controls (e.g. different handling methods), small sample sizes leading to low statistical power, qualitative rather than quantitative measurement of marking effects, inadequate study duration and the opinion that statistically significant differences are too small or uncommon to be biologically important (Murray & Fuller 2000).

Before marking is incorporated into a research programme, the effects of capture, handling and the marking procedure should be quantitatively assessed in the laboratory and then in the field. There may be effects or pressures on marked animals that are present in the field but do not exist under laboratory conditions (e.g. predation pressure). Both short- and long-term effects should be assessed in a separate preliminary trial for each population to be marked.

The effects of marking should be assessed: if the method is new or new to the population under study; if the method is a modification of an existing method; if recapture rates of marked animals are low; or if researchers suspect that the marking method is affecting marked animals. In addition, marking effects should be assessed separately for each age group and sex. There is a need to standardise marking techniques for particular species in order to lend credibility to comparative studies or to studies carried out by various researchers over time (Ferner 1979). Systematic assessments of the effects of marking will also elucidate the anticipated durability of the mark, and the type of recapture effort required for subsequent identifications.

It is the responsibility of the researcher to conduct the evaluation most relevant to the study objectives and the welfare of the animals under study. Such evaluations may involve comparing a chosen parameter before and after marking, or may compare marked and unmarked animals using a quantitative biological measure. Evaluations may include biomechanical, metabolic or physiological measures, or comparisons of behaviour, reproductive success or

survival rates. In addition, researchers have a responsibility to publish all the results of such studies, to provide other investigators with critical information on which to base future decisions about marking wildlife.

Because it is not possible to study wild animals without disturbing them to some degree, researchers have an ethical obligation to minimise, or at the very least quantify, the effects of the investigation. It is not acceptable for researchers to simply assume that capture, handling and marking have no effect on the animals under study, either because the method has been used in another population, or because there are no 'apparent effects'. The effects of capture, handling and marking should be separately evaluated for each species and population under study, and strategies should be specifically devised to minimise those effects. Unidentified effects of marking are not only potentially detrimental to the animals involved, but can also invalidate the data collected, which can have repercussions for threatened populations under conservation, as well as for the advancement of knowledge.

## APPROPRIATE METHODS FOR MARKING NEW ZEALAND WILDLIFE

As outlined above, methods appropriate for marking one group of animals may be unsuitable for another. Brief summaries of the most appropriate methods for marking amphibians, reptiles and marine mammals are provided below. Later sections present more detail on group-specific considerations for the use of each marking method. The discussion of methods for marking marine mammals is divided into two sections: cetaceans (whales, dolphins and porpoises) and pinnipeds (seals, sea lions and walruses). Because the two groups differ significantly in anatomy, physiology, behaviour and habitat, methods appropriate and useful for marking one group may not be relevant to the other.

### **Amphibians**

In general, external tags are difficult to apply and may affect the behaviour, appearance and survivability of frogs. Because of the very small size of New Zealand frogs, there are concerns about the use of implanted devices (e.g. Passive Integrated Transponders). There is still great contention over the suitability of toe clipping for the identification of frogs. This method is currently used to mark some native frog populations in New Zealand. However, with the introduction of chytrid fungus and predicted population decreases, a less invasive marking method may be preferable.

There is a growing trend towards using natural markings to identify New Zealand frogs. Natural markings have been successfully used to identify individual Hamilton's frogs (*Leiopelma hamiltoni*) on Stephens Island. This population is well suited to natural marking identification, owing to its small, defined nature. Natural markings may be less useful in the identification of the other three native frog species. Some Hochstetter's (*Leiopelma hochstetteri*) and Maud Island frogs (*Leiopelma pakeka*) have dark forms, and the pigmentation may obscure identifying markings. Populations of Hochstetter's and Archey's frog (*Leiopelma archeyi*) are more widespread and less clearly

defined, making individual identification from natural markings more difficult and time-consuming. The New Zealand Department of Conservation is currently investigating the use of natural markings for identifying individual Archey's frogs.

The present volume provides technical information and also addresses specific methodological issues relating to marking of amphibians in New Zealand. For additional discussion relevant to the marking of amphibians the reader should consult the companion volume (Mellor et al. 2004)

## **Reptiles**

Reptiles are successfully marked using a variety of methods. Painting is often used for short-term studies, and has not been shown to affect survivorship. Tagging may be less appropriate owing to the associated risks of increased conspicuousness to predators, snagging on vegetation and changes to behaviour. More permanent methods such as branding, tattooing and scale clipping are primarily used to mark snakes, because of the difficulties with attaching other devices. Toe clipping is permanent in reptiles, and is still the most common method for marking lizards and tuatara (*Sphenodon* spp.). No reliable evidence of detrimental effects of toe clipping has been reported so far, but very few studies have systematically evaluated the possibility of such effects. Passive Integrated Transponder (PIT) tags can be useful for studying reptiles. However, many of New Zealand's reptiles are long lived, and little is known about the long-term effects of intra-abdominal implantation of PITs, especially in small animals. In addition, the potential for this method is somewhat limited by current technology (e.g. short reading distances).

The present volume provides technical information and also addresses specific methodological issues relating to marking of reptiles in New Zealand. For additional discussion relevant to the marking of reptiles the reader should consult the companion volume (Mellor et al. 2004).

## **Cetaceans**

Cetaceans are difficult to mark because of their anatomy, marine habitat and wide-ranging lifestyles. External visual markers, such as streamers and tags, are not particularly effective and often cause significant tissue damage in smaller species. The growing trend in cetacean identification is to recognise individuals by their natural markings. It is important that international databases of recognisable individuals be established, as cetaceans cross many study regions during their migrations. Satellite-telemetry has increased the potential for tracking the movements and behaviours of cetaceans. The effects of transmitter packages on hydrodynamic drag, behaviour and energy expenditure should be systematically evaluated and carefully considered for each population so marked, in order to balance the use of the method against the value of the information obtained.

The present volume provides technical information and also addresses specific methodological issues relating to marking of cetaceans in New Zealand. For additional discussion relevant to the marking of cetaceans the reader should consult the companion volume (Mellor et al. 2004).

## **Pinnipeds**

Pinnipeds are marked most often by tagging, painting, dyeing or hot branding. Paint and dye marks are visible from considerable distances and are often durable enough for short-term studies. Tag loss is a serious issue in pinniped studies, especially in those estimating population size, and loss rates should be calculated for every population under study. Concerns about hot branding have not yet been resolved, although several authors advise that carefully executed hot branding is not likely to have significant effects on the daily life of the animals. Hot branding is often less satisfactory than other marking methods owing to the variability in wound healing and the legibility of the resulting marks. Satellite- and radio-telemetry can provide important information on pinniped behaviour that could not otherwise be obtained. However, the effects of transmitter packages on hydrodynamic drag, behaviour and energy expenditure should be systematically evaluated and carefully considered for each population so marked, in order to balance the use of the method against the value of the information obtained.

The present volume provides technical information and also addresses specific methodological issues relating to marking of pinnipeds in New Zealand. For additional discussion relevant to the marking of pinnipeds the reader should consult the companion volume (Mellor et al. 2004).

# Methods

This book is not intended to be a field manual of marking methods. Accordingly, only a brief description of each method is provided to facilitate understanding of the implications of using that method to mark wildlife in New Zealand. For full methodologies readers should refer to the related reference material cited herein.

The methods described below have been classified according to mark durability, rather than ranked by their potential to cause animal welfare problems, for several reasons. The ranking of methods on animal welfare grounds would be complicated and subjective, and we do not believe that enough information exists at the present time to classify marking methods on welfare grounds alone. In addition, the potential welfare problems would differ according to species, environment and other factors, making an overall classification system virtually impossible. Finally, wildlife practitioners, for whom this book is primarily written, will want to focus on the method first and then consider the associated animal welfare implications. Therefore, the methods are broadly categorised as *temporary*, *semi-permanent* and *permanent* (Table 1).

For each method, general information is given, along with relevant notes on materials and techniques, advantages and disadvantages of the method, considerations for its use in all wildlife species, and remarks on the applicability of the method to the species covered in this book (amphibians, reptiles and marine mammals).

TABLE 1. IDENTIFICATION METHODS.

TEMPORARY	SEMI-PERMANENT	PERMANENT
Paints or dyes	Tags	Hot, freeze or chemical branding
Streamers, adhesive tapes, trailing devices	Neck collars, harnesses, bands	Tattooing
Hair/fur removal	Nocturnal lights	Passive Integrated Transponders (PIT)
Fluorescent powders	Telemetry (radio-, satellite-, bio-) and archival data recorders	Visible implant fluorescent elastomer tags (VIE)
Radioisotope marking		Tissue removal: ear notching; toe, disc and web clipping
		Vital stains
		Natural marking identification

## TEMPORARY MARKS

Temporary marks are defined as those which are required to last only a short period relative to the lifespan of the animal (Neitfeld et al. 1994) (Table 2). These marks tend to be more visible from a distance because they use contrasting colours and bold characters and symbols. They are also used if more permanent methods of identification are expected to affect the animal or compromise the quality of the data, or if no other options are viable for the study (Neitfeld et al. 1994).

## Painting

Painting can be of immense value for short-term studies as it can allow identification without repeated recapture of the marked animals. Paint can be applied to the surface of the integument or hair, and is usually lost over time through wear, skin sloughing or hair shedding. The durability of paint marks depends on the animal's environment and behaviour, as well as characteristics of the paint itself.

Paint marks applied to the dorsal surface of the animal often facilitate easy observation. Such marks can be applied by hand using stencils and brushes, or more remotely with brush-tipped poles or by throwing paint-filled balloons. Groups or individuals may be identified by using different combinations of mark location, colour and symbols. Conspicuous paint marks are often used in conjunction with more permanent, less visible methods such as toe clipping (Simon & Bissinger 1983) or tattooing.

Painting materials include model paints, felt pens, tattoo inks, cattle marking wax, indelible pencil pigments and correction fluid (e.g. 'Twink'). Caution is required when selecting a paint for use on animals. Some paints and solvents may be toxic and, if absorbed through the skin, could cause illness or death of the marked animal. Therefore, paints with non-toxic pigments, bases and solvents must be used. If the toxicity of a particular product is unknown, the researcher has a responsibility to review the literature and evaluate the paint in laboratory trials before using it in the field (British Columbia Environment Resources Inventory Committee 1997).

Paint marks are used to temporarily identify many wildlife species (Table 2). However, the application of paint to thickly furred animals is not advised. Paint tends to cause clumping and matting of fur and can lead to fur loss or problems in the underlying skin, or to excessive ingestion due to grooming (Taber 1956; Bailey et al. 1973). In addition, paint should not be applied to amphibians because their skin is moist and highly absorbent, and plays an important role in gas and water exchange (Dorit et al. 1991).

TABLE 2. TEMPORARY IDENTIFICATION METHODS<sup>1</sup>.

METHOD	SPEED OF APPLICATION	COMPLEXITY	COST
Paints and dyes	Fast	Low	Low
Streamers, adhesive tapes, trailing devices	Intermediate-Fast, depending on method	Low-Intermediate, depending on method	Low
Hair/fur removal	Intermediate-Fast	Low	Low
Fluorescent powders	Fast	Low	Low
Radioisotope marking	Slow-Fast, depending on attachment method, vehicle, isotope	Intermediate-High, depending on attachment method, vehicle, isotope	Intermediate-High, depending on attachment method, vehicle, isotope

<sup>1</sup> Ranks in columns (e.g. slow, intermediate and fast) are qualitative, comparative scores for the parameter listed for the methods in the table. Table continues on p. 19

## Dyeing

When applied to hair or fur, dyes and bleaches tend to produce longer-lasting changes than paints. Dyes impregnate the hair with colour, whereas bleaches remove pigment; both create a contrast with the original coat colour. Mark durability depends mainly on hair shedding, but some dyes also fade.

Dyes are commonly used to mark the pelage of pinnipeds (Table 2). They have also been incorporated into tank water or injected directly to stain larval amphibians, methods which could also be included under the heading *Vital stains* (see, p. 38).

## Attached devices

Streamers and coloured or reflective tapes have been attached to a variety of animals to increase their visibility for a short time. Such devices are usually chosen to contrast with the natural colour or texture of the animal, and are generally attached to the integument or hair using non-toxic glue. The bond degrades over time, thereby freeing the animal of its mark. Streamers and tapes can be made of a variety of materials including fluorescent plastic, polypropylene, polyurethane, nylon-coated vinyl and vinyl tubing. In addition, trailing devices (e.g. spools of thread) can be used to track animals over short distances or periods of time (Dole 1965). Mark durability depends on the device's material and method of attachment, and the environment and habits of the animal, and can range from a few days to several months. The larger the attached device, the more likely it is to encumber the animal or be lost.

Such devices have been attached to amphibians, reptiles and marine mammals (Table 2). However, water friction makes mark retention problematic in marine mammals (Neitfeld et al. 1994).

## Hair removal

This method is applicable only to animals with sufficient hair. Hair/fur can be removed by clipping or shearing. Chemical depilatory pastes can also be used to remove hair but the agents may be irritating to the skin (Gentry 1979). Hair

SHORT-TERM STRESS	INFLUENCE OF OPERATOR	VISIBILITY	APPROPRIATE SPECIES
Low: some handling	Low	High	Terrestrial and marine mammals, reptiles, invertebrates
Low-Intermediate, depending on method	Intermediate	High	Birds, marine and terrestrial mammals, reptiles, amphibians
Intermediate: handling, machine noise, vibration	Low	Moderate-High	Haired/furred terrestrial mammals, pinnipeds
Low: handling	Low	Moderate-High, depending on vegetation, ambient light	Small nocturnal mammals, reptiles, invertebrates
Low-High, depending on attachment method, vehicle, isotope	High	Low	Small or nocturnal terrestrial mammals, reptiles, amphibians, invertebrates

Continued from p. 18

removal is particularly effective if the under-fur is a contrasting colour (Scheffer 1950). The disadvantage of this method is that the resulting marks are only retained until the next moult. The temporary nature of the marks is offset by the ease of application, high visibility and painlessness of hair/fur removal. However, it is important to note that hair removal may take more time to complete than other temporary methods, thereby increasing the handling times and potential stress experienced by the animals.

Pinnipeds are good candidates for identification by hair removal (Table 2).

### **Fluorescent powders**

Nocturnal animals present particular problems for marking, as the animals must be identifiable in the dark. One group of methods involves the application of fluorescent pigments to the surface of animals. The pigments can be applied by passive uptake (e.g. brushing against an applicator), dusting of live-trapped animals or pressurised application of powder (e.g. Fellers & Drost 1989; Mikesic & Drickamer 1992; Butler & Graham 1995; Schlaepfer 1998; Stark & Fox 2000; Sandidge & Brandt 2003). Fluorescent pigments can be visualised under ultraviolet (UV) light, although some can also be seen under white light.

Animals dusted with fluorescent pigments leave a trail of fluorescence that lasts until the following night. Researchers can then use UV lamps to detect these trails (Fellers & Drost 1989; Mikesic & Drickamer 1992). The pigment is still detectable on the animals during subsequent nights, but they may no longer leave trails. The amount of vegetation cover, precipitation, ambient light and pigment colour can influence trail detection (Mulligan 1988).

In contrast, pressurised application usually results in fixed pigment marks, and marked animals do not leave trails. Such methods can produce more precise marks and can often be used to identify individuals. Fluorescent pigments can also be injected under the skin, but only animals with transparent skin can be marked in this way (see, *Visible Implant Fluorescent Elastomer tags*, pp. 35-36).

The materials required (fluorescent pigment and a UV lamp) are relatively easy and cheap to acquire. There are many different fluorescent materials that can be used to mark animals. However most, if not all, are designed primarily for other purposes. For example, Day-Glo<sup>®</sup> fluorescent powders are paint bases that have been used to mark wildlife. They come in a variety of colours and are considered to be non-toxic. Other powders have been shown to have toxic effects on marked animals, and should not be used. For example, a dual UV-wavelength, invisible-detection powder containing cadmium borate was used to mark invertebrates, and significantly increased mortality rates in marked populations (Reinecke 1990). Therefore, it is most important that fluorescent pigments are tested for toxicity and other negative effects before they are used on wild populations.

Many pigments show up as brightly coloured under white (ambient) light, making marked animals more conspicuous during the day, as well as being easily visualised at night under UV light (Fellers & Drost 1989; Butler & Graham 1995). Therefore, it may be appropriate to select colours that correspond with surrounding vegetation or the organism's natural colour. Alternatively, some materials are not coloured or have been bleached, and at low concentrations may be inconspicuous in ambient light. Some fluorescent colours are less bright

under UV light (e.g. blue), or less long-lived than others (Fellers & Drost 1989; Reinecke 1990). In addition, some colours are difficult to distinguish under UV light; for example, red and orange are very similar. A torch could be used to help resolve colour differences at night (Fellers & Drost 1989).

Fluorescent powders have been used to track reptiles, and their application has been attempted in amphibians (Table 2). Such methods are not likely to be suitable for use in marine mammals.

### **Radioisotope marking**

Radioactive material can be applied in various ways to study small, camouflaged, retiring or nocturnal animals, which would otherwise be difficult to study. Radioisotopes are of particular value for tracking organisms too small, or otherwise unsuited to carry telemetric equipment (Ferner 1979; Thompson 1993). Radioisotope marks cannot be detected by the senses of the animals that are labelled, nor do they increase the conspicuousness of animals to their predators or prey.

Each radioactive isotope has an energy emission profile that allows detection and can cause tissue damage (radio-toxicity). The extent of tissue damage is a function of the level of radioactivity and the energy profile of the isotope. The choice of radioisotope depends on availability, type of radiation emitted, radio-toxicity, half-life of the isotope, distance of detection required and the duration of the study (Linn 1978). Researchers must determine the minimum effective amount of radioisotope required for appropriate detection (Karlstrom 1957). The optimum half-life of a radioisotope is calculated to be about two-thirds the length of the study (Lachelt 1954). A balance must be struck between the level of radioactivity sufficient for detection, and the minimum damage caused by exposure to the radioactive material.

Table 3 shows radioisotopes that are used to study wild animals (Neitfeld et al. 1994). Radioactive tags are detected using a Geiger-Muller counter or—for increased sensitivity—a scintillometer. Electronic filter systems can differentiate low-energy background radiation from high-energy experimental radiation (Linn 1978). Detection can be done by the researcher or can be automated. In addition, continuous recording systems are available (e.g. Inglis et al. 1968).

Radioactive material can be incorporated into externally attached wires, pins, capsules or tags, or attached to leg bands, collars or harnesses. Radioisotopes can also be injected into the body, which often requires anaesthesia. Inert implanted radioisotopes are not metabolically active, and are not incorporated into the tissues (Linn 1978). However, close proximity of radioactive material to tissues increases the risk of tissue damage. Radioactive material to be implanted can first be placed within a small capsule to increase its distance from tissues and, for external applications, a screen (e.g. brass) can be placed between the material and the animal.

Metabolisable radioisotopes can be implanted in the same manner as inert tags, or by forced or natural feeding. These marks are incorporated into the tissues by the metabolic processes of the body. The biological half-life and behaviour of different isotopes varies in the body. Some isotopes are well dispersed throughout the body, while others become concentrated in certain tissues (e.g. iodine in the thyroid gland). This specific localisation can be used to study a

specific physiological function, but it may result in high local doses of radiation and subsequent tissue damage. Since metabolically active radioisotopes may be voided in urine and faeces and can be passed on to offspring, they can be used to the study of movement and dispersal of labelled animals, as well as their reproductive success (Linn 1978).

Unfortunately, behaviour may be severely affected by the radio-toxic effects of radioisotopes. There are serious doubts about the normalcy of behaviours or movements of an animal suffering from radiation damage. In fact, there is a real possibility that the life of the marked animal may be shortened by such effects (Pendleton 1956). Although radioisotope marking does not allow the identification of individual animals, it has been suggested that individuals could be recognised by injecting small quantities of beta-emitters into different body locations according to a code (Pendleton 1956). However, this is likely to result in significant tissue damage, which could affect behaviour and survivorship.

Exposure to radioactive material is also potentially hazardous for research workers, and radiation levels must be monitored using personal detection units. Under the New Zealand Radiation Protection Act (1982), any researcher using radioactive material must hold a licence and comply with the relevant Codes of Safe Practice. In addition, all persons involved in the study must have special training and be supervised by the licence-holder. The cost of equipment, licencing and setting up a laboratory to handle radioisotopes is high, and transport and safety procedures can be laborious.

Owing to the long half-lives of some isotopes, there may be a risk of environmental contamination. Such contamination can expose members of the public and non-target animals to radiation. For example, in aquatic environments, released isotopes may be absorbed by other organisms (Pendleton 1956). Researchers must,

TABLE 3. RADIOISOTOPES THAT HAVE BEEN USED TO MARK WILD ANIMALS.

ISOTOPE	HALF-LIFE	TOXICITY	METHOD OF USE	NOTES
Antimony-124	60 days	Medium high	-	Penetrates rock walls, some beta emissions, beta radiation reduced if sits for 3 weeks after activation
Cadmium-115	43 days	High	Injected	-
Cobalt-60	5.25 years	High	Implanted, or used in capsules	Very toxic, readily traced due to high gamma energies
Gold-198	2.7 days	Medium high	Implanted	Biologically inert
Iodine-131	8.04 days	Medium high	Injected, used in capsules, injected into baits	Cheap, convenient, low gamma energies
Magnesium-54	312 days	-	Injected	-
Phosphorus-32	14.3 days	Medium low	Injected	-
Selenium-75	121 days	Low		Low gamma emissions, decays by electron capture, so no charged particles are emitted
Sodium-22	2.6 years	Medium high	Aqueous NaCl	Good for larger animals and longer studies, high gamma energy, some beta radiation
Tantalum-182	115 days	Medium low	Implanted	Biologically inert, high gamma energies, low beta emissions
Zinc-65	245 days	Medium low	Injected, fed	-

Information taken from Health and Welfare Canada (1976); Linn (1978); Neitfeld et al. (1994: p. 149).



Examples of metal (above) and plastic (below) tags and bands available for marking wildlife. From National Band & Tag Co. ([www.nationalband.com](http://www.nationalband.com)).



therefore, also carefully consider the potential danger to the public and the environment, and minimise the risk of accidental exposure to radiation (Ferner 1979).

Because of the potential danger posed to researchers, the public, the environment and the marked animals, the use of radioisotopes is contraindicated unless it would allow collection of critical information that could not be obtained by other methods (Pendleton 1956). The relative age of the published reports on radioisotope marking reflects the fact that this method is no longer commonly used to mark wildlife, presumably because of the inherent dangers and difficulties of using radioactive material in the field.

Radioisotopes have been used in the past to track small or nocturnal amphibians and reptiles (Table 2).

## SEMI-PERMANENT MARKS

Semi-permanent methods of identification are designed to last from days to months or years, but most marks are lost within the lifetime of the animal. Therefore, researchers should select materials and attachment methods appropriate to the desired study duration. Semi-permanent methods such as tags, collars, harnesses and bands can be used exclusively to differentiate between marked and unmarked animals, but they are usually coupled with additional information to allow identification of individuals, and are also commonly used to attach telemetric and other equipment (Table 4). Such devices often need to be removed, or are designed to fall off, to facilitate recovery of data and/or to avoid hindrance to the growth and development of the animals. Investigators are obliged to monitor the condition of marked animals and, if necessary, remove the mark at the end of the study (American Society of Mammalogists 1987).

### Tags

Tags are made from a variety of materials—most commonly metal or plastic—and are usually augmented by alphanumeric codes for individual or group recognition. Tags can be applied to ears, webs, flippers, fins, jaws and toes, depending on the anatomy of the animal. In general, there is a trade-off between a tag's visibility and its negative effects on the wearer; larger tags are more visible, but affect the wearer more. The durability and retention of a tag depend on factors such as the tag's material, size, shape and placement location, as well as wearer characteristics which include anatomy, behaviour, habitat and infection rate. In addition, the proficiency of the operator and method used (e.g. whether a hole is cut or punched first) will also influence tag retention. Tags may also be used as attachment vehicles for radioactive marks or telemetric equipment (for tracking animals).

Metal tags are commonly made of aluminium, stainless steel, titanium, Monel (a nickel-copper alloy) and other alloys. Tags used in a marine environment should be made of Monel, stainless steel or titanium to minimise corrosion from salt water (Scheffer 1950). Metal tags are less visible than tags made of coloured plastic (some of which can be used to identify animals from a distance), and

researchers often have to recapture the animals to read them. Metal tags, although less visible to human observers, are also less obvious to predators or prey.

Plastic tags come in a range of colours, some of which fade quickly in natural environments. For example, blue tends to fade and is often unrecognisable in 2 years (Testa & Rothery 1992). However, coloured plastics that are stable under UV light are now available and their use is recommended for longer studies. The disadvantage of using coloured tags is that they may disrupt the animal's camouflage or act as predator attractants (British Columbia Environment Resources Inventory Committee 1997).

Tags have been used to identify amphibians, reptiles and small cetaceans, and are the most common method for identifying pinnipeds (Table 4).

### Collars, harnesses or bands

Collars, harnesses and waistbands are used primarily as vehicles for the attachment of telemetric transmitters, nocturnal lights or radioactive marks. Leg and arm bands are usually inscribed with identifying symbols or codes, or are augmented with identifying tags. The retention of collars, harnesses and bands depends on their material and design, and on the habitat and characteristics of the wearer (such as behaviour, age and sex). Plastic, leather, vinyl, nylon, metal, metal beaded chain, rubberised machine belting and other materials can be used to fashion collars, harnesses and bands. Metal bands must be made of lightweight, rustproof material, and are usually composed of aluminium or alloys.

A correct fit is imperative, as tight equipment can cause skin damage and infection, which can progress to necrosis and even loss of a limb. Neck collars should not restrict feeding, circulation or breathing (Neitfeld et al. 1994). In contrast, equipment too loosely secured puts the animal at risk of entanglement

TABLE 4. SEMI-PERMANENT IDENTIFICATION METHODS<sup>1</sup>.

METHOD	SPEED OF APPLICATION	COMPLEXITY	COST
Tags	Intermediate-Fast, depending on location	Low-Intermediate, depending on location and species	Low-Intermediate, depending on tag material
Neck collars, harnesses, bands	Slow-Fast, depending on method	Intermediate-High, depending on method	Low-High, depending on equipment
Nocturnal lights	Slow-Fast, depending on method of attachment	Low-High, depending on method of attachment	Low-High, depending on method of attachment
Telemetry (radio-, satellite-, bio-), archival data recorders	Slow-Intermediate, depending on method of attachment	Intermediate-High, depending on method of attachment and equipment	Intermediate-High, depending on method of attachment and equipment

<sup>1</sup> Ranks in columns (e.g. slow, intermediate and fast) are qualitative, comparative scores for the parameter listed for the methods in the table. Table continues on p. 25

and/or strangulation. The eventual removal or release of devices must be considered to avoid irritation or constriction. In addition, devices attached to young or growing animals should be designed to expand or break away. Amphibians and reptiles require special consideration as they continue to grow throughout their lives.

Many collars and harnesses are secured using metal parts such as rivets, bolts or buckles. It is extremely important that corrodible or hard parts do not come into contact with the surface of the animal, as they can cause damage to the underlying skin, either by rubbing directly or by electrolysis. Care must be taken to ensure that pressure on blood vessels is minimised, since impaired circulation can lead to infection and sloughing of the skin (Sheldon 1949).

Leg-, arm- and waist-bands have been used to identify a variety of amphibians and reptiles. Collars and harnesses are occasionally used on pinnipeds and dolphins (Table 4). However, attachment of external devices to marine mammals is problematic, owing to the increases in hydrodynamic drag and subsequent changes in behaviour and energy use that they produce (Tanaka et al. 1987).

### Nocturnal lights

Chemical, electrical or radioactive light sources can be attached to animals to track them visually at night. Depending on the light source, animals can be tracked for hours to months or years. Such devices can be attached directly to the animal using non-toxic adhesives or attached using neck collars, harnesses or tags. The detection distance depends on the device and viewing method (e.g. binoculars or night vision goggles) and may vary from a few metres to about 1 km.

Visible light can be produced chemically by mixing dibutyl phthalate and dimethyl phthalate liquids and sealing them in glass spheres. The spheres are glued to the animal's fur or integument. Varying the proportions of the mixture

SHORT-TERM STRESS	INFLUENCE OF OPERATOR	VISIBILITY	APPROPRIATE SPECIES
Intermediate: handling, some pain, infection risk	Intermediate-High, depending on location	Low-High, depending on location and tag size	Terrestrial and marine mammals, some reptiles and amphibians, fish
Intermediate-High, depending on species and method: handling, possible anaesthetic use	Intermediate-High, depending on method	Moderate-High, depending on device, material, location	Terrestrial mammals, birds, some small cetaceans, pinnipeds, some reptiles and amphibians
Low-High, depending on method of attachment: handling, possible anaesthetic use	Intermediate-High, depending on method of attachment	Moderate-High, depending on distance, light intensity, viewing method	Nocturnal terrestrial mammals, reptiles, amphibians, some invertebrates
Intermediate-High, depending on method of attachment: handling, possible anaesthetic use	High	Low-Moderate, depending on attachment method	Any animal large enough to carry equipment without detrimental effect

Continued from p. 24

controls the brightness and duration of light emission, and various colours can be produced (Buchler 1976).

There are also light sources with attached batteries, including miniaturised light-emitting diodes (LEDs) (Carpenter et al. 1977; Wolcott 1977). Light intensity, colour and blinking sequence can be varied to identify individuals. The size of the battery and intensity of the light source influence the lifespan and visibility of such markers.

Betalights consist of radioactive material that decays inside a capsule, causing the phosphor-coating to emit light. All the harmful beta radiation is absorbed by the phosphor, and none escapes to affect the bearer of the light (Linn 1978). Betalights have ranges from 50 m to 1 km depending on the shape, size and viewing method, and a lifespan of 15–20 years (Kruuk 1978; Thompson 1982). Combinations of different colours and light intensities can increase the number of individuals marked.

Nocturnal lights are used primarily to track terrestrial mammals, but they have also been successfully used on amphibians, reptiles and amphibious invertebrates (Table 4).

## **Telemetry**

Telemetry refers to the interception of energy radiated from a device attached to an animal, with the objective of remotely collecting data on the animal's location, behaviour and physiology, and characteristics of the environment surrounding it. Telemetry allows investigators to maintain some distance between themselves and the animal, which can then be studied with minimal effect on its behaviour. Telemetric methods are particularly useful for collecting information about animals that elude direct observation, either through their wide-ranging habits or remote location (MacDonald 1978).

Energy forms that can be used to transmit data include acoustic, electric, magnetic and electromagnetic (e.g. visible light, radio- and micro-waves). In wildlife biology, information is most commonly transmitted using high frequency radio-waves (radio-telemetry). Radio-telemetric equipment consists of a transmitter, a power source and a transmitting antenna, all of which must be attached to the animal. This requirement limits the size of animal that can carry a transmitter package. Transmitters vary in size, mass, longevity and range characteristics, with battery capacity being the primary limitation to transmission longevity and strength (Cochran & Lord 1963). Signals can be emitted continuously or at intervals, and the emission schedule will affect battery usage and operational lifespan.

Transmitter packages can be attached externally (using adhesives, sutures, collars, harnesses, bands or tags), or implanted internally. The general recommendation is that the transmitter weight should not exceed 10% of the animal's body weight (Heyer et al. 1994). However, researchers should strive to make transmitter packages as small as practically possible, to minimise the effects on the bearer. Implanted or force-fed transmitters must be covered in a biologically inert coating. Some transmitters can be placed internally with the power source attached externally. By having an external power source, the internal package size is reduced and batteries can be replaced more easily in long-term studies. In addition, small solar-powered transmitters are available that require no

additional power supply, but their operation depends on weather and varies with behaviour. Such designs would obviously be inappropriate for the study of nocturnal or subterranean species.

External attachment devices and radio-telemetric equipment may alter the appearance of the marked individual enough to affect its interactions with conspecifics, predators and prey. The continuing miniaturisation of equipment will allow the bulk of attachment devices and transmitters, and thereby their effects on appearance, to be reduced (British Columbia Environment Resources Inventory Committee 1997). For example, transmitters weighing less than 1 g have become available (e.g. Carter et al. 1999). However, smaller transmitters usually have reduced battery lives and transmission ranges.

Radio signals are detected by receiving antennae, which can be hand-held, or carried on land vehicles, ships, planes or satellites. Receivers can be tuned to the specific frequency of each transmitter, thus allowing tracking of individual animals. For more details or technical information on the transmission and reception of telemetric data see reviews such as Amlaner & MacDonald (1980); Priede & Swift (1992); and Richards et al. (1994).

The advantages of traditional (manual reception) radio-telemetry include the simplicity and low cost of transmitters and the ability to obtain detailed real-time information on movement. Disadvantages include the relatively high time and labour costs involved in monitoring tagged animals. Radio-telemetry is most suitable for species with relatively small home ranges.

Bio-telemetry involves the transmission of biological information from sensors attached to the animal, without direct contact between the transmitter and receiver (Bengtson 1993). Internal physiological data (heart and respiratory rates, body temperature, electrocardiograms, blood and heat flow) can be relayed, along with behavioural data (e.g. diving time and depth) and environmental information (e.g. ambient temperature, light, salinity).

Archival data recorders are self-contained, bio-telemetric units that collect and archive data for later recovery. The greatest disadvantage of this method is that the recorders must be recovered in order to retrieve data. Attempts have been made to develop devices that will automatically detach from the animal (e.g. Baird & Goodyear 1993; Laws 1993; Westgate et al. 1995), and such research is likely to continue. There is also evidence to suggest that archival data recorders provide more accurate and detailed information than other methods (e.g. satellite-linked telemetry) (Stewart et al. 1989).

Satellite-telemetry, including GPS (global positioning systems), enables information to be relayed from the transmitter to a receiver via satellites, thereby reducing labour and other costs associated with conventional telemetric fieldwork. The ARGOS satellite system<sup>2</sup> is already available for commercial and scientific use. Such

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<sup>2</sup> This system is administered under a joint agreement between the USA's National Oceanic and Atmospheric Administration (NOAA) and the French Space Agency, Centre National d'Etudes Spatiales (CNES). The ARGOS instruments are carried aboard NOAA Polar-orbiting Operational Environmental Satellites (POES) and receive data from Platform transmitter terminals (PTTs), e.g. transmitters attached to an animal. The ARGOS instruments transmit data to telemetry ground stations in the USA and France, where it is processed and delivered to the end user (e.g. wildlife researcher). Because the ARGOS instruments are aboard a moving satellite, Doppler shift calculations can be used to give estimated fixes on the location of a PTT. For more information see Priede & Swift (1992); <http://noaasis.gov.ARGOS>; or [www.cls.fr/html/argos/welcome\\_en.html](http://www.cls.fr/html/argos/welcome_en.html).

systems are of immense value in collecting information on animals in remote areas, especially marine species (Mate 1987). An additional advantage of satellite-telemetry is the unlimited monitoring ranges (e.g. it does not need 'line of sight').

Limitations of current satellite-telemetry technology include the high cost of transmitter equipment, the need to capture the animals to apply the transmitter tags and problems associated with attachment. Limitations imposed by the ARGOS system include the amount of data that can be sent in each transmission, and the number of 'uplinks' that are possible. For successful data reception at the satellite receiver (an uplink), transmission must be coordinated with satellite passes. In the marine environment, this means that the transmitting antenna must be above water and transmitting at the same time as the satellite passes overhead. Therefore, the number of successful uplinks is determined not only by the timing of satellite passes, but also the behaviour of the marked animal. In addition, satellite-telemetry cannot offer detailed real-time information on the movement of the tagged animal (Hanson 2003a).

General disadvantages of telemetry include the cost, complexity of the equipment and limitations of the power source. In addition, study animals must often be recaptured to remove the equipment at the end of the study, or to replace batteries. Errors in telemetry stem from intrinsic system faults, animal movement during reception and topographic effects, such as reflection and refraction of the signals. All these effects can be reduced by user experience (MacDonald 1978). Future work will develop new sensors for bio-telemetry and improved methods of attachment and tag recovery. Non-invasive, remote methods of attachment and release of transmitter equipment need to be developed. At present, telemetric study is restricted to animals large enough to carry the transmitter and battery. The continued miniaturisation of equipment, especially batteries, will facilitate the use of transmitters on smaller animals in the future (Laws 1993; British Columbia Environment Resources Inventory Committee 1997).

Telemetry can be used to track any animal large enough to carry transmitter equipment without detrimental effect, and transmitters have been attached to amphibians, reptiles, cetaceans and pinnipeds (Table 4).

## PERMANENT MARKS

Permanent methods tend to create marks that are less readily visible, and often involve tissue damage (permanent or temporary) (Table 5). Despite their designation, there is no guarantee that these marks will be permanent, and variables including animal species and age, environment and operator experience can strongly influence the permanence of marks (Neitfeld et al. 1994). Permanent marks can be advantageous if they eliminate the need to recapture animals for remarking and/or identification, and can be used in conjunction with more visible, temporary methods. They can also be used to evaluate the rates of mark loss for other methods, e.g. tagging.

## Hot branding

A permanent brand is the result of tissue damage that occurs when excessive heat or cold is applied to the skin at a rate that exceeds the tissue's ability to dissipate it fast enough to avoid cell destruction (Pope 1993). Brands can have symbolic shapes to identify groups or individuals and, when successful, can produce highly visible, long-lasting marks. The objective of hot branding is to promote the formation of scar tissue that has few viable hair follicles (i.e. the area is bald) or is visibly different from the surrounding skin. Correctly applied hot brands can last the lifetime of an animal; successfully branded animals do not have to be handled again for re-marking. However, depending on the size of the animal and the mark, branded animals may have to be recaptured for subsequent identification. Although variation exists between species, hot brands are always applied for a shorter time than cold brands, and the success of hot branding can be determined shortly after marking.

Branding tools are made of steel or iron, and can be heated or activated by three methods: battery, electricity and open flame (e.g. wood fire, propane gas flame). The size and shape of the irons will depend on the species being marked. However, certain numerals and letters are easily confused, especially when branding is incomplete or poorly executed. Examples of numbers that may be confused include: 8 and 3, 6 and 5, 9 and 0 (Battaglia 2001).

Electric irons are expensive and often have two or three characters clustered on one handle. This can make the irons too small and close together for the handle to be rocked for good application. In addition, the time between successive brand applications increases because of the additional time required for the irons to reheat (Battaglia 2001). However, electric irons automatically reach the correct temperature for branding, thereby reducing the need for subjective judgement, that is required when using other heat sources.

The major disadvantage of hot branding is lack of consistency in the resulting marks. Operator experience can markedly affect success with regard to the permanence and legibility of a mark. A major problem is judging when the irons are hot enough to achieve a clear, lasting brand. In addition, the correct duration of application will differ with species, age and size of the animal, location of the brand and the presence of hair or fur. Finally, animals must be well restrained, immobilised or anaesthetised in order to achieve clear brands.

Experimental work suggests that hot branding causes more immediate pain and discomfort than freeze branding (Schwartzkopf-Genswein, Stookey, De Pasille et al. 1997; Schwartzkopf-Genswein et al. 1998). It may be appropriate for researchers to use analgesics to ease such discomfort. In addition, hot branding is aesthetically unpleasant, both for the researcher and the public. Although the heat will initially sterilise the wound, the risk of subsequent infection is higher than with some of the less invasive methods of marking, owing to the severity of the tissue damage and the time required for wound healing.

Pinnipeds are commonly marked by hot branding, and this method has also been used to mark amphibians and reptiles (Table 5). However, great caution is required when hot branding amphibians, because of the delicate nature of their skin and its physiological importance (e.g. branding can cause fatal water loss) (Ferner 1979).

## Freeze branding

Freeze branding selectively destroys the pigment-producing cells (melanocytes) in the hair follicles, resulting in the production of white hair or de-pigmented skin that contrasts with the original coat/skin colour. If properly applied, freeze branding produces long-lasting, clear and highly visible marks. Many large animals do not need to be recaptured for identification (Newton 1978). In addition, the local anaesthetic effect of refrigerants leads many researchers to believe that freeze branding is less painful than hot branding (Scheffer 1950; Schwartzkopf-Genswein, Stookey, De Pasille et al. 1997; Schwartzkopf-Genswein et al. 1998).

Freeze branding must be carefully tested in each species to determine the optimum application time required to kill the melanocytes without destroying the hair follicle (Scheffer 1950). Applying the brand for too long ('over-branding') will produce extensive skin sloughing and may result in a bald scar, fringed by white hair (Taylor 1949). 'Under-branding' may produce a few white hairs or a de-pigmented area, but the mark will be illegible (Battaglia 2001). The application time required to produce de-pigmented hair or skin depends not only on the species involved, but also the phase of the growth cycle of each follicle. In addition, the time and temperature required to achieve a legible

TABLE 5. PERMANENT IDENTIFICATION METHODS<sup>1</sup>.

METHOD	SPEED OF APPLICATION	COMPLEXITY	COST
Hot brands	Fast	Low-Intermediate, depending on heat source and amount of hair	Low
Freeze brands	Slow-Intermediate, depending on species	Intermediate-High, depending on method	Intermediate
Chemical brands	Slow-Intermediate, depending on method	Intermediate-High, depending on method	Intermediate
Tattoos	Slow	Intermediate	Intermediate
Passive Integrated Transponders	Intermediate-Fast	High	High
Visible Implant Fluorescent Elastomer tags	Intermediate	Intermediate	Low
Tissue removal	Fast	Low	Low
Vital stains	Intermediate	Intermediate	Intermediate-High, depending on stain and recovery method
Natural marking identification	Slow-Fast	High	Low-Intermediate, depending on equipment

<sup>1</sup> Ranks in columns (e.g. slow, intermediate and fast) are qualitative, comparative scores for the parameter listed for the methods in the table.

Table continues on p. 31

brand depends on: pressure of application; colour (amount of pigmentation) and hydration status of the animal; amount of under-fur; hair density; anatomical site; age of the animal; and amount of subcutaneous fat (Hooven 1968).

Freeze branding irons are made of steel, stainless steel, brass or copper. Copper and copper alloys are reported to be most efficient for heat transfer (Hooven 1968). The refrigerant materials required for freeze branding can be dangerous and impractical in the field, and safety equipment is often required to use them. Liquid nitrogen can be used to supercool irons for freeze branding, but it is expensive and difficult to transport and store. The advantage of using liquid nitrogen is that it maintains a constant temperature (-196°C).

Alternatively, solid carbon dioxide (dry ice) mixed with alcohol (e.g. 99% isopropyl alcohol) can be used to supercool branding irons. Dry ice is more readily available, easier to store and is reported to produce better results than liquid nitrogen (Newton 1978). Irons supercooled in dry ice and methanol drop to temperatures from -67°C to -77°C. Aerosol coolants can also be applied to the skin using stencils to produce symbols (Tanaka et al. 1987). Examples of aerosol coolant systems are pressurised Freon, or combinations of chlorodifluoromethane and dimethyl ether.

SHORT-TERM STRESS	INFLUENCE OF OPERATOR	VISIBILITY	APPROPRIATE SPECIES
Intermediate-High: handling, pain, infection risk	Very High: application duration, temperature of branding iron	Intermediate-High, depending on location, size, success of brand	Terrestrial mammals, pinnipeds, reptiles
Intermediate: handling, delayed pain, infection risk	Very High: application duration, temperature of branding iron	Intermediate-High, depending on location, size, success of brand	Terrestrial mammals, pinnipeds, small cetaceans, reptiles, amphibians
Intermediate: handling, skin irritation, infection risk	High: application duration, chemical	Intermediate-High, depending on location, size of brand	Terrestrial mammals, amphibians
Intermediate-High: handling, pain, infection risk	High: location and depth of ink application	Low	Terrestrial mammals, pinnipeds, reptiles, amphibians
Intermediate-High: handling, pain, infection risk	Intermediate-High: location and depth of transponder	Not visible	Terrestrial mammals, pinnipeds, birds, reptiles, amphibians, fish, invertebrates
Intermediate-High: handling, pain, infection risk	Very High: location and depth of elastomer implantation	Intermediate, depending on location and depth of implant	Larval and transparent-skinned amphibians, fish
High: handling, pain, infection risk	Low	Low-Intermediate, depending on method	Reptiles, amphibians, some terrestrial mammals and pinnipeds
Low-High, depending on method of administration and data recovery	Low	Low-Intermediate, depending on method, species, visibility of targeted structure	Terrestrial and marine mammals, larval amphibians
Low-Intermediate, depending on handling required	Very high	Low-High, depending on marks, size of animal	Any animal with stable, distinguishing natural markings

Continued from p. 30

The animal should be restrained, and if necessary, the branding site clipped to remove hair and under-fur. The area should be cleaned of dirt and dander, and wetted with alcohol to prevent the iron from sticking, and tearing the skin when it is removed (Macpherson & Penner 1967a). The irons should be cooled in the refrigerant until they reach the correct temperature, indicated when the refrigerant stops boiling. Cold irons must be applied for longer periods of time than hot irons; freeze branding can take from 10 seconds to 5 minutes per brand (Farrell 1966). The most appropriate application time and pressure for each species should be determined in captive animals first.

The major disadvantages of freeze branding are inconsistency in the marks produced, and an inability to determine whether branding has been successful until some time afterwards. It is also extremely difficult to determine the application time that will produce a legible mark without damaging the skin and hair follicles. Movement by the animal during application can result in a smeared or blurred brand and streaks may occur if too much refrigerant remains on the iron when it is applied. When animals age, pigment loss occurs naturally, as old melanocytes become exhausted or inactivated. This natural depigmentation could obscure freeze brands in older animals (Taylor 1949). In addition, dirt may disguise de-pigmented hair, and freeze branding white animals requires the production of bald brands.

Freeze branding has been used to mark amphibians, reptiles, pinnipeds and some small cetaceans (Table 5).

### **Chemical branding**

Certain chemicals can be applied to the skin of animals to produce marks which contrast with the surrounding hair or skin. The current theory is that such compounds inhibit melanin production in the hair follicle, leading to the production of white hairs or de-pigmented skin (Schwartzkopf et al. 1994). The compounds can be injected into the dermis or applied directly to the epidermis. The successful production of long-lasting legible marks depends on the chemical used and how long it is applied. However, de-pigmenting chemicals are often extremely irritating to the tissues.

Chemical branding for marking wildlife is not common, but has been used on amphibians (Table 5).

### **Tattooing**

Tattooing is one of the most permanent methods for marking animals. However, the durability of a tattoo depends on the species and age of the animal, as well as the application quality and the depth and location of the mark. The trade-off for their permanence is that tattoos are not readily visible from a distance, so that animals generally need to be recaptured for identification. Virtually unlimited numbers of animals can be individually identified with tattoos. They are often used in conjunction with other, more conspicuous marks, allowing identification when the less durable mark is lost, and can also be used to assess loss rates of semi-permanent marks, e.g. tags.

Before tattooing, the site should be cleaned with alcohol, as infection or warts can result if the tattoo is placed on dirty skin. There are two ways to introduce pigment into the skin of an animal. Firstly, forceps, pliers or hammer

instruments are used to pierce the skin, and indelible dye, ink or paste is then rubbed into the pinprick wounds. Forceps or pliers pierce the skin in patterns of letters and numbers when squeezed together, e.g. ear tattoos. Hammer systems are swung onto larger animals to create a pattern of pinpricks into which dye is rubbed. Tattoo paste or ink must be rubbed in well to ensure a long-lasting mark.

Secondly, electro-vibrator systems or needles that both pierce the skin and inject the dye can be used. Human tattoo guns are an example of the electro-vibrator system. These tools are used simply to 'write' an identifying code into the skin. Woodbury (1956) noted that the free ends of numerals such as 2, 3, 4, 5, 6, and 9 should be extended to aid in distinguishing them from each other. Dye can also be injected subcutaneously or intra-dermally using a needle. Although the size of the animal may limit the amount of information that can be applied, tattooed dots can also be applied in various locations to form a code for identification of smaller animals (e.g. Measey & Tinsley 1998).

There are two potential problems with tattooing animals. Firstly, the dye must contrast with the normal skin pigmentation. Tattooing materials include picric acid, methylene blue, yellow eosine, rhodamine and various black inks and dyes (Konig 1989). Black or red ink should be used on non-pigmented skin, while yellow or white ink should be used on pigmented skin. Secondly, loss of tattoo legibility can occur due to dye diffusion or degradation by UV light (British Columbia Environment Resources Inventory Committee 1997). Modern inks are longer lasting than old inks, reducing the risk of fading.

Tattooing has been used to mark amphibians and reptiles, and is often used to assess tag loss in pinnipeds (Table 5). Furred animals can be tattooed on naked parts, such as the soles of their feet, inguinal region, or inner lips or ears (Leclercq & Rozenfeld 2001).

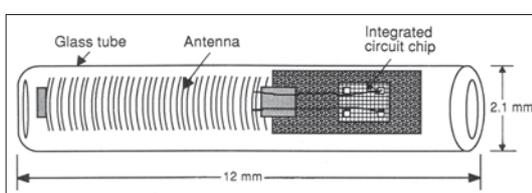
### Passive Integrated Transponders

Passive Integrated Transponders (PITs) are small electronic units encased in biologically inert glass capsules (Bridle 1973; Prentice et al. 1990; Paschke 1995; Jansen et al. 1999). With a diameter of 2 mm and length of 12–32 mm, PITs are suitable for identification of a wide variety of animals. They do not require a continuous power source (e.g. battery); when the tag is held in an electromagnetic field the microchip transmits its own unique identification code to an electronic reader (Prentice et al. 1990).

Each PIT is programmed with a unique code, allowing virtually unlimited numbers of animals to be individually marked. PIT tags can be read through soft and hard tissue, salt and fresh water, glass, wood and plastic, but are difficult to read through metal. Extreme heat or cold (i.e. temperatures ranging from -90°C to +60°C) does not appreciably affect detection or reading of PIT tags, and because they do not rely on a power source, PITs can theoretically operate indefinitely (Prentice et al. 1990). The relative permanence of PIT tags means that they are appropriate for long-term studies.

PITs are most commonly injected subcutaneously or intra-abdominally, but can also be swallowed (within boluses) or attached as part of an external tag (Rossing 1999). In most

Diagram of a typical Passive Integrated Transponder Tag.  
From Prentice et al. 1990.



cases injection can be completed in less than 1 minute plus the time required to organise the equipment and register the code (Klindtworth 1998). Although tags can be implanted in a variety of places, location has a major effect on the retention and migration of the tag and should be carefully researched before PITs are used in the field. Manipulation of the PIT away from the point of insertion is a major factor in reducing post-injection tag loss (Klindtworth 1998). In addition, gluing, adhesive taping or suturing the injection site may prevent tag loss, especially if a scalpel incision has been made. Newly inserted tags should be checked with a reader to ensure that they are working properly before the marked animal is released.

The PIT reader is used to generate the electromagnetic field, as well as receive the transmitted code, which it displays on a screen (Prentice et al. 1990). The distance of tag detection varies with transponder and antenna specifications, strength of the electromagnetic field and orientation of the transponder relative to the receiving antenna. Identification can generally be achieved only by recapturing the PIT-tagged animal owing to the short reading distances currently available. Most hand-held readers can detect tags only from 5–8 cm away. Fixed readers have an average detection range of about 18 cm, although some may detect tags up to 100 cm away, depending on the size of the transponder and electromagnetic field strength (Klindtworth 1998). These distances have improved and will probably continue to do so, eventually allowing remote identification of more mobile species.

As well as marking individuals, PITs also allow automatic monitoring of free-living animals passing near the antenna of a reader. Readers can detect relatively fast passages (e.g. at speeds up to 3.6 m/s), but the tag must pass within 7–18 cm of the antenna. Such systems allow collection of data over long periods of time with relatively little effort, and may be particularly useful for remote study sites (Harper & Batzli 1996).

The greatest advantage of PIT tagging is the high retention rates of internally injected tags. Many reports cite retention rates of 95% to 100% (e.g. Camper & Dixon 1988; Prentice et al. 1990; Rao & Edmondson 1990; Elbin & Burger 1994; Freeland & Fry 1995; Galimberti et al. 2000). Although the incidence of tag loss is low, such loss is difficult to assess, because the mark is not externally visible. If an implanted tag is lost or undetectable, there is no way to tell whether an animal is marked or unmarked, let alone identify the individual. Researchers may palpate the area where PIT tags were implanted, but this can sever the connections between the fibrous capsule and the surrounding tissue and may result in increased PIT migration, and possibly tag loss (Jansen et al. 1999).

Tag loss is primarily attributed to faulty implantation or to an inability to detect tags in large animals (Prentice et al. 1990). Transponder failures can occur because of damage to the capsule during implantation, or PIT loss immediately after injection (Conill et al. 1996; Sutterluety 1996). The level of experience of the operator has a major influence on tag loss (Geers et al. 1997). Inability to detect a PIT is most often due to migration of the tag within the animal. Not only does migration make detection difficult, but moving transponders can also be a risk to internal organs (Lambooy & Merks 1989). The degree of migration depends on the size and location of the implant, implantation method, tissue reaction and the age and species of the animal. Tags placed in areas associated

with movement are more likely to migrate (Jansen et al. 1999). Therefore, tags are generally inserted into areas on the skull, around the ears or into the body cavity itself. The problem of migration has been reduced in recent years by the addition of bondable sheaths to the transponder capsules (Rao & Edmondson 1990; Park & Wieser n.d.).

The primary disadvantage of PIT tagging is the high initial cost. Each PIT tag costs significantly more than plastic or metal tags, or tattooing and branding equipment. In addition, relatively expensive readers must be purchased (Hammer & Blankenship 2001). If a research group can afford only one reader, identification of animals can be time-consuming (Harper & Batzli 1996). However, the high initial costs may be offset by lower labour costs and low transponder replacement costs (Jansen & Eradus 1999). Equipment costs are also likely to decrease as technology advances. Other disadvantages include the fact that if the reading equipment fails in the field, animals cannot be identified, and that migratory animals marked with PITs cannot be visually identified by researchers at distant locations.

Advances in transponder technology include the development of sensor transponders, which have the ability to instantaneously measure physiological parameters. Simple sensors for temperature are already available, but sensors that detect heart rate and physical activity are more difficult to develop since an uninterrupted power source is required for continuous measurement (Jansen & Eradus 1999). Ongoing research into the miniaturisation of batteries and transponders, and alternative power sources, will increase the potential for implanting transponders with sensors.

PIT tagging has been used to identify amphibians, reptiles and pinnipeds (Table 5).

### **Visible Implant Fluorescent Elastomer tags**

Visible Implant Fluorescent Elastomer (VIE) tags consist of two bio-compatible elastomer materials that are injected under the skin as a liquid and cure into a pliable solid. Colour elastomer and a curing agent are mixed in a 10:1 ratio and injected using hand-held syringes or air-powered injectors (Davis & Ovaska 2001). The flexible nature of the compound, and its tendency to occupy available space rather than displace and irritate surrounding tissues, means that VIE tags are retained better than rigid internal tags (Northwest Marine Technology Inc n.d.).

Five fluorescent colours (red, yellow, green, orange and pink) and five non-fluorescent colours (black, blue, brown, white and purple) are available from Northwest Marine Technology Inc. Both fluorescent and non-fluorescent colours are visible under ambient light, which may make marked animals more conspicuous to predators or prey. The non-fluorescent pigments can be difficult to visualise under darkly pigmented skin, and should be used only in translucent tissue, when detection will occur under bright ambient light. While fluorescent pigments are also visible under ambient light, their detection is greatly enhanced with the use of a fluorescence enhancing technique such as blue or UV light (see figure p. 67). The combination of different colours and tag locations allows identification of individual animals; however, VIE tags are most often used for batch identification.

The success of VIE tagging depends heavily on the proficiency and experience of the operator. If a tag is injected too deeply, the mark may not be easily seen on subsequent occasions. Alternatively, if the tag is too shallow, or material is left trailing out of the injection wound, the tag can work its way out of the body leading to mark loss. Mark loss is the major disadvantage of VIE tagging. Misidentification is highly likely because often there is no indication that marking has occurred, e.g. there is no injection hole or scarring (Davis & Ovaska 2001). This can lead to population overestimates, as the number of unmarked individuals relative to marked individuals appears to be higher than it really is.

To allow recognition of mark loss, Davis & Ovaska (2001) recommended that the same number of marks per animal be used at a given study site. In addition, tags should be inserted into positions that are widely spaced on the body, as some subcutaneous migration of marks does occur. Marks may also break into two or more dots, so only one dot or line per position should be used. For maximum tag visibility, tags should be inserted into relatively translucent body parts, such as ventral or lateral surfaces. Finally, certain colours are difficult to differentiate. For example, green and yellow tags look similar under UV light, so only one of these should be used per site.

Also available from Northwest Marine Technology Inc. are Soft Visible Implant Alphanumeric (VIAAlpha) tags. VIAAlpha tags are pliable fluorescent tags bearing letters or numbers about 1 mm high. The tags are injected into translucent skin, and have been used to individually identify fish and salamanders. They are visible under ambient light, but detection is greatly enhanced using blue or UV light. A hand lens ( $\times 10$ ) may also be required to read the inscribed symbols. VIAAlpha tags are reported to be well retained and long lasting (Haw et al. 1990; Niva 1995; Measey et al. 2001).

The advantage of the VIE system is that the amount of elastomer material required is small, so that the method can be used in small animals. Moreover, the materials are relatively inexpensive. However, at present VIE and VIAAlpha materials are only available from Northwest Marine Technology Inc. VIE tags are most useful for marking groups of animals, while VIAAlpha tags can be used to easily identify individuals. Marked animals must usually be recaptured to read VIE tag codes and VIAAlpha inscriptions.

VIE and VIAAlpha tags are visualised through transparent skin and are, therefore, suitable only for animals like amphibians.

### **Tissue removals**

This identification method is based on the removal of tissue in coded sequences. Each ear, toe, disc or web location is assigned a code and the combination of removals provides a single identification number (e.g. Martof 1953; Donnelly 1989; Hero 1989). Large numbers of individuals can be marked using tissue removal codes. However, the more individuals to be marked, the more tissue removals are required per animal. Toe and disc removals are only performed on small animals, primarily because blood loss from them is low or well tolerated.

All tissue removal methods are extremely easy, fast and cheap to perform. Tools for removing tissue include nail clippers, scissors, ear punches or notchers. Equipment should be kept extremely sharp as bruising and tearing can occur when tools become blunt. Tools should also be kept very clean to prevent the transmission of diseases and to minimise the risk of infection (Society for the Study of Amphibians and Reptiles 1987). The low complexity of tissue removal methods means that operator experience has minimal influence on marking success, unlike other systems such as branding and PIT implantation. In addition, removed tissues can also provide valuable additional data on age and genetics.

Tissue removal marks cannot be lost in most species, but there is the possibility of regeneration in some amphibian species (salamanders, some anurans). There is also a real risk of infection or protracted healing when removals involve severing skin, muscle and bone (Lemckert 1996).

Although the marking procedure itself is simple, reading the identification codes is more difficult and the potential for misidentification is great. The coding system should be well documented so that future identification is as accurate as possible. In addition, natural tissue loss may confound identification systems. Researchers should endeavour to include naturally lost tissues in their codes, to minimise the number of additional removals. Identifying animals marked with tissue removal codes is time-consuming and requires practice. In addition, animals must almost always be recaptured to read identification codes properly.

Unfortunately, the possible effects of tissue removal on the health, behaviour and well being of the subject animals have been little studied and are poorly understood (Society for the Study of Amphibians and Reptiles 1987). For this reason, scientists must be diligent in their preparatory research and be able to quantify the effects of their marking method on the subject animals. For instance, the effects of toe clipping should be determined by studying captive animals before the method is used in the field.

Toe clipping is the most common method for marking amphibians and reptiles. Ear notching should not be performed on species with specialised ears, e.g. otariid seals.

### **Autotransplantation**

Autotransplantation refers to the process of grafting an individual's own skin from one location to another on its body. Individual recognition is possible using various combinations of locations and graft numbers. However, this method is difficult to use for species with naturally irregular integument patterns. Skin autografts often fail owing to graft rejection, and the procedure is often impractical for use in the field (Mrozek et al. 1994). In addition, the use of anaesthetic is generally necessary, and significantly increases the risk to the animals. The high risk of infection may make this method inappropriate for vulnerable populations.

This method is appropriate only for species with natural variation in integument pigment, and its use has been reported only in amphibians, e.g. Rafinski 1977; Verhoeff-de Fremery & Vervoordeldonk 1982; Mrozek et al. 1994.

## Vital stains

Certain chemicals can be used to mark anatomical structures in living animals. Vital stains can be injected intravenously or administered orally, and are primarily used to determine age and to study metabolic processes. They allow measurement of the growth of stained tissues (e.g. teeth, bones, hair, claws, gut wall) between the time of stain administration and subsequent inspection. For example, in some species, certain hard tissues are deposited in annual layers. The administration of a vital stain at a known age will allow subsequent layers to be counted, and a determination of present age to be made.

Some vital stains can be visualised within a live animal, but many require extraction of the tissue or euthanasia of the animal and necropsy to recover the marked tissues (Schevill 1974). Vital staining can provide information that may be impossible to collect otherwise, e.g. age in cetaceans (Mitchell & Kozicki 1975). However, researchers must evaluate potential vital stains to ensure that they are non-toxic, and do not adversely affect the behaviour or health of the marked animal.

Examples of vital stains include tetracyclines, quinacrine, rhodamine B, alizarine red and lead acetate. Tetracyclines combine with newly deposited calcium ions and fluoresce yellow when a bone or tooth is exposed to UV light (Best 1976; Neitfeld et al. 1994). Such staining is limited to areas of new growth, and tooth extraction or euthanasia of the animal is required to examine the hard tissues. This type of marking is not useful for large-scale marking or the identification of individuals, and is of limited use in field studies as the equipment and procedures required to perform readings are fairly sophisticated (Erickson et al. 1993).

Rhodamine B can be administered orally and marks the gallbladder, gut, faeces, urine, and oral and urogenital openings (Lindsey 1983). It can also be used as a systemic marker and may produce fluorescent banding in actively growing tissues such as claws and hairs (Johns & Pan 1981; Lindsey 1983). The marks may be visible 24 hours after stain administration, and persist for several weeks. The advantage of this method is that necropsy is not required to observe the marks.

Theoretically, any animal can be marked with a vital stain; however, staining that requires euthanasia of the animal for data recovery may be inappropriate for vulnerable species (see, *Dyeing*, pp. 42–43 for vital staining of larval amphibians).

## Natural marking identification

Although not technically a marking method, identification of animals by their natural markings is commonly used in wildlife biology, and is becoming increasingly popular for identifying individuals in vulnerable populations. Photographic recordings, sketches, coded descriptors or a combination of all three have long been used to keep track of individual features in a variety of animals (e.g. Carlstrom & Edelstam 1946; Stamps 1973; Scott 1978; MacConkey 1999).

Natural marking identification systems require that the animals have a few distinguishing characters. The first step is to search the animal from end to end

and list all the characters found. Features used for identification include: sex and size of the animal; colour; presence or absence, size, shape, location and configuration of a mark or group of marks; and individual idiosyncrasies such as scars, deformities or behavioural oddities. Oddities should be used only to supplement physical features, as they may not be unique or stable (Pennycuick 1978).

Next, the number of characters required for reliable identification, and the average amount of information obtained from each character should be determined (see Pennycuick 1978). The information content and identifying value of each character will vary. A character that is rare within a population will be of greater value for identification than one that is common. Characters that are unlikely to change over the animal's lifetime should be used preferentially. For example, genetically determined pigmentation patterns and wrinkle patterns are very stable. In contrast, marks caused by physical damage may fade with time, and new marks may occur. Therefore, caution must be exercised when including scars in identifications. Graded characters should also be avoided, as subjective judgement is required and is likely to differ between observers (Pennycuick 1978).

Identifying animals using natural markings is never entirely reliable. For instance, a researcher cannot be certain that an individual is the only one so marked in the population, as a particular combination of markings may be repeated. For complete reliability, artificial marks must be employed. However, the probability of pattern duplication can be predicted. Odds of 100:1 are generally considered reliable. The amount of information required to achieve these odds depends on the complexity of the pattern, as well as the size of the population. In general, greater pattern complexity is required to identify an individual in a large population than in a small one (Pennycuick 1978).

Identification using natural markings may make catching or disturbing the animals unnecessary if characters can be recognised at a distance. In addition, natural identification is likely to be well received by the public as it is non-invasive, does not cause pain and does not alter the appearance of the animal. These methods will have no effect on the behaviour and survivability of the animals, except perhaps through repeated capture and handling where these are necessary for identification.

Natural marking identification can be laborious and time-consuming, and requires training and experience. In addition, pattern mapping is open to observer bias and the characters and scoring methods must be carefully outlined to avoid misidentification. For these reasons, the method is most useful for intensive studies of small populations. In such experiments, a few well-trained observers can get to know the population, and may be able to confidently identify individuals quickly. These methods are also useful to identify control groups in order to test the effects of other marking methods on subject animals.

Natural identification may require animals to be handled for longer periods of time than other methods. However, advances in photographic technology have allowed accurate representation of identifying characters without the laborious job of sketching each animal. Polaroid and digital cameras may decrease handling times, and help avoid observer bias, but the equipment is still

relatively expensive. Pattern mapping is labour-intensive, even with the use of photographs. However, advances in computer technology are aiding the development of systems wherein a digital photograph can be loaded into a program that can code identifying characters very quickly, reducing labour costs and observer bias.

Variations in natural markings are commonly used to identify amphibians, reptiles, cetaceans and occasionally, to identify individual pinnipeds.

Continue to next file: [MarkingwildlifeA.pdf](#)

# Amphibians

New Zealand is home to four unique frog species, as well as three introduced frog species. The native frogs (Family: Leiopelmatidae) are generally small, secretive, camouflaged (cryptic) and mostly silent. In contrast, the introduced species (*Litoria* spp.) are more commonly seen, as they are often brightly coloured and highly vocal.

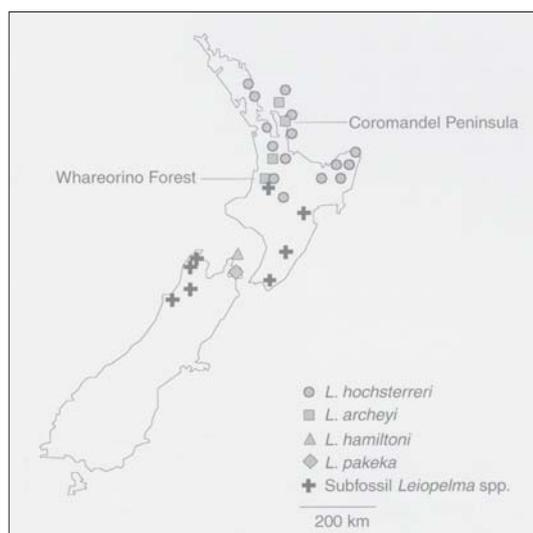
All four native frog species are currently considered to be under threat (Hitchmough 2002).<sup>3</sup> Hamilton's frog (*Leiopelma hamiltoni*) and the Maud Island frog (*L. pakeka*) exist as populations found at only one location each. Hamilton's frog is classified as Nationally Critical, with only a few hundred members existing in the wild (Newman 1990, 1996; M. Tocher, DOC, pers. comm. 2003). The population of Maud Island frogs, although less vulnerable (Nationally Endangered), is still small. Archey's frog (*L. archeyi*) is listed as Nationally Critical, as populations have been severely depleted since the arrival of the pathogenic chytrid fungus in New Zealand. Hochstetter's frog (*L. hochstetteri*) is slightly less vulnerable (listed as Sparse), having larger numbers and a greater scatter of populations. Hochstetter's frog is semi-aquatic, while the other three species are terrestrial.

Leiopelmatid frogs are unique and may be difficult to mark for several reasons. They are relatively long-lived and slow to mature, with Archey's frogs living for 17 years or more and some Hamilton's frogs surviving for over 20 years (Bell 1994). Marks must, therefore, be long-lasting for longitudinal studies of these species (Whitaker & Alspach 1999). Leiopelmatid frogs never achieve snout-vent lengths (SVL) more than 50 mm, which precludes the attachment of most equipment or the application of large marks such as brands or tattoos. Large marks may also interrupt the frogs' disruptive colourations, i.e. patterning that does not match the outline of the body. This cryptic colouration is important

for avoiding predation, and should not be interrupted by identifying marks (Eggers 1998). The small size of New Zealand native frogs may also complicate the use of PITs and other implanted markers, owing to the large size of the transponder relative to the animal's body. For instance, a 12-mm-long PIT would not be suitable for an animal less than 50 mm long.

Another serious consideration when using any marking method to study amphibians in New Zealand is the recent emergence of the fungal infection chytridiomycosis. The chytrid fungus is waterborne and pathogenic to post-metamorphic anurans. Infected frogs exhibit unusual posture, fail to flee upon approach during daylight hours and have difficulty righting themselves. Infection may be transmitted by juveniles, healthy carrier adults and

Distribution map of extant and extinct species of *Leiopelma* frogs in New Zealand (after Worthy 1987; Bell 1994; Bell et al. 1998). From Holyoake et al. 2001.



<sup>3</sup> Refer to Molloy et al. (2002) for information about New Zealand's classification system for threatened species.

waterfowl, and by humans on boots, clothes or field gear. Translocation of apparently healthy tadpoles by the public and the pet trade may also have aided the spread of chytridiomycosis to new localities within New Zealand (Waldman et al. 2001). Quarantine and hygiene procedures are now in place on island sites to decrease human spread of the chytrid fungus.

Not only direct transmission, but also the stress resulting from human interference, may increase the vulnerability of native and introduced frogs to infection. Individuals may suffer immunosuppression due to capture, handling or marking, and may subsequently become more vulnerable to infection. Such effects are illustrated by the fact that handled populations of frogs and toads succumb to common bacteria that are normally harmless, such as *Aeromonas hydrophila* (Waldman et al. 2001). This is yet another reason for researchers to select handling and marking methods that minimise stress to the animals involved.

## TEMPORARY MARKS

### Painting

Only one report of paint application to anuran amphibians has been found. Moore (1954) marked common toads (*Bufo bufo*) with dabs of blue oil paint. The paucity of reports probably reflects the difficulty of applying paints to the moist and highly absorbent skin of amphibians. Amphibian skin plays important roles in thermoregulation, water regulation and gas exchange (Dorit et al. 1991). Even non-toxic paints could conceivably have detrimental effects if absorbed in high concentrations through amphibian skin. The possibility that painting a large skin area could detrimentally affect exchange functions of the skin should also be considered. However, salamanders have been temporarily marked with felt ink pencils (Woolley 1962). The marks lasted at least 1 month if acetic acid or ammonium hydroxide were used to remove slime from the skin before application. Woolley (1962) did not report any negative effects of marking or slime removal. However, slime plays an important role in moisture retention and gas exchange, and its removal may be deleterious to amphibians (Clarke 1997).

New Zealand native frogs generally have cryptic colouration, thereby allowing them to merge visually with their environment. It is likely that the application of paint marks could adversely affect their ability to avoid predators or secure prey. Consequently, survival and reproductive outcomes may be altered through the effects of paint marking.

### Dyeing larval amphibians

New Zealand native frogs emerge from the egg as tailed adults, bypassing the tadpole phase (Eggers 1998). Although larval marking methods are not necessary for studying Leiopelmatid frogs, they may be appropriate for studies of the introduced *Litoria* species and will be discussed briefly in this context. General considerations for marking larval amphibians are their small size, delicate tissues and impending metamorphosis. In addition, larval amphibians are highly preyed upon and, ideally, marking methods should not increase their conspicuousness. Most larval marking methods cannot be used to identify individuals.

Larval anuran amphibians have been stained using Neutral red (Herreid & Kinney 1966; Guttman & Creasey 1973). The larvae were immersed in solutions of one part Neutral red stain to 25–50 000 parts pond water. Tadpoles developed bright red venters and retained the stain for up to 10 days. Guttman & Creasey (1973) reported an immediate mortality rate of 9% in green frogs (*Rana clamitans*), which may be unacceptable for endangered frog species. Dyed and non-dyed wood frog tadpoles (*R. sylvatica*) were found to survive equally well in laboratory studies (Herreid & Kinney 1966). However, the question remains as to whether dyed tadpoles in the wild would be more susceptible to predation. In addition, Travis (1981) found that staining larval barking treefrogs (*Hyla gratiosa*) with dyes such as Neutral red may have affected the subsequent growth of the larvae.

Organic stains such as Oil red O and Oil blue M were used to dye mineral oil that had been mixed with petroleum jelly and injected into leopard (*R. pipiens sphenoccephala*) and bullfrog (*R. catesbeiana*) larvae and eastern tiger salamander larvae (*Ambystoma tigrinum tigrinum*) (Seale & Boraas 1974). Dye was injected subcutaneously with a 22-gauge needle, leaving a mark about 0.5 cm in length. The marks were placed in the tail fin cavity, adjacent to the tail musculature. If an excess of either compound was injected, the undulations of the larval tail caused the marks to be ejected. No infection, mortality, impairment of movement or retardation of growth was reported in larvae observed in the laboratory for up to 1 year. All species retained their marks for the duration of the larval stage. During metamorphosis, the dye-solvent mix was resorbed with the tail, with no apparent effects on the adult.

All larval dyeing methods appear to be time limited, most do not allow individual identification and the marks may have significant effects on the larvae's conspicuousness to predators and on growth and survival.

### **Attached devices**

In a short-term study of frog behaviour, Dole (1965) glued a bobbin to an elastic band secured around the waist of a leopard frog. When the frog moved, a trail of nylon thread unravelled from the bobbin, marking where the frog had been. The thread lasted from 1 hour to 7 days, depending on the activity of the frog. When full of thread, the device weighed about 8.5 g and may have shortened the frog's jumping and swimming abilities. Frogs were seen to have difficulty entering crevices, which may have affected their ability to avoid predators. This method had a noticeable effect on the frogs, and data resulting from such a study are not likely to represent the normal behaviour of leopard frogs.

Thread bobbins were also used to track the movements of American (*Bufo americanus*) and California (*B. boreas*) toads (Tracy & Dole 1969; Dole 1972). Heyer (1994) noted that thread bobbins are useful only for tracking large terrestrial anurans (> 60 mm SVL), and only for distances of about 50 m. General problems associated with the use of thread bobbins include that fact that animals often become entangled, and can drown when they enter water. In addition, the animal cannot escape when the thread runs out, as the end is tied to the bobbin. Finally, the elastic waistband may irritate the skin on the hips and thighs (Heyer 1994).

Frazer (1966) developed a technique for marking common toads with coloured beads sewn through the skin with braided nylon or cotton thread. Small coloured glass beads (1 mm long) were sewn onto the dorsum, with up to 5 beads easily attached to each animal. Braided nylon was found to be difficult to knot, resulting in poor retention of beads. Even when cotton thread was used, the beads were easily lost, and returns were poor. In addition, certain beads lost their colour.

African clawed frogs (*Xenopus laevis*) in the laboratory were also marked using a similar technique (Nace & Manders 1982). The beads were attached under anaesthetic by passing surgical wire (28–32 gauge) through a fore (females) or hind (males) limb, including the humerus or femur. If the loop of wire included only the muscle and skin, the device gradually pulled out. The beads were positioned on the inside of the forelimb to stop snaring. The beads were attached using a colour code (e.g. white = 0, black = 1 etc.) to assign each animal a unique identifying number. No infection or other adverse effects were observed in 13 frogs that carried the devices for 3 or more years. Juveniles were also marked; however, the loop had to be made large enough to accommodate growth. This method was found to be extremely successful under laboratory conditions, but was not tested in the field.

Patches of reflective tape were attached to the heads of bullfrogs using cyanoacrylate tissue cement (Robertson 1984). These reflective patches remained in place for 16–41 days. Such a method may be suitable for vulnerable species, as a light source is required to visualise the patches. Therefore, marked animals would not be more obvious in ambient light. However, the patches may render nocturnal species more conspicuous under moonlight.

Attached devices are generally fixed to amphibians using non-toxic adhesives, and are only suitable for short-term studies. Problems generally relate to changes in appearance, which in turn may increase the animal's conspicuousness to predators or prey, or alter intraspecific interactions.

### **Fluorescent powders**

Taylor & Deegan (1982) applied dry fluorescent powder under pressure to larval green frogs and adult eastern newts (*Notophthalmus viridescens*). They found most animals retained their marks for at least 5 months in the field. A similar method was used to individually identify terrestrial salamanders (*Plethodon jordani*, *P. glutinosus*) (Nishikawa & Service 1988). Fluorescent powder was applied at 25–40 psi from about 1 cm from the surface of the animal. The pressure forced pigment molecules to penetrate the skin, leaving a mark 2–5 mm in diameter. A maximum of four marks at coded positions were applied to each animal. The dust-filled lesions were completely healed in 2 weeks, and 1 year later 80% of the marks were still visible under UV light. In fact, some marks lasted up to 2 years in the field. Animals measuring 12–95 mm SVL were successfully marked, but juveniles with an SVL less than 20 mm were considered to be too fragile to mark. In a related study using the same method, 3% of marked arboreal salamanders (*Aneides lugubris*) died when the air pressure tore open the body cavity (Nishikawa & Service 1988).

Schlaepfer (1998) attempted to mark small terrestrial leaf-litter frogs (*Eleutherodactylus podiciferus*) in Costa Rica, using the pressurised application of fluorescent powder. Adult frogs (10–24 mm SVL) were marked

with an inert fluorescent powder applied to the hind leg using pressurised air (100 psi) from 0.5 cm away. Juveniles (less than 10 mm SVL) were considered too fragile to spray. The procedure resulted in fluorescent yellow marks 3–4 mm in diameter. Only five frogs (of 68 marked) were recaptured, three of which had yellow spots still visible in ambient light 3 weeks after marking. The marks on the other two frogs had faded to light grey, but were still visible under UV light.

Schlaepfer (1998) found that spraying was more difficult, and appeared to be more harmful to the animals, than toe clipping. The equipment was cumbersome, impractical in the field and relatively expensive. In addition, frogs had to be held tightly for marking, and approximately 15% suffered leg dislocation due to the air pressure. One frog was literally blown away by the blast and died immediately. Between 30% and 50% of marked frogs appeared stunned after marking, and recovery (assessed as the ability to right the body, ability to jump when touched) was incomplete after several minutes. Schlaepfer concluded that the small size of the frogs made this method inappropriate. This experiment is an excellent example of field evaluation of a potential marking method to determine its suitability for a particular species. No subsequent reports of pressurised powder application to frogs have been found.

Windmiller (1996) adopted a slightly different approach to tracking frogs with fluorescent powder. Windmiller notes that the direct application of fluorescent powder to amphibian skin is problematic because the particles adhere strongly to the moist skin and might interfere with transcutaneous gas exchange. To avoid this problem, Windmiller attached lengths of yarn dipped in fluorescent powder to the mid-dorsum of juvenile green frogs and bullfrogs with cyanoacrylate glue. The yarn tags weighed 0.5–1 g each, and were applied to froglets as light as 3 g. The yarn tag shed a fluorescent trail allowing the frogs to be tracked with a UV lamp at night. Fluorescent trails as long as 150 m could be detected, but many became imperceptible after 50 m. In addition, 30% of marked frogs lost their tags prematurely in the field. Mild skin discolouration was found where the tags had been glued, but no skin lesions occurred. Furthermore, no mortality or other detrimental effects were found in laboratory trials.

Although fluorescent powder marking provides a unique method for tracking amphibians at night, there are some serious drawbacks. Due to the role of amphibian skin in gas and water exchange, direct application of powder is problematic. In addition, it appears that pressurised application of fluorescent powder can cause serious harm to small amphibians. For the small native frogs of New Zealand, the direct or pressurised application of fluorescent powders is therefore not likely to be acceptable on animal welfare, conservation or practical grounds.

## Radioisotope marking

Radioisotope marking has commonly been used to study amphibians in the past (e.g. Madison & Shoop 1970; Semlitsch 1981; Kleeberger & Werner 1982; see review in Ashton 1994). However, there are many reports of the detrimental effects of radioisotopes on the study animals. Karlstrom (1957) showed that Yosemite toads (*Bufo canorus*) subcutaneously tagged with only a few microcuries ( $\mu\text{Ci}$ ) of  $\text{Co}^{60}$  suffered exposure to doses of radiation far exceeding the expected  $\text{LD}_{50}^4$  value for anurans. Radioactive toads removed from the wild population and kept in captivity died within months of tagging. Autopsies revealed extensive haemorrhage in the vicinity of the lead-coated capsule containing the radioactive material. Therefore, a toad exposed to a detectable dose of this radioisotope may not be expected to survive a long-term study.

Ashton (1975) used  $\text{Co}^{60}$  wires (35–50  $\mu\text{Ci}$ ) to tag plethodontid salamanders and observed ulcers around the tags, which eventually opened, exposing the tag. A population of leopard frogs exposed to a single, 1000 roentgen (R) dose of X-rays suffered 81% mortality within 6 weeks (Patt & Swift 1948). Steaner (1950) found 50% mortality at 6 weeks for the same species exposed to 700 R of X-rays. For species undergoing conservation efforts, methods yielding these types of outcomes would be counterproductive.

The additional information that could be gained by using radioisotope tags rather than other methods is expected to be minimal. The application of radioisotopes to vulnerable or threatened amphibian species is inappropriate, as the related tissue damage is highly likely to affect behaviour and survivorship. Moreover, potential contamination of New Zealand's environment would be unacceptable to many people. One of New Zealand's native frogs is semi-aquatic (Hochstetter's frog), and isotopes may be reabsorbed by other organisms in the environment (Pendleton 1956).

## SEMI-PERMANENT MARKS

### Tags

A variety of tagging systems have been used to study amphibians in the past. Savage (1934) used paper tags to temporarily mark anurans. Raney (1940) adapted fish jaw tags for use in bullfrogs. However, Stille (1950) showed that the loss of jaw tags in Fowler's toads (*Bufo woodhousii fowleri*) was significant and that the tags were often lost due to sloughing of the jawbone. This process would cause considerable irritation to the animal, and may result in decreased feeding and other behavioural changes (Griggs et al. 1998). Consequently, jaw tags are no longer used in amphibians.

Elmberg (1989) attached Floy® fish fingerling and streamer tags to the knees of 637 common frogs (*Rana temporaria*) over 8 years. The tags were attached with vinyl elastic thread, and tag loss in the first year after tagging was 10–15%. Most losses were attributed to poor application technique. This method can be used on any frog with knees narrower than the upper and, especially, the lower legs.

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<sup>4</sup>  $\text{LD}_{50}$  is the dose of radiation or a toxic substance that is lethal to 50% of the group of animals.

Tags are no longer commonly used to mark amphibians. The contrasting colours or shiny surface of the tags, which make them attractive as research tools, are also expected to increase the conspicuousness of the animal to predators or prey. In addition, there is a risk of irritation to the delicate skin, or constriction of tagged limbs, depending on how the tags are attached. Such risks are especially pertinent to amphibian marking, as these animals continue to grow throughout their lifetime. Identification of tagged amphibians may still require recapture and handling in order to read the small inscriptions. In addition, the small size of New Zealand's native frogs precludes the use of most tagging equipment. Today, other methods are available that may be more appropriate for marking frogs in New Zealand.

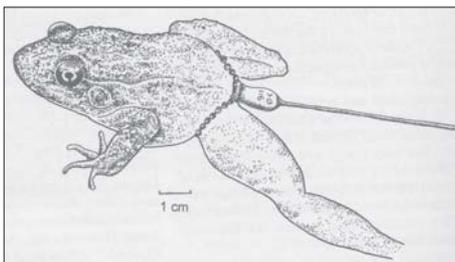
### Bands and waistbands

Aluminium bird bands have been adopted by amphibian researchers, and placed around the toes or thighs of frogs. When bands were put around toes, the two ends were pressed together with pliers to the point where the toe webbing was pierced, but circulation was not restricted. These bands were reportedly fixed indefinitely with no apparent reduction in movement (Kaplan 1958). However, researchers must consider the risk of skin irritation or infection when banding frogs.

Often equipment is attached to amphibians using waistbands made of plastic tubing or latex rubber straps (Kluge 1981). Emlen (1968) used a nylon waistband that was 13 mm wide and painted with black numerals for individual identification in a study of male bullfrogs. The waistbands were recognisable from 8–12 m away, but numerals only from 4–6 m. Emlen reported no differences in behaviour, mortality or emigration rates or weight gains. The bands had to be replaced seasonally owing to soiling and staining, which necessitated recapture.

Rathbun & Murphey (1996) evaluated the use of a belt of beaded chain (metal) for radio-tracking frogs. The belt was sprayed with black enamel paint to reduce reflection, and attached around the waist of red-legged frogs (*Rana aurora draytonii*). Beaded chain was chosen because it is flexible, lacks sharp edges and rolls over the skin with little friction. A transmitter weighing 1.8 g was attached to the belt using epoxy. Fitting the belt required two people, one to hold the frog and one to fit the band. A proper fit was crucial to avoid the formation of indentations and small sores in the skin. Even so, 13% of frogs in the field developed small sores on the sides of their waists as a result of the chain belt. In addition, the researchers found that the chain had to be replaced at every capture owing to corrosion and wear, which increased the amount of handling required.

Beaded chain radio-belt applied to an adult red-legged frog (*Rana aurora draytonii*). From Rathbun & Murphey 1996.



Rathbun & Murphey (1996) concluded that the radio belt did not significantly affect frog weight. However, 24 functioning units (of 89 deployed) were shed by frogs and later recovered. In addition, three belted frogs died, presumably after underwater bulrush shoots became threaded between the belt and the frog, trapping and drowning the animal. This hypothesis was confirmed in a subsequent study by the same authors, which showed a 25% mortality rate due to bulrush shoot drowning (Rathbun & Murphey

1996). Although this method cannot be said to be free of effects on the behaviour and survivorship of the frogs, the authors believed it to be an improvement over other radio-tagging techniques used on amphibians (also see *Telemetry*, pp. 48–51).

Because of the delicate nature of their skin, amphibians are especially susceptible to skin irritation and haemorrhage associated with wearing bands and waistbands. Banding systems should be specifically designed for the particular species and environment. By altering the design of an attachment system to match the species involved, problems such as skin abrasion can be reduced (Griesemer et al. 1999). Bands and waistbands may affect amphibians by virtue of increased weight, by snagging on vegetation, or by hindering the animal's movement, feeding or attempts to escape predation. Other risks include skin damage due to electrolysis at the coupling of the band (Harker et al. 1999) and the possibility of increased conspicuousness to predators and prey.

### **Nocturnal lights**

Only one report of the use of nocturnal lights for identification of amphibians has been found. Windmiller (1996) attached chemical lights to juvenile green frogs and bullfrogs using cyanoacrylate glue. Small tubes filled with Cyalume® fluid, weighing 0.5–1.9 g, were glued to the dorsal skin of the frogs. Different colours of Cyalume® enabled differentiation of subgroups, and the fluid remained bright for at least 12 hours. Depending on vegetation density and ambient light, the marked frogs were visible up to 80 m away. Windmiller urges caution, as skin contact with Cyalume® fluid is fatal to bullfrogs and possibly other frog species. In addition, caution is required when using cyanoacrylate glue on amphibian skin, as it can cause severe and sometimes fatal skin lesions in some amphibian species, e.g. spotted salamander (*Ambystoma maculatum*).

Small LEDs have been used to identify and track amphibious crabs (Wolcott 1977), and it is conceivable that similar systems could be used on amphibians. Evidence to date suggests that the use of such optical light sources does not increase detection of the marked animals by predators (Neitfeld et al. 1994). However, marked predators may have more difficulty capturing prey (bats: Barclay & Bell 1988). There are no reports of adverse effects of nocturnal lights on the behaviour of marked animals; however, such effects have not been systematically assessed. Such systems are unlikely to be used to identify New Zealand native frogs as our frogs are diurnal and rely on cryptic colouration to avoid visual predators.

### **Telemetry**

Radio-telemetry is often unsuitable for the study of amphibians due to the small size of the animals (British Columbia Environment Resources Inventory Committee 1997). However, with the continued miniaturisation of telemetric components, it is possible to attach tracking equipment to amphibians without violating the recommended maximum transmitter weight-to-body weight ratio of 10% (Heyer et al. 1994). Radio-telemetry has been used to study migration, habitat use, foraging and hibernation behaviour in amphibians (e.g. Sinsch 1989; Seitz et al. 1992; see review in Richards et al. 1994). Such methods are

generally useful only for short-term studies, primarily because of the size/weight limitation on the power source (longer studies require larger power sources for transmitters). Because amphibians are often secretive or subterranean, telemetry may be the only way to gain meaningful data on their movements and habits.

Radio-telemetric equipment is most often attached to frogs using a waistband (see, *Bands and waistbands*, pp. 47–48). Rathbun & Murphey (1996) attached radio transmitters weighing 1.8 g to red-legged frogs using beaded (metal) chain waistbands. Radio transmitters were attached to mountain yellow-legged frogs (*R. muscosa*) using the same methods (Matthews & Pope 1999). In this study, only frogs with SVLs greater than 55 mm were telemetered to minimise the potential effects of transmitter weight (1.5 g). Similarly, Holenweg & Reyer (2000) attached radio transmitters only to pool (*R. lessonae*) and edible (*R. esculenta*) frogs having an SVL greater than 40 mm. These transmitters were attached with a waistband made of synthetic thread encased in a silicon tube. Bull & Hayes (2001) fitted radio transmitters to Columbia spotted frogs (*R. luteiventris*) using 6-mm-wide satin ribbon.

Loss of externally attached radio transmitters often results from equipment breakage or failure, or from an improper fit which leads to shedding of the waistband. Rathbun & Murphey (1996) reported significant transmitter losses from red-legged frogs wearing beaded chain waistbands. Transmitter failure before the predicted exhaustion of the battery was attributed to water leakage into the transmitter, transmitter shedding or removal of the bearer from the study area by predators (Rathbun & Murphey 1996). Twenty-four radio transmitters (of 89 deployed) were shed by frogs and were still functioning when they were later recovered.

Bull & Hayes (2001) reported that 16 of 47 tagged Columbia spotted frogs that lost their transmitters had slipped their satin ribbon waistbands and five had abrasion wounds. More males than females were lost from this study owing to skin abrasion or equipment slippage. Matthews & Pope (1999) reported five of 24 tagged mountain yellow-legged frogs lost their transmitters, while three frogs suffered small abrasions. However, no entanglement or wedging in tight spaces was reported in this study. Holenweg & Reyer (2000) reported one Ranid frog (*R. lessonae* or *R. esculenta*) with a swollen leg, from which the transmitter was removed immediately. In addition, three frogs (pond and edible frogs) lost transmitters in the first few days after attachment. Five transmitters stopped working, and five frogs were preyed upon. Of 36 animals originally tagged in September/October (northern Autumn) only six could be tracked through until the following March (northern Spring) (Holenweg & Reyer 2000).

The effects of externally attached radio transmitters on the behaviour of the subject animals are largely a function of the size and weight of the animal and equipment, and the method of attachment. Effects on behaviour are not often reported in amphibian studies, not because they don't occur, but primarily because researchers have not systematically evaluated the possibility of such effects. Bull & Hayes (2001) did not comment on the potential effects that transmitters attached with satin ribbon waistbands may have had on the movement of Columbia spotted frogs. However, in a comparative study of radio transmitter attachment methods, Bull (2000) found that the satin ribbon waistbands caused less skin abrasion than arm bands made of carpet thread.

Rathbun & Murphey (1996) commented that the long antenna trailing from radio transmitters attached to red-legged frogs did not appear to affect the animals. Matthews & Pope (1999) concluded that transmitters had little or no effect on frog behaviour because they observed similar movements and habitat use in 582 frogs marked with PIT tags in the same study area (Pope 1999). However studies of small mammals and birds suggest that the attachment and wearing of radio-telemetric equipment can have effects on food consumption, grooming, behaviour and activity levels (Boag 1972; Banks et al. 1975; Amlaner 1978; Leuze 1980; Webster & Brooks 1980; Ormiston 1985; Koehler et al. 1987; Pouliquen et al. 1990; Harker et al. 1999).

Radio-telemetric equipment can also be implanted into amphibians, but the success of this method is limited in frogs because of their thin skin (Werner 1991). Lamoreux et al. (2002) implanted paraffin- or beeswax-coated radio transmitters (10 mm × 25 mm) into 27 green frogs. Each transmitter package, weighing 2.9–4.4 g, was inserted through a 12–14-mm-long incision in the ventrolateral abdominal wall (Lamoreux & Madison 1999). The muscle and skin layers were then sutured. Lamoreux et al. (2002) reported that seven of 27 frogs implanted with radio transmitters were lost from the study. Two animals were preyed upon, one died from implantation surgery, one was lost until the following spring (321 days later) and three were lost for unknown reasons. Seitz et al. (1992) implanted transmitters into the abdominal cavity of common frogs. The transmitters exceeded the recommended 10% of body weight for some of the smaller frogs, but the authors observed no obvious changes in behaviour in laboratory trials.

The effects of implanted radio transmitters on the bearer's behaviour warrant consideration, even though problems such as snagging do not arise. Implanted material, especially in small animals, can adhere to internal structures, causing damage (Amid et al. 1994; Aiello 1998). Smaller internal spaces mean that implants come into greater contact with visceral structures, even if the recommended body-to-implant ratio is maintained. Implants have been found to affect behaviour and mortality rates in small animals (Koehler et al. 1987; Knights & Lasee 1996; Baras et al. 2000). Lamoreux et al. (2002) concluded that implanted radio-tags did not affect green frogs. They based this conclusion on the fact that similarly sized frogs caught in a drift fence at the same site and then either toe clipped or tattooed (not implanted) were found to make forays of the same frequency and duration, and gained similar mass to radio-implanted frogs. However, there are no reports of long-term amphibian-specific studies on the effects of implantation.

Radio transmitters can also be force-fed to amphibians. Ingested transmitters were used to track common toads and common frogs (Oldham & Swan 1991; French et al. 1992). Oldham & Swan (1991) reported that the transmitters had negligible effects on appetite, and that they remained in the stomachs of the frogs for an average of 6 days and the toads for 13 days. However, ingested transmitters have had significant effects on behaviour and survivorship in some reptile species, and this method is now strongly discouraged (Fitch & Shirer 1971; Lutterschmidt & Reinert 1990; British Columbia Environment Resources Inventory Committee 1997). French et al. (1992) noted that because ingested transmitters lack external antenna, it is difficult to differentiate transmitter signals from background noise.

Radio-telemetry can be successfully used for short-term studies of amphibians, and is especially useful for secretive or subterranean species. However, researchers must determine the most appropriate method of attachment for each species and environment, and must attempt to quantify and minimise any negative effects on the animal. Comparative studies using different methods would be useful in determining the potential effects of transmitter attachment. Transmitter weight-to-body weight ratios must be maintained below 10% (well below if possible). Therefore, only amphibians of sufficient size can be radio-tagged. External transmitters must be fitted correctly, and equipment must be removed, or designed to fall off. To date, there have been no long-term studies of the effects of internal implantation on amphibians. This is an area requiring attention.

## PERMANENT MARKS

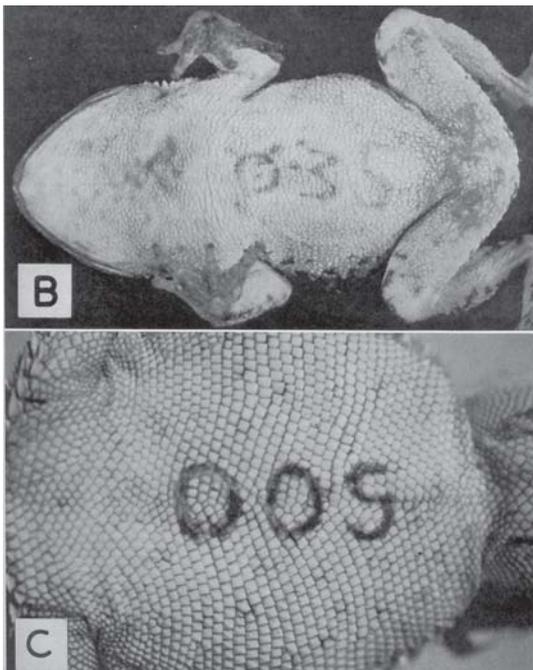
### Hot branding

Amphibian skin plays important roles in thermoregulation, water regulation and gas exchange (Dorit et al. 1991). Because of the skin's importance and delicate nature, hot branding must be used with great caution. If the resulting wound is too large, body fluid loss may be excessive and the frog could die of dehydration (Ferner 1979). This is especially pertinent to small individuals, such as New Zealand's native frog species, which have higher surface area-to-volume ratios. Branding requires skill and improves with operator experience. As poor technique can lead to suboptimal marking and harm to the animal, including damage to underlying muscles and organs (Ferner 1979), researchers must be competent with the techniques before attempting to mark wild populations. In addition, brands should never be applied to the ventral pelvic patch because this site is important in the water physiology of anurans (Donnelly et al. 1994).

Hot branding was used to mark 311 Gulf Coast toads (*Bufo valliceps*) and 159 Ranid frogs (bullfrogs and leopard frogs) (Clark 1971). Hoskins Chromel® A resistance wire (20% chromium, 80% nickel) was shaped into numerals, which were heated with a portable propane torch. Three numerals were applied to the ventral surface of each animal. Recaptured toads had a glossy brown, horny layer over each numeral, and no loss of body fluid at the brand site was observed. This layer had disappeared on most toads after 2 weeks. One brand was legible for 21.5 months on a toad, but no information on the durability of frog brands was given. Recapture frequencies and intervals were similar for branded and toe-clipped frogs, suggesting that the two methods had similar effects on survival (Clark 1971).

Taber et al. (1975) used a similar method to mark hellbender salamanders (*Cryptobranchus alleganiensis*). Many of the 1.5-cm-high brands lasted throughout the 2-year study. Some, however, faded and rebranding was required before the end of the study. It may not be appropriate to use such

Hot branded numerals on Gulf Coast toads (*Bufo valliceps*). From Clark 1971. Reprinted with permission of the American Society of Ichthyologists and Herpetologists.



an invasive marking method when it cannot be considered reliable for the duration of a study.

Ehmann (2000) devised a code system of small dot brands placed at various positions on the body of amphibians and reptiles. The system is similar to that used for toe clipping, with each body site assigned a code, e.g. the number of marks on the right shoulder might correspond to ID numbers 1-9, then the left shoulder might correspond to 10, 20, 30 etc. The number of marks at another site would correspond with 100, 200 etc. and another to 1000, 2000 etc. The codes from each body site are combined to give the animal a unique identification number. In Ehmann's micro-branding system, sites most frequently used (e.g. units, 10s, 100s) should be placed on more robust body sites (e.g. torso, upper limbs). The use of larger sites also facilitates easier branding and optimises visibility for re-identification. An application of less than 1 second was considered adequate and left a fresh brand with two bridges of intact dermis through the spot. The author believed these tissue bridges helped the healing process by holding the wound closed and possibly acting as grafts to encourage new skin growth in the branded area. Applying brands to distal areas must be precise as repeated branding may damage the blood vessels on the trailing side of the leg. This method has been applied to frogs (species not specified); however, no attempt has been made to evaluate the impact of the brands (Ehmann, 2000).

Because small animals such as frogs must usually be captured and handled for re-identification, it makes sense to reduce the size of the marks to a practical minimum. Because of the speed of micro-branding, handling and marking stress may be decreased. Infection risk and scarring is expected to be minimal (Ehmann 2000). Large numbers of individuals can be marked in sequence. In addition, one person alone can mark the animals, and the equipment required is portable and convenient to use. The coding system precludes the use of larger and more ambiguous numerals and characters. However, the use of dispersed dots may disrupt the cryptic patterning or social signalling of the animal (Murray & Beacham 1990), which is particularly pertinent to New Zealand's native frogs. Another disadvantage of using a coded micro-branding system is the high risk of mistakes when reading the codes. In addition, identification is more time-consuming and is likely to improve only with experience.

Hot branding should be attempted on amphibians only if there is no better alternative. The delicate nature of amphibian skin, risk of fatal fluid loss and risk of disrupting cryptic colouration make hot branding undesirable for marking New Zealand's vulnerable frog populations. In addition, the success of hot branding is heavily dependent on operator proficiency, such that inexperience can lead to deleterious effects on the animals.

### **Freeze branding**

Freeze branding has been reported to work well on smooth skinned frog species like Ranids, but was not found to be effective on Bufonids and other species with granular skin surfaces (C. Smith, pers. comm. in Halliday 1995).

Daugherty (1976) freeze branded tailed frogs (*Ascaphus truei*) using insulated copper wire immersed in dry ice for 30 minutes of initial cooling. The frog's light coloured ventral side was branded for about 10 seconds, and the branding

tools re-cooled for 30–60 seconds between brands. Brands were observed to last more than 2 years in the field. However, the marks gradually lost pigment, until 1 year later, the viscera were often visible through the integument. Measey & Tinsley (1998) used the same methods to freeze-brand African clawed frogs, and found that all numerals could be easily distinguished 2 years later. Measey (2001) also reported that freeze brands are long lasting on African clawed frogs under field conditions, but that the success of branding could not be determined immediately after application.

Tailed frogs generally have an SVL of less than 50 mm, meaning that only two numbers could be applied in Daugherty's 1976 study. This size constraint limits the number of animals that can be identified. Daugherty noted that freeze brands might not remain visible in frogs marked immediately after metamorphosis, especially in those species with rapid post-metamorphic growth. In such animals, pigment may migrate into the branded area within 2 years, obliterating the identifying mark.

Measey et al. (2001) freeze branded caecilian amphibians (*Gegeneophis ramaswami*) using 1.5-second applications, after first gently drying the animal. Brands were faintly visible immediately after application, and clearly visible 15 minutes later. After 24 hours, the skin had blistered and the marks were legible for the 4-month duration of the study. No signs of infection or other ill effects of freeze branding on the marked animals were reported. In contrast, Klewen (1982) found that the use of copper stamps cooled in dry ice caused severe injury or death in several salamander species (*Salamandra atra*, *Triturus alpestris*, *T. vulgaris*). Klewen found that even applications of 0.5 seconds (the shortest duration used) caused significant damage. Damage only became evident 2 days after marking, and subsequent healing was slow (2–8 weeks). The author concluded that freeze branding should not be used to mark these salamander species.

The major disadvantage of freeze branding compared with hot branding is that the success of the application cannot be determined at the time of branding. If the animals are released immediately after marking, the researcher cannot be sure that the procedure has been effective. In addition, the method may not be permanent in amphibian species (e.g. Verhoeff-de Fremery & Vervoordeldonk 1982). There are no consistent results indicating the optimal application times for freeze branding amphibians. Researchers should test their methods on captive or closely allied species. For New Zealand native frogs, the tailed frog (*Ascaphus truei*) is the closest living relative on which testing could occur (Newman 1996). Most operators note that freeze branding in the field has practical limitations. Liquid nitrogen is difficult and dangerous to transport and evaporates quickly in tropical climates (Measey et al. 2001). In addition, operator proficiency has significant influence on freeze-branding success. Other methods produce marks that are equally benign and permanent, but do not have the logistical problems associated with freeze branding.

### **Chemical branding**

Thomas (1975) marked Hylid frogs using 75% silver nitrate mixed with 25% potassium nitrate. This compound is usually used for veterinary cauterisation. Tracing narrow lines on the dorsal surface of the frogs caused brown marks to

appear immediately. Two weeks later, these marks had faded to a light brown that contrasted well with the background colour of the frogs. The marks were distinguishable for over 9 weeks, and Thomas reported no evidence of harm to the animals. However, one frog accidentally got the mixture over most of its back and died 5 days later. This raises concern about the potential for smaller applications of the compound to have sub-lethal effects on behaviour and fitness.

One advantage of chemical branding is that if the mark is placed on the dorsum, then recapture is not always necessary for identification. However, the method is appropriate only for species with a background colour that will contrast with the light brown mark. The method is therefore recommended only for short-term use on dark coloured amphibians. Once again, this method may interrupt the cryptic colouration of New Zealand native frogs and increase their conspicuousness to predators.

### **Tattooing**

Kaplan (1958) was the first to mark amphibians by inserting ink into their skin. Kaplan incorporated India ink into scarified skin on the ventral surface of frogs. Numerals were etched into the skin with a hypodermic needle and filled with ink mixed with glycerine (the latter to aid spreading). Originally, Kaplan had injected the India ink directly into the skin, but this caused excessive swelling. The modified technique caused only temporary, localised inflammation and the resulting mark was reported to be permanent.

Shirose et al. (1993) also applied India ink to the ventral surface of bullfrogs. However, these tattoos were applied using a veterinary tattoo gun fitted with six needles. All of the tattooed frogs were also marked by removing two phalanges of one toe, in order to evaluate the permanency of the tattoos. There was only one report of a tattoo being lost in the 2-year study. In addition, no increase in mortality was reported either from toe clipping or tattooing (Shirose 1990). Purple India ink marks applied with a Panjet needle-less tattoo gun to male common toads were reported to be unreadable within 20 days of application (Brown 1997).

Male bullfrogs were tattooed with unique alphanumeric codes on their ventral surface for identification of individuals during the chorusing season (Judge & Brooks 2001). No anaesthetic was used during the procedure. Relaxation of the belly muscles brought on by local anaesthetic makes tattoo application more difficult and time-consuming, which may increase the stress experienced by the frog. No local infections were observed, and no frog was considered to have died as a result of the tattooing treatment (Judge & Brooks 2001). The authors considered that the pain associated with tattoo application was likely to be less intense and shorter lived than that resulting from hot branding or tissue removal. Released males had returned to chorusing 1–3 hours after tattooing (Judge & Brooks 2001).

A Panjet inoculator was used to apply Alcian blue dye at eight coded locations on the ventral surface of the limbs of common toads, common frogs and African clawed frogs (Wisniewski et al. 1980; Brown 1997; Measey & Tinsley 1998). The Panjet gun was held about 5 mm from the surface of the frogs. However, since toads have thicker, more granular skin than frogs (especially females), the Panjet inoculator had to be held right against the skin to obtain a satisfactory

mark (Wisniewski et al. 1980). There were very few injuries in Panjet-marked toads, but on two occasions (0.05%) the force of the jet tore a small hole in the skin allowing air and water to enter the leg (Wisniewski et al. 1980). No inflammation was observed after Panjet dye marking in common frogs or toads, and marking with 1–3 Alcian blue dye marks had no significant effect on the body condition or survivorship of wild common toads (Brown 1997).

Alcian blue dye marks were retained for at least 2 years on all common frogs and common toads so marked in laboratory and field trials (Brown 1997). Measey & Tinsley (1998) found several Alcian blue tattoos that were still legible on wild African clawed frogs 14 years after application. However, Brown (1997) noted that all Alcian blue dye marks faded to some extent over time. Other problems with the coded tattoo method include the fact that, usually, only the original operator could find the coded marks on subsequent recapture, and that failure to detect one or more marks led to spurious identification (Measey & Tinsley 1998).

Measey et al. (2001) also used a Panjet innoculator to mark caecilian amphibians (*Gegeneophis ramaswami*). Alcian blue dye was applied under pressure through a small aperture held 5 mm away from the skin. Panjet marks were immediately visible and remained so throughout the 4-month study period. Each mark took about 5 seconds to apply, and no signs of infection or modification of behaviour were observed. Panjet tattooing was deemed to be the simplest and fastest method of administering marks to caecilian amphibians.

In contrast, Hendrickson (1954) reported unsatisfactory results after tattooing *Batrochoseps* salamanders. Tattoo marks spread through the sub-dermal spaces and were not permanent. This may be related to the depth of dye placement, and illustrates just how dependent the success of tattooing can be on operator proficiency.

Tattooing is considered to be the most permanent method for marking animals, including amphibians. However, the species and dye or ink chosen can affect the permanence of the marks, as can operator proficiency. The equipment required is simple and there are few reports of detrimental effects. The small size of many amphibians may limit the usefulness of tattoos for individual identification; however, coded systems may allow larger numbers of individuals to be identified (Measey et al. 2001). Tattoos must be applied to the lighter ventral surface of amphibians, meaning that capture is required for re-identification. In addition, dorsally placed or coded tattoo marks may disrupt the cryptic colouration of New Zealand native frogs.

### **Passive Integrated Transponders**

PIT tags are useful for marking amphibians owing to small tag size, the absence of a visible mark, their reliability of function and high retention rates (Prentice et al. 1990). Donnelly et al. (1994) recommended that PIT tags be implanted into the dorsal lymph sac of anurans rather than intra-abdominally, to avoid damage to internal organs. PIT tags can also be implanted subcutaneously. However, subcutaneous implants may be lost or malfunction more easily than intra-abdominal implants. Intra-muscular injection is not considered viable in amphibians, owing to insufficient muscle volume and the heavy strain implants place on the musculature (Lehmann 1996).

Camper & Dixon (1988) implanted PIT tags into Hurter's spadefoot (*Scaphiopus holbrookii burteri*), Texas (*Bufo woodhousei velatus*) and Gulf Coast toads. PITs were implanted intra-abdominally in 4 toads, and subcutaneously in 14 toads. All wounds were sealed with glue. The PITs were found to be highly efficient (99% reading success), with 92% of first pass readings successful. Migration of the transponders was found with 75% of intra-abdominal implants and 64% of sub-cutaneous implants (67% of total), but this movement did not affect first pass readings in any case. However, migration of PITs raises concern about damage to internal structures (see below). The authors found that the size of the implant tool prohibited tagging amphibians with an SVL less than 80 mm, but implant tools have since improved. Holenweg & Reyer (2000) successfully marked free-living pond and edible frogs with an SVL of 40 mm.

PIT tags were implanted into African clawed frogs under anaesthetic (Mrozek et al. 1994). An incision was made in the middle of the back, parallel to the spine and the transponder injected subcutaneously into the dorsal lymph sac and secured with one suture. Attempts to inject the transponder without a prior incision failed in this species owing to the skin's resistance to needle insertion. Two out of the five transponders were not readable as they were lost through the puncture hole after injection, owing to poor procedure. The three correctly inserted transponders worked well for 41 months.

Captive cane toads (*Bufo marinus*) were marked with PIT tags, injected parallel to the urostyle (Freeland & Fry 1995). This location was chosen because, in this species, the large parotid gland precludes insertion into the dorsal lymph sac. One tag (of 21 inserted) was rejected; rejection was likely due to faulty implantation. Some tags were found to have a tendency to move towards the site of implantation as the needle was withdrawn. In all cases, the tags continued to provide accurate results for 43 days.

Christy (1996) carried out field trials to assess the efficacy of using PIT tags to mark free-living *Litoria* frogs. Tagging took less than 5 minutes per individual, and healing was complete in 3-5 days. No evidence of infection or trauma was observed at the injection site. No behavioural changes or other adverse effects were evident in the free-living populations, but such effects were not systematically evaluated. The author recommended that metamorphosing frogs with an SVL less than 40 mm should not be PIT-tagged until the researcher is proficient and confident with the technique.

Brown (1997) marked common frogs and common toads by pinching a flap of skin on the dorsal surface and injecting a PIT tag under the skin. The tag was then gently rubbed down the back until it lay beneath the skin at the base of the spine between the back legs. In two studies (8-22 months long), all captive frogs and toads retained their marks. In addition, 100% of PIT tags were retained in wild male common toads, and the tags were found to have no effect on body condition or survivorship. Brown (1997) attributed the high retention rates to careful positioning of the tags.

Trenham et al. (2000) marked California tiger salamanders (*Ambystoma californiense*) using PIT tags and by toe clipping. They found that PIT tags implanted in juveniles were either lost or induced mortality more often than did toe clipping.

The use of PIT tags in amphibians eliminates the need for excessive handling after initial marking, as marked animals can be scanned from a short distance. The Trovan<sup>®</sup> Conventional Scanner can read tags from 20 cm away, while the Destron<sup>®</sup> Portable ID Reader can read them from 50–60 cm away. Portable scanners are capable of reading tags through most materials (e.g. rock, plant material) and from any direction (Freeland & Fry 1995), allowing identification of secretive or subterranean amphibians without contact or disturbance of their shelter sites. However, PIT tagging is not considered useful for behavioural studies where individuals must be recognised from further distances. Christy (1996) concluded that PIT tagging is an effective method for identifying, but not locating, free-living frogs, as the reader must be in close proximity to the tag for detection.

PIT marking does not detract from the appearance of the animal. This is important for public perception, and especially so for animals such as New Zealand native frogs, which primarily use visual crypsis (camouflage) to avoid predation, and have strong environmental and cultural significance. PIT tags can also be used to identify dead animals, which may be important for studies of predation and disease prevalence. The recent epidemic of Chytrid fungus in New Zealand frogs suggests a use for PIT tags to help track the spread of disease among frog populations. Alternatively, the invasive nature of PIT implantation could exacerbate the spread of infection.

Theoretically, PIT tags have indefinite lifespans, making them useful for studies of relatively long-lived animals, such as New Zealand's native frogs. However, PIT tags are relatively large compared to these native frogs (all species have an SVL less than 50 mm). Fascola et al. (1993) noted that even newts with masses of less than 2 g could be successfully implanted with PIT tags. However, other authors consider some amphibians to be too small to safely mark with PIT tags (e.g. Lehmann 1996; Measey et al. 2001).

Even if the recommended body-to-implant weight ratio is maintained, the increased weight burden may affect smaller animals. In addition, there is the potential for internal injury if implanted tags come into contact with internal organs, which itself is more likely in smaller intra-abdominal spaces. Moreover, adhesion to visceral structures has been found to affect growth, reproduction, behaviour or survivorship (Smith 1980; Koehler et al. 1987; Amid et al. 1994; Knights & Lasee 1996; Tillmann et al. 1997; Aiello 1998; Baras et al. 2000).

In contrast, no effects on body weight, food consumption, general health, behaviour or survivorship of the bearer were evident from other studies on intra-abdominal implants in small animals (Rao & Edmondson 1990; Ball et al. 1991; Reichling & Tabaka 2001). Christy (1996) found no noticeable change in mass, mobility or feeding, and no unusual behaviour in any of six captive striped marsh frogs (*Limnodynastes peronii*) implanted with PITs posterior to the axilla. Upon necropsy, two of the six tags had adhered to the outer peritoneum, while four remained floating in the abdominal cavity. Unfortunately, the majority of such studies are relatively short and the long-term effects of intra-abdominal implants are not well known. In addition, interspecific differences can be marked.

Before PIT tags are used to identify frogs in New Zealand, the long-term effects of intra-abdominal and subcutaneous implantation should be evaluated, either

in captive individuals, or closely related species (e.g. tailed frog). The small size of all native species may preclude the use of intra-abdominally implanted PITs, but they may be useful for identifying the larger exotic species. If proven safe for native frogs, PIT tags would be useful for identifying individuals, estimating population changes and tracking the effects of chytridiomycosis in New Zealand, without detracting from the appearance of these unique frogs.

### **Visible Implant Fluorescent Elastomer tags**

Visible Implant Fluorescent Elastomer (VIE) tags and Soft Visible Implant Alphanumeric (VIAAlpha) tags are commonly used in fish studies and are also used to mark amphibians. These tags are particularly useful to identify salamanders, which are very difficult to mark owing to their small size, their sensitive and slippery skin and their ability to regenerate toes (Davis & Ovaska 2001).

Ireland (1973) marked ringed and gray-bellied salamander larvae (*Ambystoma annulatum* and *Eurycea multiplicata*, respectively) using fine-grained fluorescent pigments in a paste applied to the larvae with a heated probe. The probe burned the outer epithelial layers leaving a 1-mm scar that regenerated within 15 days, incorporating the pigments. In laboratory trials, 30% of gray-bellied salamander larvae had lost their fluorescent marks after 15 days. After 70 days, 50% had lost their tags. Retention times in larval ringed salamander were even shorter.

Woolley (1973) used subcutaneous acrylic polymer injections to mark cave (*E. lucifuga*) and dark-sided (*E. longicauda melanopleura*) salamanders. Two parts Liquitex<sup>®</sup> acrylic polymer and one part water were injected into the lateral caudal region with a 22-gauge needle. The resulting marks were 7–10 mm in diameter and could be applied in a variety of colours. The marks were found to be stable in both species for the duration of the 19-month study, and allowed identification from 3–5 m. Parameters such as movement in water and on horizontal and vertical cave surfaces were assessed. No adverse effects on the marked animals were observed. In addition, the food selection, temperature preference, substrate selection, phototrophic response, wavelength and relative humidity preferences of marked and unmarked salamanders were compared. No significant differences between the groups were found. Woolley (1973) found that the method allowed quick recognition in the field with a subsequent reduction in handling. However, individual identification was not possible using this method.

Larval anurans have also been marked using acrylic polymer injections (Cecil & Just 1978). Anholt et al. (1998) also used fluorescent elastomer marks in anuran larvae and reported 15% loss of the marks in the first 8 days after injection.

Davis & Ovaska (2001) injected VIE tags into western red-backed salamanders (*Plethodon vehiculum*) in both laboratory and field trials. Each animal was individually identified using a combination of three elastomer colours and six body positions for injection. In the laboratory trial, only one of 17 salamanders had lost its mark after 16 months. In the field trial, five salamanders (of 69 recaptured) had lost one of the three marks, and a further 13 had ambiguous marks. No mortality or weight loss was associated with elastomer tagging in the laboratory study.

In a related study, western red-backed salamanders and Pacific treefrogs (*Hyla regilla*) were marked with three VIE tags per animal (K.E. Ovaska, Biolinx Environmental Research Ltd., pers. comm. 2004). In the 11 month laboratory trial, there was no VIE-related mortality in either species. However, 11% of the salamanders and 22% of the frogs lost one of their elastomer tags within 11 months of application. In a mark-recapture field study, 10% of recaptured salamanders had either lost one of their elastomer tags, or the tags were inserted too deeply to be visible.

Movement patterns between elastomer-tagged and toe-clipped western red-backed salamanders were found to differ in the field trial (Davis & Ovaska 2001; K.E. Ovaska, Biolinx Environmental Research Ltd., pers. comm. 2004). On average, elastomer-tagged animals used a higher number of different cover objects than toe-clipped animals. The use of more cover objects was taken to indicate more movement in this species. This result implies either that toe clipping discouraged movement by the salamanders or that elastomer-tagged animals moved more. Toe-clipped animals exhibited movement patterns similar to the unmarked reference group. However, only a small number of unmarked animals were recaptured, and all had natural deformities of the feet or toes that could have affected their movements in similar ways to the toe-clipped animals.

Measey et al. (2001) found VIE tags to be highly successful for marking caecilian amphibians (*Gegeneophis ramswamii*). The animals were first anaesthetised, and 0.05 ml of prepared elastomer was then injected subcutaneously. Each mark took about 1 minute to apply. Measey et al. (2001) also implanted VIAAlpha tags into caecilians. The tags they used were 2.8 mm × 1.2 mm and less than 0.1 mm thick, and each carried a unique combination of letters and numbers in 1-mm-tall black characters. The tags were injected into anaesthetised caecilians to a depth of 5 mm. Tags were visible immediately after injection and the injection site had healed 11 days later. A hand lens (×10) was sometimes required to read the characters. Application of each tag took 5 minutes, which makes the method time-consuming compared to others. However, single tags allow long-lasting identification of a large number of individuals.

Nauwelaerts et al. (2000) found VIE tags implanted in the translucent skin between the toes to be very successful for long-term individual recognition of edible frogs. Eight months after implantation, 100% of the tags were retained, although some reduction in the size of the tags was evident after 4 months. The advantages of the system include the large number of individuals that could be marked, high mark retention, mark visibility at night under UV light and the low volume, weight and cost of the tags.

Most VIE tag losses are considered to be a result of improper application, e.g. material was left protruding from the injection wound (Davis & Ovaska 2001). However, these authors reported that there was no reduction in tag loss with increasing experience of the operator. The elastomer marking process requires longer handling than does toe clipping, taking more than 2 minutes on average compared with 40 seconds to toe clip.

Because VIE marks often migrate subcutaneously, Davis & Ovaska (2001) recommended using the same number of tags in each animal at any particular study site. This would allow identification of lost or displaced tags. Accurate detection of fluorescent tags may require the use of a long-wave UV lamp in a

darkened box (see figure p. 67). However, Measey et al. (2001) found that VIE marks on caecilian amphibians were visible in strong sunlight immediately after injection and for the duration of the 4-month study. Such visible marks may make tagged animals more conspicuous to predators or prey.

VIE and VIAAlpha tags are most commonly used to mark salamanders owing to their ability to regenerate clipped toes. However, these tags have also been used successfully on anuran amphibians. Amphibian species not capable of regenerating toes are usually marked using easier methods, e.g. toe clipping. Alternative methods to VIE and VIAAlpha tagging are likely to be easier and safer for use in New Zealand's vulnerable frog populations.

### Tissue removals

Many researchers believe that if carried out carefully, toe clipping is the most cost-effective, reliable and least stressful marking method available for amphibian species. In addition, clipped toes can provide valuable additional information on the age and genetic structure of a population without further tissue removal. Despite the concerns of many researchers about the effects of toe clipping on the subject animal, it remains the most common marking method for amphibians and reptiles.

Toe clipping was first used by Hamilton (1934) to study the growth rates of American toads. Martof (1953) developed a numbering system that allows the individual identification of 6399 individuals in series, with no more than two toes removed per foot. Both Donnelly (1989) and Hero (1989) have since devised systems that require fewer toes to be removed than Martof's system.

The success of toe clipping for identification depends on the species involved. The effects of toe removal on the particular species and population should be tested before the method is used on a large scale (Halliday 1995). Clark (1971) toe clipped several anuran species and found that the amount of blood lost differed between species. In addition, particular digits have specialised functions in different species. For example, male natterjack toads (*Bufo calamita*) utilise the first three fingers of the front feet for amplexus. In addition, the long fourth toe of the hind legs should not be removed from either sex, owing to its importance in moulting (Clark 1971). For such reasons, all studies of new populations that will involve toe clipping should incorporate a controlled experiment to quantify the effects of marking.

The possible effects of tissue removal on the health and well being of the subject animals are little studied and poorly understood (Society for the Study of Amphibians and Reptiles 1987). It is remarkable that since the practice of toe clipping began, so few of the thousands of studies using the method have noted any adverse effects of the procedure. This may be due, in part, to the reluctance of scientists to report results that indicate that their work impinges negatively on the lives of the subjects and thereby influences their data (Reaser 1995).

The potential disadvantages of using toe clipping to mark amphibians include the pain and stress of a physical mutilation and resulting risk of infection. In addition, there is the possibility of increased mortality or morbidity and

Inflammation response following toe clipping in a spotted frog *Rana pretiosa*. From Reaser & Dexter 1996.



changes in behaviour. There is also the possibility of confusion between marked frogs and those with natural toe removals, and of misidentification through incorrect code reading. Toe-clipped animals must usually be recaptured and handled for subsequent identification. Finally, some species are capable of tissue regeneration, which can confound identification using toe removal.

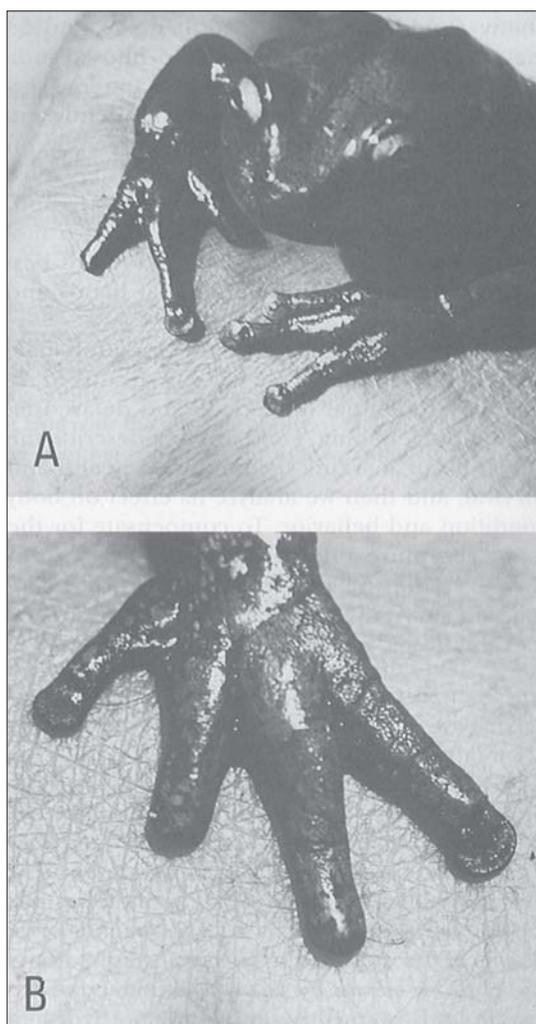
Although the effects of toe clipping are not often systematically evaluated, several authors have reported significant infection rates following toe removal. A commonly quoted study is that of Golay & Durrer (1994). These authors amputated the ends of phalanges from front and hind legs of 96 natterjack toads. Upon recapture, 18% of toads had inflammatory complications. Symptoms ranged from infection or necrosis of the stump, to necrosis of the entire foot, to metastatic infection and necrosis of the toes of other feet. In addition, 4% of recaptured toads had unrecognisable codes. Golay & Durrer (1994) concluded that toe clipping should not be used to mark natterjack toads in the future.

Davis & Ovaska (2001) found that the toe stumps of some toe-clipped western red-backed salamanders were swollen to twice their normal size and appeared inflamed. In the field, some animals had swollen toe stumps up to 240 days after marking, but these subsequently healed and the toe regenerated. The same pattern of inflammation (but not regeneration) was reported for toe-clipped

Columbia spotted frogs (Reaser & Dexter 1996). Smooth toadlets (*Uperoleia laevigata*) toe clipped in a field trial exhibited an infection rate of nearly 100%, with swollen limbs and tissue necrosis observed at the toe-clip site (Lemckert 1996). In contrast, only six of 500 Australian frogs (*Crinia signifera*) in the same study exhibited infection due to toe clipping. These infections were observed only in newly marked frogs (1–10 days after marking). These two species are of similar size, habitat and lifestyle, and the difference in infection rates illustrates the variation in a mark's effect between species and the importance of control studies in all marked populations.

There are other reports of very low rates of infection after toe clipping. Reaser & Dexter (1996) found very low infection rates (<1%) in spotted frogs, but the toe-clipped frogs were only followed for a short time, and recapture rates were very low (eight out of 122 marked). No mortality was reported, and the authors concluded that toe clipping spotted frogs is acceptable, at least in the short term. Van Gelder & Strijbosch (1996) found no inflammation in common toads over a 10-month period. Even in specimens in poor physical condition, wounds healed within 1 week and no residual inflammation was found. However, the method involved covering the stump with epidermal tissue, either by cutting the bone further back than the skin, or by pulling the skin up over the stump. This procedure aided healing in the toads, as epidermal migration is known to be a limiting factor in wound healing in anurans (Kuhn 1994).

Hands of two different individuals of *Hyla labialis*. A, immediately after the removal of two discs; B, with two healed fingertips, several months after disc clipping. From Luddecke & Amezcuita 1999. Reprinted with permission of the American Society of Ichthyologists and Herpetologists.



Although the procedure takes longer to perform and is likely to be more painful and stressful as it is being carried out, the improved healing may reduce longer-term pain and stress. Van Gelder & Strijbosch (1996) concluded that toe clipping using the described method is a reliable marking method for common toads.

Changes to the behaviour and survivorship of toe-clipped amphibians have also been reported. The best known is the Clarke (1972) study on the effects of toe clipping on survival in Fowler's toad. Clarke removed one to two toes per foot using Martof's identification code, and made 828 recaptures of 463 toads. Clarke found that the probability of recapturing a marked toad decreased as the number of toes removed increased. This result implies that there were fewer toe-clipped toads present in the population than non-toe-clipped toads, and that toe clipping affected the survivorship of Fowler's toads. The differences in recapture probability continued after the clip wounds had healed, indicating to Clarke that the decreased survivorship was most likely due to the physical absence of toes, rather than to the presence of open wounds.

Reaser (1995) presented several criticisms of the Clarke (1972) study. Reaser noted that the correlation between recapture and toe clips is taken as evidence that the missing toads have died as a result of toe clipping. However, rather than dying, the missing toads may have relocated outside the study area, perhaps because of the stress of marking but perhaps for other unrelated reasons. Alternatively, the increase in mortality could be due to some factor other than toe clipping. Clarke's study was conducted on a golf course, where mowing and biocide application were common causes of mortality in toads. Clarke gives no indication of having randomised the number of toes removed to control for possible area effects. Marked subpopulations would then be subject to different mortality pressures. Van Gelder & Strijbosch (1996) noted that Clarke would not have established the negative correlation between survivorship and number of toes clipped if he had clipped only 2–4 toes per animal, as is common in most marking schemes.

McNally (1979) found that toe clipping caused temporary disruption in the breeding routines of two *Ranidella* frog species. Weight loss was reported in leopard frogs after toe clipping (Daugherty 1976; J.C. Underhill, pers. comm. in Honegger 1979). Spotted frog tadpoles marked by cutting a series of notches into the tail reportedly suffered higher mortality than tadpoles stained with Neutral red (Turner 1960). Turner suggested that the increased mortality was probably due to decreased movement capabilities and therefore, increased susceptibility to predators. Humphries (1979) found that 14 of 30 species of Australian frogs exhibited reduced survivorship with increasing number of toes clipped. This author attributed the decrease to a loss of mobility, which reduces the frogs' ability to escape from predators. Humphries also found that frogs measuring less than 40 mm SVL were more severely affected by toe clipping than larger ones. This should be considered when marking New Zealand native frogs, all of which have an SVL of less than 50 mm.

In contrast, there are other reports giving little or no evidence of adverse effects of toe clipping on behaviour or survivorship in amphibians (e.g. Castellano & Giacoma 1993; Reaser & Dexter 1996; Schlaepfer 1998). Van Gelder & Strijbosch (1996) found that toe clipping had no influence on the amount of food consumed by common toads, or on their mean mass. Working

on the same species, Van Gelder & Rijdsdijk (1987) found no difference in the proportion of recaptured toads missing two to four toes. Even repeated toe clipping at short intervals did not appear to disrupt the male mating behaviour of Johnstone's whistling frogs (*Eleutherodactylus johnstonei*) (Ovaska & Hunte 1992). Standaert (1967) found only a slight, statistically insignificant depression in the growth of newly toe-clipped carpenter frogs (*R. virgatipes*). He concluded that toe clipping had only a slight and temporary effect on this species of frog.

Lemckert (1996) compared Australian frogs with two, three or four toes removed, and found no significant relationship between the number of toes removed and the number of captures over time. Similarly, Luddecke & Amezcua (1999) studied the effects of disc-clipping on Andean frogs (*Hyla labialis*) and found no significant relationship between the number of discs removed and body condition index, probability of recapture or behaviour. The authors concluded that none of the parameters measured decreased significantly in relation to the number of discs removed and therefore that disc clipping of Andean frogs was harmless when performed correctly.

Based on observations of natural toe and limb loss, some researchers believe amphibians cope well with tissue removals. During field studies, Van Gelder & Strijbosch (1996) found common toads that had lost an entire hand or foot, or even a leg due to natural causes. Some of these animals were recaptured more than once in consecutive years, suggesting that missing some toes does not seriously affect the survival of a toad. However, the consequences of such injuries to fitness, reproductive success and behaviour are unknown, and could be important in vulnerable populations.

Toe clipping is not universally the most appropriate method for marking amphibians. Some urodele amphibians (salamanders and newts) are capable of toe and limb regeneration, with regenerative capabilities and times to mark loss varying between species (Davis & Ovaska 2001). Salamanders are still marked by toe clipping, but inhibitory compounds are often applied to the stumps to discourage regeneration (Heatwole 1961; Efford & Mathias 1969).

Regeneration has also been reported in some species of anuran amphibians but the regenerative abilities of only a limited number of genera have been well studied. Owing to the primitive nature, and close familial associations of New Zealand native frogs to species with known regenerative abilities, it is pertinent to consider the possibility of regeneration in New Zealand's native frogs (Scadding 1980). Eggers (1998) observed a clear example of partial re-growth of digits following previous injury in an Archey's frog. However, Eggers also noted that regeneration of toes in Archey's frogs is rarely complete, and would likely result in a defective stub or recognisably altered digit. Dr Ben Bell, who has been involved in long-term field studies of New Zealand frogs, reported that he has not seen any evidence of toe regeneration in Leiopelmatid frogs (B. Bell, Victoria University, Wellington, pers. comm. 2004) In addition, Bruce Waldman considered it unlikely that the failure to recapture toe-clipped Hochstetter's frogs on the Coromandel Peninsula (Whitaker & Alspach 1999) was due to complete toe regeneration (B. Waldman, Canterbury University, pers. comm. 2001). Don Newman also reported never having observed toe regeneration in Maud Island frogs (D. Newman, DOC, pers. comm. 2001).

The three species of introduced frogs in New Zealand are members of the Hylid tree frog family, members of which are known to have regenerative capabilities (Singer et al. 1967). Kristiina Ovaska reported significant regeneration of toes, complete with toe-pads, seven months after toe clipping Pacific tree frogs (K.E. Ovaska, Biolinx Environmental Research Ltd., pers. comm. 2004). However, as 'Hylid tree frogs' may be an artificial grouping, there is no guarantee of regeneration in the species present in New Zealand (Cogger & Zweifel 1998). If *Litoria* frogs found in New Zealand are capable of regeneration, toe clipping would not be a permanent method of identification. Tree frogs may be disadvantaged by toe removal, as the specialised structures of the toes may be essential for functions such as climbing, clinging, locomotion or reproduction.

Most of the recent studies conducted on New Zealand native frogs have used toe clipping for identification (e.g. Newman 1996; Eggers 1998; Pledger 1999; Whitaker & Alspach 1999; Holyoake et al. 2001). Handling and toe clipping of New Zealand native frogs must be performed within 4 minutes, in accordance with Massey University Animal Ethics Committee requirements (Eggers 1998). There have been no reports regarding the effects of toe clipping on the behaviour or survivorship of toe-clipped amphibians in New Zealand. However, the Department of Conservation Animal Ethics Committee has approved toe clipping for use on Maud Island and Stephens Island populations.

Slaven (1992) marked Hochstetter's frogs on the Coromandel Peninsula by removing only distal phalanges of toes using scissors. Subsequent surveys revealed a steady decline in recovery rates of marked frogs, from 3.8% in 1994 to 0.8% in 1996 and to 0% in 1998 (Whitaker & Alspach 1999). Hochstetter's frogs were thought to be sedentary, but the results of these studies may contradict this supposition; it is possible that marked frogs dispersed from the area of marking. Alternatively, toe clipping Hochstetter's frogs may increase mortality. Either way, the result would be a decreased probability of recapture.

Whatever the reason, toe clipping is no longer used to mark this population of Hochstetter's frogs because of the high concern for the conservation of this species. In addition to the possible effects on the frogs' mortality or movement, the low rate of recoveries means that the marking method is not making an important contribution to understanding the dynamics of the population. In such situations researchers cannot, in good conscience, continue to use a method that provides little benefit, while potentially causing negative welfare effects, and possibly decreasing the fitness or survivability of the animals involved.

The effects of toe clipping on behaviour and survivorship should be assessed in each New Zealand native frog population before the use of this method is continued. The vulnerability of these populations means that any detrimental effects of toe clipping could be devastating to the probability of the species' survival.

### **Autotransplantation**

Rafinski (1977) used autotransplantation to mark European alpine newts (*Triturus alpestris*). Pieces of skin, 3 mm × 3 mm, were removed from the orange belly and darker dorsum of the animals and the grafts exchanged. The animals were anaesthetised during the 3-minute procedure, and no adhesives

were required to attach the grafts. Grafting was successful in 95% of the several hundred newts so marked. Rafinski reported recognition of marked newts in the field 3 years after grafting, and believed that autotransplantation is probably permanent in this species.

Skin autotransplantation was also used to mark captive African clawed frogs (Verhoeff-de Fremery & Vervoordeldonk 1982). The grafts were attached to the new site using cyanoacrylate glue, and successful grafts appeared to be permanent. The authors reported infection rates of only 3%, with 5% rejection a few days after the procedure. Aseptic conditions, such as those required for successful autotransplantation, may be impossible to achieve in field studies (Mrozek et al. 1994).

The risk of infection, requirement of anaesthetic and its impracticality in the field may make this method inappropriate for vulnerable populations.

### **Vital stains**

No reports of vital staining adult amphibians have been found (see, *Dyeing larval amphibians*, pp. 42–43). For vulnerable populations, euthanasia for recovery of vital stain information is not likely to be acceptable.

### **Natural marking identification**

Natural markings have commonly been used to identify individual amphibians (e.g. Hagstrom 1973; Andreone 1986; Doody 1995). Pattern mapping is widely accepted for many amphibian species as a reliable, non-invasive method of identification (Reaser 1995) (see figure p. 67). For some amphibian species, skin pattern is not a reliable way to identify individuals, as patterns may change with age (J. Reaser, pers. comm. in Halliday 1995; J. Baker, pers. comm. in Halliday 1995). Researchers must be sure that pigmentation patterns are stable before using them for individual identification. Natural markings on amphibians are best documented with the animal anaesthetised and colour-photographed underwater (Donnelly et al. 1994). However, the use of anaesthetic increases the risk to the animal, and may not be appropriate for use on vulnerable populations.

In New Zealand, pattern mapping has already been used to identify individual Hamilton's frogs on Stephens Island (Newman 1982, 1990). Photographic records allow differentiation of the few hundred individuals in the population. Each Hamilton's frog has a unique pattern of black markings along the upper lip that allows it to be recognised readily (Newman 1982) (see figure p. 68). Pattern mapping provides an ideal way to identify individual frogs on Stephens Island because of the small size and contained nature of the population. In addition, the use of such a non-invasive method is considered appropriate for one of the most vulnerable frog populations in the world.

Although all Leiopelmatid frogs have black markings along the sides of their bodies and legs that could be used for individual identification, pattern marking may be less useful for the other three native frog species. Some Hochstetter's and Maud Island frogs have a uniformly dark morphology that obscures the dark markings on the body. Hochstetter's frogs are relatively widespread and their populations are not well defined, making recognition of individuals by their natural markings more difficult and time-consuming.

Likewise, populations of Archey's frogs are not clearly defined; however, recent trials indicate that it is feasible to use natural markings to identify individuals of this species (for a thorough review of the use of natural markings for identifying Archey's frogs, see Bradfield 2004). In addition, the recent development of a multi-imaging device allows rapid documentation of natural markings that can be used to identify individual frogs. This instrument has already been used successfully to identify individual Archey's frogs (Avi Holzapfel, DOC, pers. comm. 2004). The device<sup>5</sup> includes a mirrored stage that makes it possible to record four different image angles of each frog in a single digital photograph (see figure p. 68). Its use will reduce the lengthy handling times previously required to identify New Zealand native frogs using their natural markings.

Natural marking recognition is likely to be the most acceptable method of identifying New Zealand native frogs wherever it is feasible. Although recognition by natural markings may require repeated recapture and longer handling times, it is non-invasive and does not change the appearance of the animal. This means that cryptic colouration is not disrupted and the frogs are not more conspicuous to predators. Considering the vulnerability and ecological and cultural significance of New Zealand frogs, natural marking identification is likely to be viewed favourably by the general public and conservationists.

## S U M M A R Y

In general, external tags are difficult to apply and may affect the behaviour, appearance and survivability of frogs. Because of the very small size of New Zealand frogs, there are concerns about the use of implanted devices (e.g. PITs). There is still great contention over the suitability of toe clipping for the identification of frogs. This method is currently used to mark some native frog populations in New Zealand. However, with the introduction of chytrid fungus and predicted population decreases, a less invasive marking method may be preferable.

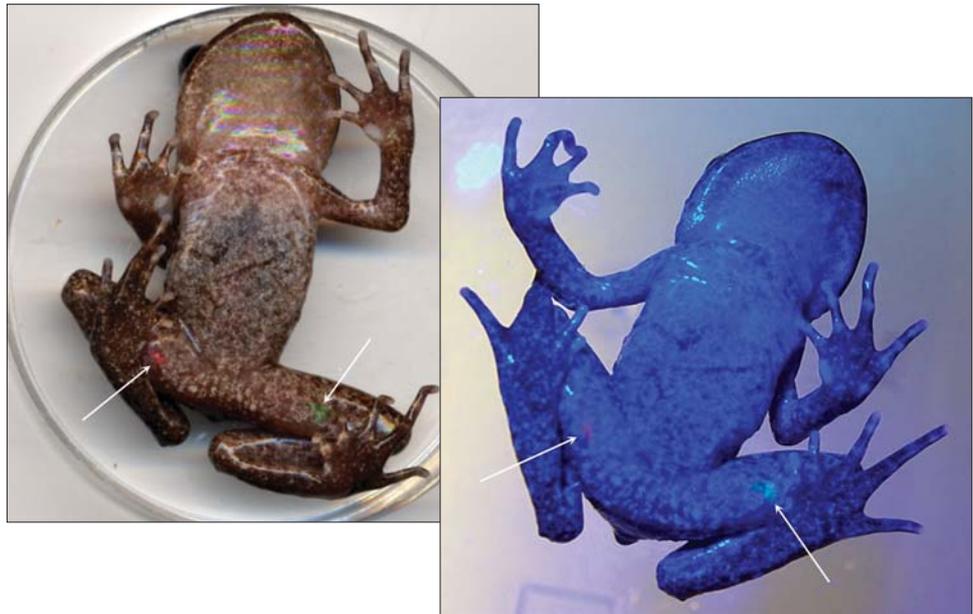
There is a growing trend towards using natural markings to identify New Zealand frogs. Natural markings have been successfully used to identify individual Hamilton's frogs on Stephens Island. This population is well suited to natural marking identification, owing to its small, defined nature. Natural markings may be less useful in the identification of the other three native frog species. Some Hochstetter's and Maud Island frogs have dark forms, and the pigmentation may obscure identifying markings. Populations of Hochstetter's and Archey's frog are more widespread and less clearly defined, making individual identification from natural markings more difficult and time-consuming. The New Zealand Department of Conservation is currently investigating the use of natural markings for identifying individual Archey's frogs.

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<sup>5</sup> Developed by the New Zealand Department of Conservation, with support from Waikato University and MWH Environmental Consultants.

Maud Island frog (*Leiopelma pakeka*) (snout-vent length = 45.5 mm) marked with Visible Implant Fluorescent Elastomers (VIE, Northwest Marine Technology Inc.) on the ventral surface of the thighs. Red, right thigh; green, left thigh (viewed with white light, top; UV light, bottom). Photographs taken approximately 20 months after marking.

PHOTOS: PHIL BISHOP, UNIVERSITY OF OTAGO.

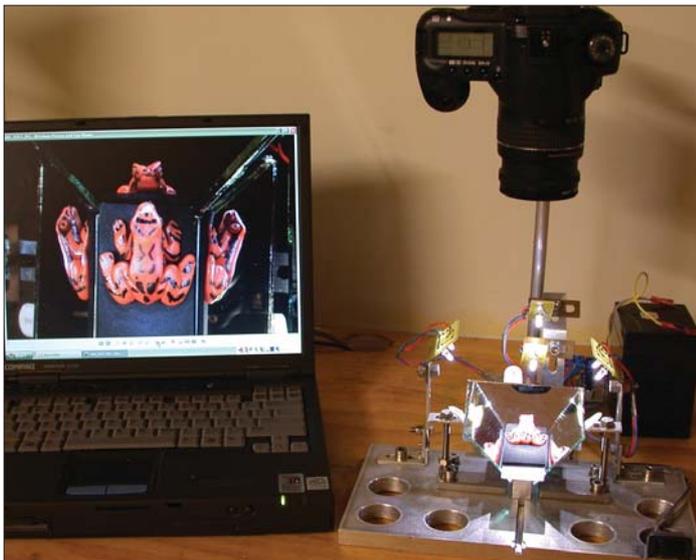


Variation in natural markings on the ventral surface of crested newts (five taxa from *Triturus cristatus* superspecies) which have been used for individual identification.

PHOTO: L.A. VAN DER LAAN. From Arntzen & Wallis 1999.



Two Hamilton's frogs (*Leiopelma hamiltoni*) from the Frog Bank individually identified using their natural markings. Note difference in skin colour and pattern, particularly along upper lips. PHOTOS: DON NEWMAN, DOC.



Multi-imaging device records four different image angles of each frog in a single digital photograph, allowing rapid documentation of natural markings which can be used to identify individual frogs. Archey's frog (*Leiopelma archeyi*.) PHOTOS: AVI HOLZAPFEL, DOC.



# Reptiles

New Zealand is home to two lizard families: Gekkonidae (geckos) and Scincidae (skinks), as well as two species of tuatara (Sphenodontidae) (Gaze 2001), all of which are protected under law. These reptiles are found in a wide variety of New Zealand habitats, although many species are now restricted to predator-free offshore islands. New Zealand's lizards are unique, most notably because they bear live young rather than lay eggs. The egg-laying skink (*Oligosoma suteri*) is the exception. Most of the lizard species found in New Zealand are threatened, with three species (and one subspecies) listed as Nationally Critical (Hitchmough 2002).

Geckos in New Zealand range from 55 mm to 160 mm in snout-vent length (SVL). There are two groups of geckos, the grey-brown (*Hoplodactylus* spp.) and the green (*Naultinus* spp.) geckos. The grey-brown geckos are generally nocturnal, and have wide pads on the under surface of their toes that assist in climbing smooth vertical surfaces. In contrast, the green geckos are diurnal, and have slender toes for grasping twigs and climbing among foliage. Green geckos are less likely to lose their tails than grey-brown geckos as the tail is used for grasping during climbing (Cogger & Zweifel 1998). New Zealand geckos produce a maximum of two young per year; this slow reproductive rate limits the speed of population recovery.

New Zealand skinks vary in size, having SVLs from 48 mm to 140 mm; however, the most common species have an average SVL of about 60–80 mm. Skinks can be differentiated from geckos by their shiny skin, flat overlapping scales and indistinct necks.

Tuatara are long-lived (up to 60 years) and exhibit sedentary behaviour, late sexual maturity and low reproductive rates. Tuatara can grow to SVLs of up to 280 mm. They have been legally protected since 1895, and wild tuatara are now entirely restricted to 35 offshore islands (Gaze 2001). Two species are recognised, *Sphenodon guntheri* (Brothers Island tuatara) and *S. punctatus*. There are two subspecies of *S. punctatus*: the northern tuatara (*S. punctatus punctatus*) and an unnamed subspecies, the Cook Strait tuatara (Gaze 2001). Both species (and subspecies) are currently listed as Threatened, however, populations are considered stable (Hitchmough 2002).

There are several characteristics of New Zealand reptiles, and reptiles in general, that should be taken into consideration when selecting a marking method. Firstly, all New Zealand reptiles are capable of shedding their tails (autotomy). Therefore, New Zealand reptiles should never be marked on the tail. In addition, tail colourations and patterns should be used only to supplement other, more stable features when identifying individuals by their natural markings. Secondly, all reptilian species rely, to a greater or lesser degree, on behavioural thermoregulation (e.g. basking in the sun) to modify their body temperature. Thermoregulation affects the ability of reptiles to perform certain behavioural and physiological processes such as digestion and predator evasion (Wang & Adolph 1995; Fair & Henke 1999). Any method that affects the animal's ability to thermoregulate in a normal manner could have

detrimental effects on behaviour and survival. Finally, reptiles shed their skin at regular intervals, meaning that any marks applied to the epidermis will be lost periodically. Shedding is a time of increased vulnerability (Lewke & Stroud 1974), and marks that interfere with or induce skin shedding could have negative effects on behaviour or survival.

Throughout this section, most of the information refers to lizard species. However, the information is also applicable to tuatara. In general, the word 'lizards' will be used to encompass all New Zealand reptiles, rather than 'reptiles', as reptilian groups such as snakes, turtles and tortoises are not discussed.

## TEMPORARY MARKS

### Painting

Paint can be extremely useful for the short-term identification of reptiles (see figure p. 88). However, as lizards regularly shed their skin, paint marks must be replaced periodically. Conspicuous paint marks have often been used in conjunction with a more permanent, less visible mark such as toe clipping, to enable remote identification without human interference (Simon & Bissinger 1983). Water-based paints can be useful under laboratory conditions, are easily removed and have not been found to cause damage or necrosis to the tissues (Lopez et al. 2003). Longer-lasting oil-based paints are generally used in field studies. Quick-drying model airplane paint, often used to mark terrestrial reptiles, can be messy and difficult to apply in the field (Boone & Larue 1999).

Patterson (1992) tested a range of pigment markers on Otago and grand skinks (*Oligosoma ottagense* and *O. grande*, respectively). Pilot<sup>®</sup> silver marker pen, Chromacryl<sup>®</sup> white acrylic paint, nail varnish, Paper mate<sup>®</sup> correction fluid and sheep marker dye were used to mark the skinks. The correction fluid was visible for longer than any of the other markers, lasting an average of 16 days. Silver marker was visible for 12 days, white paint and sheep dye for 9 days and nail varnish for 8 days. Temporary marks were usually lost owing to abrasion with rocks or skin shedding (the latter occurs once per summer in these species). No ill effects were observed, and the author noted that the smallest amount required for identification should be used in order to minimise possible long-term effects.

Flannagan (2000) used a non-toxic, xylene-free, silver ink pen to make temporary markings on the dorsum of goldstripe (*Hoplodactylus chrysosireticus*) and Duvaucel's (*H. duvaucelii*) geckos on Mana Island, New Zealand. All lizards marked in the winter months retained their marks for 160 days, while lizards marked in the summer lost their marks within 83 days. Evidently growth and sloughing occur more frequently during warm months than winter months in these populations.

Southern prairie lizards (*Sceloporus consobrinus*) were marked using blue or white latex paint (Tulip<sup>®</sup> Pearl Fabric Paint) (Quinn et al. 2001). Nine captive male lizards were painted with two patches (covering about 80% of the dorsum) and one dot on the head or the base of the tail. No measurable effects on

Rainbow skink  
(*Lampropholis delicata*)  
marked dorsally with  
xylene-free silver pen.  
Natural toe loss (rear left  
foot) and regenerating tail  
also used for individual  
identification.

PHOTO: JOANNE PEACE,  
UNIVERSITY OF AUCKLAND.



survivorship or behaviour of the captive males were observed, and the paint remained in place for the duration of the 4-week study. Ten of 12 captive striped plateau lizards (*S. virgatus*) marked with xylene-based paint pens (Faber-Castell™) retained their marks over a 4-week study (Quinn et al. 2001).

A fast-drying, oil-based reflective paint (Scotchlite™ Brand Reflective liquid series 7200) was used to mark green iguanas (*Iguana iguana*) (Rodda et al. 1988). A thin coat of the paint was dull and weakly translucent in daylight, but reflected brightly under artificial light at night. Small spots (1 cm in diameter) of yellow, silver or white paint were used to aid in the relocation of these arboreal animals at night, and under a strong artificial light such marks could be seen up to 100 m away. The paint marks were retained for up to 2 months or until the skin was shed. White and silver could not be distinguished from a distance. Although yellow paint could be distinguished from the other colours, it was less reflective, especially when wet.

The greatest disadvantage of painted marks, apart from their temporary duration, is the fact that painting lizards makes them more conspicuous not only to humans, but also possibly to conspecifics and visually oriented predators such as birds. Simon & Bissinger (1983) evaluated whether the colour of paint used affected survivorship in mountain spiny lizards (*S. jarrovi*). Testors® model paint was used to mark the dorsal surface of the lizards with a symbol in either a conspicuous colour (yellow, red or white) or a cryptic colour (dark green, tan or navy blue). The failure to recapture or relocate a marked lizard in an intensively surveyed area was taken to indicate that it had died or emigrated. The authors found no significant difference in recapture rate among or between the cryptic or conspicuous colour groups. The primary predators of *S. jarrovi* are small snakes and birds. Snakes rely more on chemoreception for prey location than vision. Although birds are primarily visual hunters, it is possible that they do not perceive colours in the same way as humans, which could explain why the conspicuous colours apparently did not affect the survivorship of *S. jarrovi* in this study.

These results corroborate the earlier study of Jones & Ferguson (1980); they tested the effect of painting diurnal fence lizards (*S. undulatus*) with yellow and orange paint. All lizards were toe clipped for permanent identification, and half were also marked with a spot of paint at the base of the tail. The authors found no differences in the number of recaptures of painted and unpainted lizards. However, when the data for orange paint were analysed separately there was an almost significant difference between painted and non-painted lizard recapture rates. In addition, the number of tail breaks, which reflect predation pressure, was slightly higher in painted (both colours) than unpainted animals. The authors concluded that the paint markings did not seem to increase the probability of the lizards being detected by visually oriented predators such as birds. However, they conceded that they had applied the minimum amount of paint, which was probably not representative of the type of paint marks used in research, and that large paint designs may affect predation. In contrast, L.J. Vitt (pers. comm. in Simon & Bissinger 1983) lost large numbers of lizards (*Cnemidophorus* sp.) after painting.

Paint marking may also significantly affect intraspecific interactions. Males of many lizard species show conspicuous breeding colours, which may function as

social cues to females or other males. Bright orange head colour is known to be an important releaser of aggressive behaviour in some lizard species (Madsen & Loman 1987; Cooper & Vitt 1988). The experimental manipulation of the throat colour of tree lizards (*Urosaurus ornatus*) altered aggression and dominance relationships (Hover 1985). Similarly, changing the colour of the eyespot in green anoles (*Anolis carolinensis*) affected the social status of the bearer; lizards with black eyespots outranked those with green (Korzán et al. 2002). Eyespot colour also affected the hormone levels of the opponent, suggesting that the eyespot may indicate the bearer's disposition or arousal level. Such examples highlight the importance of external appearance on intraspecific signalling, and the potential for artificially applied marks to alter the interactions between conspecifics.

Small, male Algerian sand lizards (*Pssamodromus algirus*) were painted on the sides of the mouth with either orange or brown Testors® model paint (Lopez et al. 2003). The orange paint simulated the colouration of a sexually mature male, while the brown mimicked the juvenile male or female colouration. In this study, colour alone did not increase the intensity of the aggressive behaviour of larger, resident males. The larger males responded aggressively to the orange-painted males only when the scent of a dominant male was added, indicating that males of this species use pheromones as well as colouration to determine the gender or dominance status of an intruder. Similarly, painting fence lizards to mimic the blue ventral patches of sexually mature males had no effect on the behaviour of other males (Cooper & Burns 1987). However, Lopez et al. (2003) suggest that colouration may be more important in long-distance communication, outside the range of pheromones.

Only marker compounds of low toxicity to vertebrates should be used, and new compounds should be tested before field use (Patterson 1992). Boone & Larue (1999) assessed the suitability of Faber-Castell™ paint pens that contained xylene as a carrier. Xylene is known to have toxic effects on animals (D'Azevedo et al. 1996; Rana & Kumar 1997). Captive side-blotched lizards (*Uta stansburiana*) were marked with a spot of paint on the dorsum. The lizards were painted every 3–4 days over 2 weeks to simulate four recapture events. Of the 21 animals painted, five died (24%) after two or more paint applications. None of the unmarked control animals died during the study. The sleeping behaviour of the marked animals was also altered. Whereas unmarked controls continued to burrow under the substrate at night, 73% of painted juveniles and 20% of painted adults switched to sleeping above ground. Failure to seek cover at night could affect survival by increasing vulnerability to predation or through temperature stress.

Boone & Larue (1999) also observed what appeared to be skin irritation when the paint was first applied: some animals gaped; others exhibited dorso-ventral flattening or backward flexion; and still others, lethargy. Such responses were most often seen after the second application of paint, and smaller animals seemed affected more often than larger animals. Likewise, four of the five mortalities were smaller juvenile animals. The authors concluded that the xylene-based paint was associated with death and altered behaviour in side-blotched lizards, and its use could introduce bias into field experiments.

The difference in species' responses to paint materials is demonstrated in a follow-up study on xylene and latex paints by Quinn et al. (2001). Faber-Castell™ xylene-based paint pens were used to mark 12 captive striped plateau lizards. Control lizards were similarly handled but marked with water. Only two animals were repainted during the 4-week study period. No differences were found in survivorship, growth or mass between painted and control animals. In addition, no behavioural responses, such as the gaping and dorso-ventral flattening described by Boone & Larue (1999), were noted in painted animals. Reasons cited for the differences in the two studies included: interspecific differences in sensitivity to xylene, and differences in handling and painting regimes, with the side-blotched lizards being handled and repainted up to four times in 2 weeks, and most striped plateau lizards being handled and painted only once. Quinn et al. (2001) concluded that a single application of xylene-based paint had no measurable effects on survivorship and behaviour in striped plateau lizards.

Bonne & Larue (1999) also found that subpopulations of the same species collected from different locations were differentially affected by the xylene-based paint markers. One group (n = 3) did not exhibit any negative responses, while four of five marked animals from another location responded negatively. This result highlights the need for assessment of the suitability of marking materials and methods not just for the species, but also for the particular population under study.

Painting is an extremely useful method for identifying reptiles in short-term studies. However, the effects of paint materials and marking methods on physiology, behaviour, predation and intraspecific interactions must be assessed for each species and population before they are used in the field. In addition, paint marks are regularly lost when the marked animal sheds its skin.

### **Attached devices**

Attached devices are used mainly to increase the visibility of reptiles under study. Zwickel & Allison (1983) attached streamers to the backs of small New Guinea blue tongue skinks (*Tiliqua gigas*) for remote identification. Henderson (1974) tagged iguanas by tying bells around their necks with fishing line. It is likely that Henderson's method affected behaviour or risk of predation.

Buttons and beads have been sewn onto snakes (Pough 1970) and lizards (Snell 1984) (see figure p. 88). Such methods have been used to individually mark Galapagos land iguanas (*Conolophus* sp.) (Snell 1984), green iguanas (Rodda et al. 1988) and other lizards (Fisher & Muth 1989). Nylon thread was used to sew beads into the mid-dorsal skin flap of green iguanas in South America (Rodda et al. 1988). Two beads per side in hatchlings, and four beads per side in adults, allowed identification of individuals in each study area. A hole was punched through the loose skin first, and the authors noted that slack must be left in the thread to allow for subsequent growth. There were no problems with identification of bead-marked animals. About 5% of bead marks were lost in hatchlings but, because older animals had tougher skin, no beads were lost after 3 months of age. Most bead-marked iguanas could be identified remotely, using binoculars. Remote identification is probably more feasible in the relatively large and inactive green iguanas than in smaller or more active lizards, which might require recapture to be identified from coded bead marks.

Certain trailing devices can be used to track animals over short periods of time (Dole 1965). Deavers (1972) attached a 30-cm length of string with a piece of foil attached to its end around the lower abdomen of the fringe-toed lizard (*Uma notata notata*). He used this method to measure the burial depth of the lizards at night.

Attached devices may be useful for increasing the visibility of marked animals over short periods of time or for relocating animals that are individually marked with less visible permanent marks. In addition, some devices can be used to track reptiles over short distances. No systematic evaluations of the effects of attached and trailing devices on reptiles have been found. However, trailing devices are likely to affect the animal's behaviour and its ability to evade predation. In addition, many reptiles rely on cryptic colouration to avoid predation or to facilitate sit-and-wait foraging tactics (Fair & Henke 1999; Burrow et al. 2001). Any device that increases conspicuousness to the researcher is also likely to make the marked animal more noticeable to predators and prey.

### **Fluorescent powders**

Based on the success of fluorescent powders in marking mammals (e.g. Mikesic & Drickamer 1992), tortoises (e.g. Butler & Graham 1995) and other animals, this method was evaluated for tracking the movements of nocturnal lizards (Fellers & Drost 1989). Island night lizards (*Xantusia riverstiana*) were marked by dipping them, tail first, into a plastic bag containing 50 mL of powder. The lizards were held by the head to keep the powder out of their eyes, nares and ear openings, and the powder was massaged into the skin to improve retention. The powder adhered well, even though this species is smooth-skinned. Powder trails facilitated tracking for at least 5 nights; however, trails became more difficult to detect after the first 2 nights. Island night lizards are rather sedentary, and more active lizards might have to be re-powdered sooner to continue tracking. Powder tracking does not allow researchers to determine the direction of movement or number of times a route has been used.

No lizards showed any ill effects from marking, but the authors noted that the fluorescent powders were very bright under daylight, and may not be appropriate for marking conspicuous diurnal species (Fellers & Drost 1989). This concern was reiterated by Butler & Graham (1995), who noted that tortoise hatchlings marked with fluorescent powder were extremely conspicuous during the day. In addition, Fellers & Drost (1989) suggested that the powder might influence heat absorption which, if true, means that only the ventral and lateral surfaces should be coated.

Trials on western fence lizards (*Sceloporus occidentalis*) showed that powder adheres better to lizards with keeled scales, allowing these animals to be tracked for longer periods (Fellers & Drost 1989). Texas horned lizards (*Phrynosoma cornutum*) were marked using the same method (Stark & Fox 2000). Of the five Radiant® colours used, chartreuse, pink and green were found to be most detectable. Blue powder trails were difficult to find after the animal had moved about 15 m, and its use was not recommended. The authors reported that 93% of the powder-marked lizards could be tracked until the end of the day's trial. Confusion caused by the overlap of trails of the same colour

was the primary reason for unsuccessful tracking, and limited the usefulness of the method for species that stay in the same area. In addition, the limited number of available colours meant that only a small number of resident animals could be uniquely distinguished. Finally, powder blew off lizards that spent a great deal of time in open, windy areas.

Fluorescent powder marking can be extremely useful for tracking the movement of nocturnal or secretive reptile species. However, the powders are very bright under daylight, and are likely to increase the conspicuousness of marked animals to predators or prey. In addition, it has been shown that lizards may respond to visual cues outside the spectrum visible to humans (e.g. naturally occurring patches on their skin that are only visible to humans under UV light) (Fleishmann et al. 1993). Therefore, it is possible that the application of fluorescent powder could affect intraspecific social signalling of marked animals.

### **Radioisotope marking**

H. Fitch (pers. comm. in Ferner 1979) reported successfully using radioactive tantalum ( $Ta^{182}$ ) wires on a variety of reptiles: five-lined skinks (*Eumeces fasciatus*), ground skinks (*Scincella lateralis*), slender glass lizards (*Ophisaurus attenuatus*), ring-necked snakes (*Diadophis punctatus*) and worm snakes (*Carphophis amoenus*). The tag was not successful with the brown snake (*Storeria dekayi*), which is highly mobile, as the tag was soon lost in the field. Fitch recommended the use of radioactive tagging for sedentary species. Barbour et al. (1969) also studied worm snakes using radioactive cobalt tags.

O'Brien et al. (1965) tracked a female northern fence lizard (*Sceloporus undulatus hyacinthinus*) using radioactive gold ( $Au^{198}$ ). A gold wire was inserted into a piece of plastic tubing, and the tag was tied around the lizard's waist with the tubing on the ventral surface in front of the hind legs. The animal was allowed 7 hours to become accustomed to the tag, and then its location was monitored every 1–4 hours for approximately 2 weeks. When first released, the animal was detectable from about 4 m away. By the seventh day, the tag was not detectable beyond 1.5 m away, and the animal was captured and retagged. Effects of the radioisotope or tag on the animal were not reported. It is possible that the relatively close proximity of the researcher to the animal (a maximum of about 6 m away during scanning) during 2 weeks of intensive monitoring may have influenced its natural behaviour. Inglis et al. (1968) devised a remote method for automatically and continuously recording the location of small radioisotope-tagged animals. This type of system would obviate the requirement for human presence in the study area.

Radioactive tags were used to investigate the home ranges of horned lizards (*Phrynosoma* sp.) (Munger 1984). More recently, Thompson (1993) used radioactive sodium ( $Na^{22}$ ) to track stripe-tailed goanna (*Varanus caudolineatus*). Radioisotope tracking was used because previous attempts to track this species using miniature radio transmitters (weighing less than 2 g) were unsuccessful. Stripe-tailed goannas use tree hollows to hide, and the transmitters severely restricted the lizards' movement within these hollows.

Nine  $\mu$ Ci of radioactive sodium (370 kBq) were injected into the peritoneal cavity of 11 lizards, and their daily movements tracked for up to 18 days

(Thompson 1993). Laboratory studies of the same species revealed that a 15 g lizard injected with 10  $\mu$ Ci of sodium could be detected, behind wood, from 50 cm away. The author concluded that radioactive sodium was an effective method for tracking this species, but two potential problems were identified. Firstly, the time required to search for and locate lizards meant that there was a high human impact on the study area, which may have affected the lizards' natural behaviour (e.g. Sugerman 1990). In addition, there was a reasonably high probability of mistaking a radioactive scat for a lizard. No mention was made of the potential effects of the radioactivity on the lizards.

There are only a few reports of the use of radioactive material for tracking reptiles, and most of these are relatively old. It appears that other methods of identifying, locating and tracking reptiles have superseded the use of radioisotope marking. This is probably because of the deleterious effects of radioisotopes on animals, as well as the strict safety and regulatory restrictions on the use of radioactive material. In addition, advances in technology have facilitated the use of radio-telemetry for tracking small animals, largely supplanting the need for radioisotope marking.

## SEMI-PERMANENT MARKS

### Tags

Rao & Rajabai (1972) tagged Sita's (*Sitana ponticeriana*) and bloodsucker (*Calotes nemoricola*) lizards around the thighs with aluminium rings of different shapes and colours, and reported no apparent hindrance of movement or behaviour. Desert iguanas (*Dipsosaurus dorsalis*) were marked with coloured tail bands for individual identification (Muth et al. 1978). Coloured plastic bird bands glued to the tails of six-lined racerunners (*Cnemidophorus sexlineatus*) were retained for an average of 26 days (range: 4–63 days) (Paulissen 1986).

It is surprising that so few studies have reported the use of tags as a method of lizard identification. No reports directly assessing the effects of tagging on behaviour or survival have been found. This dearth probably reflects the fact that tags are generally highly conspicuous, and may affect the cryptic colouration of the bearer. In addition, tags may interfere with skin shedding, which could cause dysecdysis or necrosis of the tissue under the tag.

### Collars and harnesses

Colour-coded, plastic Insulok<sup>®</sup> cable ties were used to identify adult tree agamas (*Acanthocercus atricollis atricollis*) (Reaney & Whiting 2003). Juveniles (with a SVL less than 100 mm) were marked with silver pen because collars were considered to be detrimental to growth. Warrick et al. (1998) used nylon cable ties secured with copper wire to attach transmitters to adult blunt-nosed leopard lizards (*Gambelia sila*).

Two different methods were used to attach transmitters to free-ranging desert iguanas (Muth et al. 1978). Non-gravid lizards were instrumented using a waist collar, with the transmitter sitting on the dorsal pelvic region. Gravid females

were instrumented differently, as a waist collar would have caused abdominal constriction during egg development. A yoke-harness was attached to gravid females, with the transmitter worn between the forelegs and the antenna passing up over the back of the neck. The yoke followed the contour of the animal's body and was fitted snugly to prevent snagging. The transmitter was secured to the lizard with cotton thread passing behind the forelegs and joining the yoke at the shoulders. In addition, thread was used to stop the two sides of the yoke from spreading across the lizard's chest. This harness did not appear to interfere with locomotion, even in dense vegetation, and no differences in the behaviour between harnessed and non-instrumented animals were observed.

Richmond (1998) devised a backpack for flat-tailed and coast horned lizards (*Phrynosoma mcallii* and *P. coronatum*, respectively) to attach radio-transmitters. Similar backpacks were used to attach radio-tags to adult Texas horned lizards (Fair & Henke 1999; Burrow et al. 2001). Backpacks made of beige cotton muslin with elastic straps were dyed to match the natural substrate colour, to avoid disrupting the cryptic colouration of the lizards. The anterior strap was passed around the neck and one front leg, while the posterior strap went around the abdomen in front of the hind legs. The straps were glued to the chest and lower abdomen with cyanoacrylate gel (Burrow et al. 2001).

Fair & Henke (1999) dyed tan harnesses with black spots to simulate the cryptic colouration of Texas horned lizards. These harnesses covered approximately 50% of the animal's dorsal surface, and the authors noted that this might have altered thermoregulatory behaviour by decreasing the absorption of solar radiation. Such a decrease in absorbed energy could make horned lizards more susceptible to predation.

Collars are suitable for short-term studies only, as reptiles continue to grow throughout their lives. They must be removed, or designed to expand, if they are to be used in longer-term studies. Harnesses are useful for the external attachment of telemetric equipment. However, researchers must take care that thermoregulatory behaviour is not affected by extensive coverage of the animal's dorsal surface. In addition, harnesses and collars should fit snugly to stop snagging or premature loss.

### **Nocturnal lights**

Only one report on the use of nocturnal lights for marking reptiles has been found. Clark & Gillingham (1984) glued small tubes filled with Cyalume® fluid to the back of 20 anoline lizards (*Anolis* spp.) in Puerto Rico. The tubes measured 1.8 mm × 30 mm and were glued (Duco® cement) longitudinally to the dorsum. The lights were attached 2 hours before nightfall, and the glue was allowed to set for 5 minutes before the animals were released. Marked lizards observed before nightfall appeared to behave normally. After darkness, individuals could be located easily at their perch sites from up to 30 m away, and the lights were visible for 6 hours. The authors concluded that this method was harmless to the animals, since all devices were sloughed off within 24 hours, with no evidence of damage to the lizard's body.

Glass spheres and gelatin capsules filled with Cyalume® liquid, or pre-formed chemiluminescent light sticks have been used to study a variety of small animals (e.g. Buchler 1976; Barclay & Bell 1988; Hovorka et al. 1996). Likewise,

Betalights (Davey et al. 1980) and miniature LEDs (Wolcott 1977) have been used to observe the behaviour of animals as small as crabs. Thus, it seems plausible to use such methods on reptiles.

Chemical lights, Betalights and LEDs could be useful for behavioural studies of nocturnal reptiles over a single night. Nocturnal lights can be easily attached to reptiles using non-toxic glue, and the marks are lost as soon as the skin is shed. There may be concern that nocturnal lights increase the conspicuousness of marked reptiles to predators or prey. This possibility should be assessed for each population for which nocturnal lights are being considered.

## Telemetry

Telemetric equipment can be attached to reptiles externally using harnesses and collars, or adhesives, or by surgical implantation or force-feeding. Externally attached equipment should neither conceal nor enhance the appearance of structures such as dorsal crests and gular flaps, which reptiles use for social signalling (Ferner 1979). Force-feeding has mainly been used to instrument snakes, owing to the lack of alternative attachment options. However, several studies have found that ingested transmitters can have significant effects on the behaviour and survivorship of reptiles, and this method is now strongly discouraged (Fitch & Shirer 1971; Lutterschmidt & Reinert 1990; British Columbia Environment Resources Inventory Committee 1997).

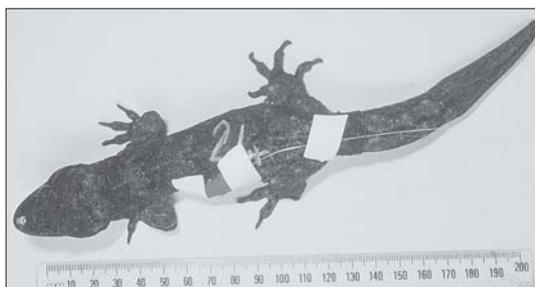
Detection durations and distances vary with species characteristics and the equipment used. Nine of 16 Texas horned lizards instrumented with transmitters in backpacks were tracked for more than 30 days (Fair & Henke 1999). Blunt-nosed leopard lizards instrumented with 4 g packages attached to neck collars had to be recaptured every 15 days to replace the batteries (Warrick et al. 1998). These lizards could be detected at distances of 50–150 m whether above or below ground. Free-ranging desert iguanas carrying transmitters in backpacks were tracked for 33–47 days before removal (Muth et al. 1978). Above ground, the iguanas could be detected up to 100 m away, with detection ranges of 70 m when they were underground in burrows. No differences were observed in the behaviour of instrumented and non-instrumented desert iguanas, and the transmitters did not appear to interfere with locomotion, even in dense vegetation.

Transmitters were attached to the tails of 55 adult frillneck lizards (*Chlamydosaurus kingii*) using glue and adhesive bandages (Griffiths & Christian 1996). The packages weighed 15 g, about 2% to 6% of the lizards' weight. Telemetered frillnecks could be detected at distances between 300 m and 1 km from the researcher. Sabo (2003) used non-toxic epoxy glue to attach radio-

transmitters to western fence lizards. The 1.3-g transmitters were attached to 17 lizards, with SVLs of 62–72 mm, including 8 gravid females. The package was glued to the dorsal surface above the pelvis, to minimise interference with locomotion. Four instrumented females died during the 5–6 week study, some from snake predation. However, as there were no control animals for comparison, it is not known whether the mortalities were related to the wearing of transmitters.

Duvaucel's gecko  
(*Hoplodactylus  
duvaucelii*) with radio-  
transmitter and paint  
markings on Lady Alice  
Island.

PHOTO: GRAHAM USSHER,  
AUCKLAND REGIONAL COUNCIL.



Sabo's (2003) study examined aspects of the nocturnal retreats chosen by western fence lizards. However, there was no mention of the possible effect of a protruding transmitter on the animal's ability to squeeze into a retreat. The presence of the transmitter may have affected the size of the retreat chosen, and therefore the outcomes of the study. Transmitters glued to stripe-tailed goannas were found to severely restrict the lizards' movement within tree hollows (Thompson 1993).

Weight constraints often limit the use of telemetry to adult reptiles. Only male tree agamas weighing more than 100 g were considered to be suitable to wear a transmitter backpack weighing 7.8 g (Reaney & Whiting 2003). Males with (n = 4) and without (n = 8) transmitters selected similar diurnal and nocturnal perch heights. Christian & Bedford (1995) attached relatively heavy transmitters (20 g) to the base of the tail of frillneck lizards. These transmitters weighed about 20% of the body weight of the average male or large female (about 400 g), well in excess of the recommended 5% to 10% (Heyer et al. 1994). However, the authors reported that the packages did not impair the locomotion of the frillnecks in any way. Transmitters weighing less than 2 g are available, which is less than the average clutch mass of many small lizards (Vitt 1977). However, smaller transmitters have limited operational lives and transmission ranges.

Temperature-sensing radio transmitters are often used to monitor the body temperature and thermoregulatory behaviour of free-living reptiles. Transmitters that included a temperature probe were attached to the base of the tail of frillneck lizards with an adhesive bandage (Christian & Bedford 1995). The probe was inserted into the cloaca to a depth of 6 cm and a stitch was put through the lip of the cloaca to secure the probe in place. Body temperature was recorded every 15 minutes and transmitted with location information. Temperature-sensing radio transmitters were implanted into the abdominal cavity of adult land mullets (*Egernia major*), the largest skinks in Australia (Klingenbock et al. 2000). Body temperature was determined each time the animal was located in the field.

Wang & Adolph (1995) attempted to determine the effects of surgical implantation on behavioural thermoregulation in adult male western fence lizards. If implantation affects thermoregulation, the data collected from telemetered animals will not reflect the normal situation. Twelve animals were surgically implanted with mock transmitters, each weighing 1.2 g. A 3-cm longitudinal incision was made on the caudal surface of the abdomen, and the implant was placed in the peritoneal cavity. Control groups included animals undergoing sham surgery (which was identical to implantation except that the transmitter was not introduced) (n = 12), and an anaesthetic-only control (n = 12).

Adult tuatara (*Sphenodon punctatus*) with radio-transmitter and backpack harness, Whale Island, Bay of Plenty.

PHOTO: ALEX EAGLES.



For the first 2 days after treatment, sham-operated lizards selected warmer environments and implanted lizards selected cooler environments than the anaesthetic controls. Three days after surgery, there were no differences in temperature selection between the three groups. The authors concluded that transmitter implantation and sham surgery had small, short-lived (but significant in the short term) effects on behavioural thermoregulation in this

species (Wang & Adolph 1995). In contrast, implantation of transmitters early in the active season caused some female western rattlesnakes (*Crotalus viridis viridis*) to resorb follicles (Graves & Duvall 1993). In addition, transmitters implanted into the peritoneal cavity of male red-sided garter snakes (*Thamnophis sirtalis perietalis*) were associated with weight loss (not observed in control males) (Shine et al. 2001).

A number of authors reported that radio-tracking gives a more accurate picture of the habitat use patterns of reptiles (e.g. Warrick et al. 1998; Burrow et al. 2001). They believe that previous work based on direct observations of lizard location had been biased towards finding lizards in open ground because of the ease of observing the animals there. The authors reported that the use of telemetry removed this bias because lizards could be located regardless of vegetative cover. Griffiths & Christian (1996) collected perching data from telemetered and non-instrumented frillneck lizards. They noted that if they had analysed only data from non-instrumented animals, they would have concluded that frillnecks perch close to the ground on small trees. Telemetry revealed that in the dry season, frillneck lizards selected larger trees, or used higher perches. The authors took this discrepancy to indicate that data from non-telemetered animals are biased towards individuals that perch in the lower branches of trees, because these animals are more visible to the researcher. Alternatively, carrying a transmitter could alter the animal's behaviour, so that it is no longer representative of the behaviour of the unmarked population.

Radio-telemetry is useful for determining the habitat use and movement patterns of reptiles, especially those species that are cryptic, fossorial, arboreal or live in dense vegetation. Telemetry may help avoid the biases associated with direct observation, as animals can be located regardless of the surrounding cover. Equipment can be attached externally using harnesses, collars or adhesives for short-term studies, or implanted, but weight constraints usually mean that, in smaller species, only adults can be instrumented. Radio-telemetry is also commonly used to gather information about the thermoregulatory behaviour of reptiles.

## PERMANENT MARKS

### Hot branding

Reptiles are branded using the same general system as amphibians. The branding tool is left in contact with the scales of the animal just long enough to produce a lasting and legible mark, but not so long that it penetrates the dermal layer. Branding produces a wound that is not open at the time of release and over which a scab forms within a few days (Clark 1971). Fluid loss is negligible in properly branded reptiles (Ferner 1979). Information on snake branding will be included in this section as the integument and, therefore, reactions to hot branding, of snakes and lizards are similar.

Brands appear to be as permanent as tissue removals in reptiles. Weary (1969) used a pyrographic needle to brand the ventral scales of red-bellied snakes (*Storeria occipitomaculata*) and common garter snakes (*Thamnophis sirtalis*). The needle was applied briefly to burn through the scales. No regeneration was seen over a

2-year period. Brown & Weatherhead (1999) branded northern water snakes (*Nerodia sipedon sipedon*) and found no loss of brand marks in their long-term recapture study. Clark (1971) used hot branding to mark green anoles, Texas horned lizards and several species of snake. A 20-mm-piece of metal alloy wire was heated and placed on the ventral or subcaudal scales. Clark found that in addition to changes in the scale configuration, branding caused changes in the pigmentation of the regenerated portions, thus enhancing identification.

Ehmann (2000) devised a system for marking small reptiles using thin, metal alloy wires to apply small dot brands at coded positions. The method was applied to skinks, geckos and snakes. The micro-branding system is similar to that used for toe clipping, with each body site assigned a code, e.g. the number of marks on the right shoulder might correspond to ID numbers 1-9, then the left shoulder might correspond to 10, 20, 30 etc. The number of marks at another site would correspond with 100, 200 etc. and another to 1000, 2000 etc. The codes from each body site are combined to give the animal a unique identification number. In Ehmann's micro-branding system, sites most frequently used (e.g. units, 10s, 100s) should be placed on more robust body sites (e.g. torso, upper limbs). The use of larger sites also facilitates easier branding and optimises visibility for re-identification. Brand application to distal parts of the hind leg must be precise to avoid damaging the blood vessels on the posterior side of the leg. In addition, brands should not be applied to the tails of lizards, as they are often lost through autotomy.

Each brand mark was left with two bridges of intact dermal tissue through the spot. Ehmann (2000) believed that these bridges aided the healing process, by holding the sides of the wound together and potentially acting as skin grafts. No blood loss occurred after branding, owing to the cauterising effect of the hot wire, and no gross evidence of infection was observed up to 21 days after marking. Lizards that had moulted displayed scars with narrow frills of epidermis. After 7 weeks, the spots had a covering of small flat scales and were highly visible. No regeneration was seen 2 years after branding (Ehmann 2000).

This micro-branding method has several advantages, including the reduced amount of damage to the integument and lowered risk of infection. In addition, micro-branding may not affect cryptic colouration patterns or increase the conspicuousness of the animal. However, the use of a coding system increases the chance of mistakes in reading the marks. In addition, it is likely that the animals would have to be handled for re-identification; additional handling is less likely if larger symbols are applied (Ehmann 2000).

Hot branding appears to be used more frequently to mark snakes than lizards, probably because of the lack of viable alternatives for snake marking. Micro-brand marks on reptiles are not highly conspicuous, which is an advantage to the animal but means that recapture is usually necessary for identification, which can be problematic in behavioural studies. In addition, the use of coded systems increases the risk of misidentification.

### **Freeze branding**

Freeze branding reptiles alters their normal pigmentation by destroying the chromatophores, but does not permanently change any other part of the integument. Lewke & Stroud (1974) used freeze branding to mark western

rattlesnakes and bull snakes (*Pituophis melanoleucus*). Two branding systems were tested. Firstly, copper branding instruments were supercooled in dry ice and 95% ethyl alcohol. The brand site was swabbed with ethyl alcohol to increase heat conduction, and the branding iron applied for 5–30 seconds. Alternatively, pressurised liquid Freon 12 or Freon 22 was sprayed into a stencil held against the skin. Only the dry ice coolant produced legible marks, with the best results produced by 20–30 second applications. These marks were visible for at least 2 years. Both types of Freon were successful in creating a de-pigmented mark when applied for 5–20 seconds. However, spraying into stencils led to distorted marks due to leakage, so that the resulting brands were not legible.

Freeze branding may induce moulting, as all snakes moulted within 3 weeks of marking (Lewke & Stroud 1974). As moulting greatly increases the vulnerability of reptiles, freeze branding may have an effect on survivorship. Other disadvantages of freeze branding reptiles are: the mark is not visible until after the first moult, and there is a high degree of inter-animal variation in the legibility and permanence of freeze brands. In addition, the background colour of the reptile must be considered, as de-pigmentation will be less effective on light coloured reptiles.

Although freeze branding may produce clear changes in pigmentation, the disadvantages are many. The inability to immediately judge the success of marking, the high inter-animal variability, the induction of moulting and the expense and impracticality of the equipment required make freeze branding less suitable as a marking method for reptiles (Patterson 1992). This is reflected in the fact that the method is rarely used in reptile research.

### **Tattooing**

Woodbury (1956) marked reptiles with a portable, battery-powered tattoo machine. The author found the marks to be permanent in snakes, but noted that the tattooing apparatus needed power sufficient to drive the needles through the scales and leave the ink underneath. Tattooed marks must be written on smooth, light coloured surfaces such as on the throat or the base of the tail, where skin pigment does not obscure the ink. The tail should not be marked if the species is capable of autotomy, as the mark will be lost with the tail.

Chalbreck (1963) and Hines et al. (1969) tattooed alligators on the under surface of the tail, where there is little pigmentation. Both groups found that the marks faded within a few months, but modern tattoo inks may last longer. Patterson (1992) considered tattooing to be unsuitable for marking New Zealand's giant skinks because the marks would not be very obvious on their dark background colour.

As with freeze branding, there are alternative methods for marking reptiles that are easier and more effective than tattooing. Tattooing is time-consuming, and requires the use of somewhat cumbersome equipment in the field. Moreover, reptiles are often lighter on the ventral than dorsal surface, and therefore must be tattooed on the underside. Although this would not increase the animal's conspicuousness or interrupt its cryptic colouration, recapture would be necessary for subsequent identification.

## Passive Integrated Transponders

Camper & Dixon (1988) conducted a study on the suitability of PITs for identifying a variety of reptiles. PITs were implanted into collared lizards (*Crotaphytus collaris collaris*), crevice spiny lizards (*Sceloporus poinsetti*), Texas spiny lizards (*S. olivaceus*) and one blue spiny lizard (*S. cyanogenys*). Of 17 PITs implanted intra-abdominally, nine (53%) migrated from the point of insertion, to various locations among the visceral organs. One lizard died 35 days after intra-abdominal implantation; the PIT was found between the lungs, with no infection or damage apparent. A further 10 PITs were implanted subcutaneously in the throat or neck of lizards. Three subcutaneous implants (30%) migrated, and two animals died several days after implantation. Whether the deaths were related to PIT implantation was unknown. All PITs were successfully read on each attempt.

Over 3.5 years, Germano & Williams (1993) marked 581 blunt-nosed leopard lizards with PIT tags. Initially the tags were implanted subcutaneously on the dorsum near the tail base (n = 52). However, the skin was found to be too tight, and the tags broke through. PITs were then injected subcutaneously into the lateral fold of skin on the torso (n = 253), but three tags were destroyed in adults, and the skin was still too tight in well-fed hatchlings. The three tags that malfunctioned were all found in male lizards; the housings were broken, probably due to male aggression. Thereafter, tags (n = 276) were injected intra-abdominally in adults and hatchlings. Of 20 tags lost during the entire study, only three had been implanted intra-abdominally. Scarring indicated that the lost subcutaneous tags had worked their way out through the point of entry, or broken through the skin. The rate of loss or malfunction of PIT tags was 8% for the first recapture and 4% overall. The authors concluded that PIT tag loss was higher than desired, but that loss might be decreased by injecting the tags into the body cavity. In addition, they noted that PITs could be implanted intra-abdominally into hatchlings having an SVL as small as 50 mm.

PITs were implanted subcutaneously under the loose fold of skin in the neck area of frillneck lizards for permanent individual identification (Griffiths & Christian 1996). Brown & Weatherhead (1999) reported no PIT loss in a long-term recapture study of northern water snakes. PITs had no detectable effect on the growth or crawling speed of neonatal *Thamnophis* snakes in the laboratory, and Keck (1994) concluded that PITs were safe and reliable for this species. Similarly, Jemison et al. (1995) found no significant differences in growth or movement of free-ranging pygmy rattlesnakes (*Sistrurus miliarius*) with intra-abdominal PIT tags versus scale clips.

PITs are generally considered to be a reliable method of identifying reptiles. Nevertheless, subcutaneous implants are often lost, and intra-abdominal implants can migrate and potentially damage internal organs, although the addition of anti-migration capsules to PITs has reduced this risk. Reptiles smaller than 50 mm from snout to vent may be too small to safely carry intra-abdominal implants. Patterson (1992) considered PIT tagging to be too traumatic for small reptiles such as New Zealand's endemic giant skinks. There appears to be little risk of harm to larger reptiles.

Subcutaneous insertion of a PIT into the left inguinal fold of a Cook Strait tuatara (*Sphenodon punctatus*) on Stephens Island.

PHOTO: LEE PAGNI, ZOOLOGICAL SOCIETY OF SAN DIEGO.



## Tissue removals

Unlike amphibians, reptiles are incapable of any form of limb regeneration (Paulissen & Meyer 2000). However, the effects of toe clipping on the behaviour and survivorship of reptile species are far from clear. Despite the concerns of many researchers about the effects of toe clipping on the subject animal, it remains one of the most common marking methods for reptiles. It is interesting to note that scale clipping is the most common method for marking snakes, and is sometimes used to mark lizards as well.

New Zealand geckos have been marked by toe clipping: 141 common geckos (*Hoplodactylus maculatus*), with 42 subsequent recaptures (Whitaker 1982); a population of Northland green geckos (*Naultinus grayi*) (Hitchmough 1982); and 382 Duvaucel's geckos on the Brothers Islands (Barwick 1982). Barwick (1982) made the toe clips at the first joint, and natural toe loss could usually be distinguished as it occurs at other points on the toe. Marked and unmarked geckos were found to be equally catchable. This author chose toe clipping owing to the slow growth and potential longevity, as well as small size, of Duvaucel's geckos.

The giant skinks of New Zealand, *Oligosoma grande* and *O. otagense*, are both listed as Nationally Endangered species owing to population declines resulting from habitat loss and predation (Hitchmough 2002). Patterson (1992) toe clipped both species, removing two distal digits from different feet with ultra sharp surgical scissors sterilised in 100% ethanol. Patterson (1992) justified the use of toe clipping on such vulnerable populations by noting that the method does not appear to adversely affect skinks, and that it is the most practical method available.

Tuatara are still marked by toe clipping in New Zealand to enable recognition of individual animals (Cree & Butler 1993; Nelson et al. 2002). However, Nelson et al. (2002) noted that there is the possibility of misidentification owing to natural toe loss in tuatara.

Bocage's wall lizards (*Podarcis bocagei*) were individually marked with toe clips (Galan 1999). Toe clipping was considered by the author to be the most appropriate method for identification of this species because the hatchlings were too small (less than 0.5 g at hatching) to allow scale clipping or the attachment of radio-transmitters. In addition, because this species sheds its skin every 4-8 weeks, temporary methods such as painting were considered unsuitable for a long-term study. Only the distal phalanx was removed, and recapture results indicated that the long-term survival of the lizards was not affected. However, an editor's footnote to this paper states 'The Ethical Committee of the Zoological Society of London considers that toe clipping is no longer acceptable as a routine procedure for marking animals' (Galan 1999).

Dodd (1993) found that toe removal did not affect the sprint speed of six-lined racerunners. Similar results were found for canyon lizards (*Sceloporus merriami*) and western fence lizards (Huey et al. 1990). However, these lizards are ground or rock dwellers, and may be less affected by the removal of toes than arboreal or wall-dwelling species. Toe loss may affect climbing species more than terrestrial species because climbing requires more substrate adhesion. Even the loss of claws was reported to reduce the ability of some geckos to cling to vertical surfaces (Mahendra 1941). In accordance with such

results, Klawinski (1991) suggested that toe clipping be avoided in studies of arboreal and wall-dwelling geckos.

The clinging ability of geckos is correlated with the area of the expanded toe pad (Irschick et al. 1996). Therefore, one might conclude that removing the toe pad could affect clinging ability. When tree dtellas (*Gehyra variegata*) were toe clipped, 39% were never seen again (Bustard 1969). Only 19% were recaptured a month after marking, but subsequent recaptures were high. This may indicate post-marking trap shyness due to toe clipping. Similarly, Bustard (1971) noted that toe clipping had an effect on arboreal Australian geckos (*Oedura ocellata*); after toe clipping, 69% of the population were never seen again.

The hypothesis that toe clipping decreases clinging ability was rigorously tested in the Mediterranean gecko (*Hemidactylus turcicus*). Paulissen & Meyer (2000) removed the middle toe of each foot at the distal phalanx and determined the maximum weight that could be held by a gecko before it fell off a wall. No significant difference was found in the amount of weight held by toe-clipped or control groups, in either adult or neonatal geckos. In addition, the distance run by a gecko on the wall was recorded and no significant difference was found between experimental and control groups. However, with identification systems such as Martof's (1953), the number of toes and phalanxes removed can far exceed those tested in this experiment. Therefore, removal of greater numbers of toes might have more impact on the clinging and running ability of geckos on vertical walls. Paulissen & Meyer (2000) noted that Mediterranean geckos use all four feet to grip, so that clipping more than one toe per foot may reduce the gripping power of that foot enough to impair the gecko's ability to run and cling. They recommend that no more than one toe per foot be clipped.

Hudson (1996) examined natural toe loss in southeastern Australian skinks (*Niveoscincus* and *Pseudemoia* spp.) and the implications of marking lizards by toe clipping. Hudson postulated that if the incidence of natural toe loss is relatively high within a population, then the effect of missing toes on survivorship must be small. It is unlikely that high frequencies of injury could persist in populations if they had a severe effect on survivorship. Natural toe loss was found to be relatively common in the skink species studied (19% to 30% of females). Not only did some individuals survive, but they also grew and reproduced for years after losing most of a limb. Four females of a *Pseudemoia* species had lost an entire foot or limb, and three of these individuals were recaptured up to a year later, having increased in size. One female also became gravid. The author believed that as toe clipping removes only one or two toes per foot, it would not affect survivorship. However, natural toe loss is common enough in these species to potentially cause misidentification of individuals marked by toe clipping.

Scale clipping is the primary method used for marking snakes and is sometimes used to identify lizards. However, scale clipping is impractical for skinks because the scales are small and lie flush with the body surface (Patterson 1992). Lizard species with large dorsal crest scales are particularly good candidates. For example, green iguanas in South America were individually marked by clipping three dorsal crest scales with scissors in a coded system (Rodda et al. 1988). Anterior scale clips were assigned numbers in the ones or tens, and numbers in the hundreds were assigned for posterior scale clips. At

least 11 intact scales were left between each coding clip. Naturally missing scales were always incorporated into an individual's code. The crest scale codes were found to be readable from a distance with binoculars and from a variety of angles. However, in certain populations (e.g. Venezuela), juveniles less than 3 months old regrew the clipped dorsal scales, obscuring the identifying marks. Rodda et al. (1988) also noted that dorsal crest clipping in hatchlings was time-consuming and error prone, as was subsequent interpretation.

Disadvantages of scale clipping for identification include the possibility of scute regeneration. This is especially true for neonate reptiles, which shed frequently, leading to the loss of marks more quickly than in adults (Carlstrom & Edelstam 1946). Even if most of the scale is clipped, the neighbouring scales can grow over and obscure the identifying clip (Pough 1970). However, Keck (1994) found that less than 5% of recaptured *Thamnophis* neonates had regenerated clipped scales. The degree and rate of regeneration will vary between species. There is also the risk of bleeding and infection if large portions of the scale are clipped (Camper & Dixon 1988).

The effects of toe clipping on reptiles are not well known, but appear to be highly specific to the species under study, as well as to the clipping system employed. Arboreal or clinging species are more likely to be affected by the removal of toes than terrestrial species. The more digits removed, the higher the likelihood of detrimental effects. In addition, specialised toes should not be removed. Scale clipping is primarily used for marking snakes, although lizards with large dorsal scales are also good candidates. Scale clipping might not be permanent, and there may be species-specific effects of clipping scales.

### **Natural marking identification**

Many reptilian species exhibit sufficient variation in pigmentation patterns to enable individual identification (see figure p. 88). Carlstrom & Edelstam (1946) used black and white photographs and sketches to record the unique dorsal patterns of viviparous lizards (*Lacerta vivipara*) and throat patterns of slow worm lizards (*Anguis fragilis*). Stamps (1973) was able to differentiate a small number of individual bronze anoles (*Anolis aeneus*) by combining information on pattern with tail regeneration status. Other species in which individuals have been identified by their natural markings include: red-headed rock agama (*Agama agama*) (Harris 1964); green iguana (Dugan 1982; Rodda et al. 1988); Mona Island rhinoceros iguana (*Cyclura stejnegeri*) (Wiewandt 1977); and Northland grey gecko (Hitchmough 1982). However, there must be evidence that those patterns remain stable over the lifetime of the individual for the method to be of value (McDonald et al. 1996).

Two populations of green iguanas in South America were identified by their natural markings (Rodda et al. 1988). The length and shape of the dorsal crest scales were used, and these features were supplemented with information on body colouration pattern and deformities. More than 90% of individuals had distinctly patterned scales, and half could be identified by these scales alone. Other features used included tail regeneration status (indicated by ring numbers), overall size and patterns of dewlap margin scales. Individuals could be identified from 40–70 m away with the aid of binoculars. However, these lizards are relatively inactive, allowing researchers adequate time to assess the

identifying features. Dorsal crest scales were found to be useful only if the population had an average of two scales, or portions of scales, missing per individual. Natural scale loss was found to be too low for individual identification in one of the South American populations (Rodda et al. 1988).

Despite its advantages, natural marking identification can take longer to perform and check than other marking methods. It may also necessitate increased handling times, which can be detrimental to reptiles. However, the non-invasive nature of the procedure may make up for the additional handling time, in terms of the stress experienced by the animal. Natural marking identification is most appropriate for small populations occupying well-defined areas. This method is particularly useful for behavioural studies of reptiles, as it is less likely than other methods to affect the behaviour of the animals.

## S U M M A R Y

Reptiles can be successfully marked using a variety of methods. Painting is often used for short-term studies, and has not been shown to affect survivorship. Tagging may be less appropriate owing to the associated risks of increased conspicuousness to predators, snagging on vegetation and changes to behaviour. More permanent methods such as branding, tattooing and scale clipping are primarily used to mark snakes, because of the difficulties with attaching other devices. Toe clipping is permanent in reptiles, and is still the most common method for marking lizards and tuatara. No reliable evidence of detrimental effects of toe clipping has been reported so far, but very few studies have systematically evaluated the possibility of such effects. PIT tags can be useful for studying reptiles. However, many of New Zealand's reptiles are long lived, and little is known about the long-term effects of intra-abdominal implantation of PITs, especially in small animals. In addition, the potential for this method is somewhat limited by current technology (e.g. short reading distances).

Spotted skink (*Oligosoma lineocellatum*) marked with Stephens Vivid® felt pen (xylol based) in an individual colour code at Motunau Island, 1967.

PHOTO: TONY WHITAKER.



Female rough gecko (*Naultinus rudis*) from Hanmer Springs, identified by natural markings.

PHOTO: DENNIS KEALL.



Otago skink (*Oligosoma ottagense*) from Middlemarch, identified by natural markings. PHOTO: DENNIS KEALL.

Captive tuatara (*Sphenodon punctatus*) marked using a unique combination of coloured beads on string threaded through the dorsal crest. PHOTO: ERIC FOX, OTOROHANGA KIWI HOUSE.



# Marine mammals—cetaceans

New Zealand is home to one species of endemic cetacean, Hector's dolphin. Two subspecies are currently recognised: Hector's dolphin (*Cephalorhynchus hectori hectori*) and the North Island Hector's dolphin (*C. hectori maui*) (Baker et al. 2002). The South Island subspecies has three regional populations, with a total population estimated at 7270 (Slooten et al. 2002), and is currently listed as Nationally Vulnerable (Hitchmough 2002). The North Island Hector's dolphin, found only on the west coast of the North Island, is geographically and genetically distinct from the South Island subspecies (Baker et al. 2002). This subspecies is currently listed as Nationally Critical, reflecting the small size, geographic and genetic isolation, and high vulnerability of the population (Hitchmough 2002). Because these small, slow-growing populations are highly localised, they are particularly vulnerable to even low levels of incidental mortality (Dawson 1991).

In addition to Hector's dolphin, one other toothed whale is threatened in New Zealand: the killer whale or orca (*Orcinus orca*). This species is classified as Nationally Critical in New Zealand waters. However, populations are stable here, and there are secure populations overseas. Many other dolphins are migrants or vagrants in New Zealand waters. Unfortunately, there are insufficient data on the various species of beaked whales to draw conclusions about the status of their populations in New Zealand waters.

Two baleen whale species are classified as Threatened in New Zealand waters: Bryde's whale (*Balaenoptera edeni*) and the southern right whale (*Eubalaena australis*). Both species are included in the 13 'great whales' protected by the moratorium on commercial whaling adopted by the International Whaling Commission in 1982 (IWC n.d). Other great whales found in New Zealand waters are the minke (*Balaenoptera acutorostrata*), blue (*B. musculus*), sei (*B. borealis*), fin (*B. physalus*), humpback (*Megaptera novaeangliae*) and sperm (*Physeter macrocephalus*) whales. Baleen whales migrate annually between tropical breeding and calving grounds, and polar feeding waters. It is during these seasonal migrations that baleen whales pass through New Zealand waters. However, Bryde's whales are probably permanent residents on the northeast coast of the North Island.

Special considerations for marking cetaceans include the longevity of most whale species, which must be reflected in the persistence of the mark. In addition, mark-related effects on reproduction and survival must be minimised owing to the low reproductive rates of cetaceans (Chapman 1974). Whales have small lymphatic systems relative to their size, which makes them vulnerable to infection (Obree 1992). Therefore, the marking method should not increase the likelihood of infection. The use of tranquillisers is considered risky, as they are known to depress respiratory rates and thermoregulatory capabilities in cetaceans. Also, sedatives cannot be used while the animals are free in the water because of the risk of drowning (Obree 1992).

Retention of externally applied marks is problematic, as cetaceans are anatomically designed to minimise hydrodynamic drag. Any protruding

equipment will increase the drag experienced by the animal during swimming. This will not only change the energy expenditure and behaviour of the animal, and potentially cause tissue trauma, but will also hinder mark retention owing to the continuous pressure of the passing water.

Finally, methods for small and large cetaceans may be discussed separately in some instances, as differences in size, behaviour and habitat may affect the suitability of a particular marking system.

## TEMPORARY MARKS

### **Painting**

Watkins & Schevill (1976) applied underwater paint to small cetaceans, and found it to be easy to use and the resulting marks highly visible, although not permanent. The dorsal surface or dorsal fin was found to be the best location for paint application, to maximise visibility in short-term studies. Paint-like crayon markers have also been used, and were found to be successful to temporarily mark manatees (*Trichechus manatus*) (Irvine & Scott 1984). Aquatic paints can be useful for short-term identification of animals that have small home ranges, as long as the materials are non-toxic and the marks do not affect social or interspecific interactions.

## SEMI-PERMANENT MARKS

### **Tags**

#### ***Large cetaceans***

Most information about whales has been collected from animals obtained during the course of commercial whaling. Traditional methods of whale marking involved killing the animal and recovering the mark. The 'drawing pin mark' was an early, unsuccessful attempt to mark commercial species (Kemp et al. 1929). This mark had a hollow, barbed metal head 6.5 cm long protruding from the centre of a flat metal disc (of 4.5 cm diameter). The face of the disc was inscribed with a serial number and return address. This device was mounted onto a wooden shaft and fitted into a modified 12-bore shotgun. The mark was designed to penetrate just below the surface of the blubber, leaving the disc flush with the skin and causing the shaft to fall away. Unfortunately, it was found that the mark only penetrated a short way into the blubber. As blubber is known to suppurate readily, there is little doubt that the whales quickly rejected the marks. No recoveries of 'drawing pin marks' were ever made (Kemp et al. 1929).

The discovery mark was first used in 1932 (Rayner 1940). Discovery marks are numbered metal cylinders, 1.5 cm in diameter and 23 cm long with a blunt lead head. The mark was fired from a 12-bore shotgun and buried itself into the whale's blubber or muscle (Norris et al. 1973). The maximum range for applying discovery marks was about 65 m. Marks were aimed at the region around the dorsal fin in baleen whales and behind the dorsal fin in sperm whales. The area behind the

flippers was to be avoided in all species. The major difficulty with this method was uncertainty about whether the mark was successfully embedded (Brown 1978). Whale marking trials in Norway showed that shots from the normal range (35–45 m) were not likely to cause obvious injury if the mark was correctly placed (Ruud et al. 1953). These authors recommended that the marks be smeared with anti-bacterial ointment before firing; however, no evidence of the value of the practice was found and it was discontinued.

Data from discovery marks are limited, being useful for age and growth information only. This type of mark is not useful for studying movement or behaviour, as the only two locations known for each animal are where marking and then capture occurred. These are often the same, as commercial harvesting usually occurred on feeding grounds (Brown 1962). Discovery marks were often overlooked in the carcass until the later processing stages. Doi et al. (1971) reported that 70% of markers were found at flensing, and less than 1% were found at boiling. This meant that even the point of capture could not be conclusively known for many marked whales, as later processing occurred at locations distant from capture sites. Another serious problem with the use of marks in conjunction with commercial harvesting is that mark recovery reflects not the actual population parameters of the species, but the intensity and location of the whaling efforts (Brown 1978). Of 4291 whales marked with discovery-type markers in 1970, only 405 (9.5%) were ever recovered (Chapman 1974). In general, the number of recaptures was not sufficient to make unbiased estimates of population size.

The original discovery mark has been modified in several ways. Because of the large number of marks overlooked during processing, streamers were attached to improve the chances of the mark being detected at an earlier processing stage (Brown 1978). Six coloured nylon threads, 2 m long and 0.5 mm thick, were attached to stream from the open end of the discovery mark tube. Colour coding of the streamers could theoretically stop double marking of individual whales. However, the threads were often lost by abrasion, or were too fine to be visible on the back of a swimming whale, and the use of nylon streamers was discontinued. Vinyl spaghetti tags have also been attached to fin and blue whales (Mitchell & Kozicki 1975). A strip of vinyl was attached to an anchor rivet behind the head of the mark by a length of Teflon-coated line. The line and streamer were coiled in a tube and released when the mark was fired into the whale's flesh. No conclusive results were reported.

Between 1950 and 1960, mark-recapture studies of humpback whales in New Zealand waters were undertaken using discovery tags. In 1966, the IWC granted humpback whales total protection, and in 1976 the sei whale also became a protected species (Horwood 1987). This meant that a non-kill marking technique was required to obtain information on whales. Tags of the types outlined above are no longer used to identify large cetaceans. Acceptable marks that can be visually recognised at long ranges have yet to be developed.

### ***Small cetaceans***

A small-bore modification of the original discovery mark was made for marking smaller cetacean species such as minke whales. Calves and adults smaller than 11 m in length were seriously injured or killed using the 12-bore mark at close range (Miyashita & Rowlett 1985). The modified mark was 15 cm long and fired

from a 0.410 shotgun. Some of these smaller marks were augmented with one white streamer to increase mark recovery (Brown 1978).

Spaghetti tags have been attached to *Stenella* spp. dolphins caught in tuna nets, or while bow-wave riding (Perrin 1975). Plastic deer tags have also been attached to the dorsal fins of small cetaceans (Norris & Pryor 1970). A cylindrical hole was punched through the trailing edge of the dorsal fin, near the tip, and the two-part tags inserted. Application of the tags was quick and could be achieved in the water if the animal was well restrained. The wounds appeared to heal around the tag shaft, and one wild animal was sighted bearing a tag 3.5 years later.

Evans et al. (1972) assessed a range of plastic discs and streamers for marking delphinid cetaceans. These marks were attached by a variety of steel barbs, nylon darts with flukes, umbrella anchors and anchor rivets. The authors reported that these marks were often visible for considerable distances at sea, and endured for periods of days to months. They considered spaghetti streamer tags to be the best method for marking large numbers of small cetaceans, primarily because the animal did not have to be captured to apply the mark. However, the authors also concluded that difficulties such as water friction, behavioural changes and tissue trauma could not be avoided when using external marks.

Irvine et al. (1982) evaluated spaghetti tags, Dalton Rototags<sup>®</sup> and plastic tags bolted through the dorsal fin in bottlenose dolphins (*Tursiops truncatus*) in the Atlantic Ocean. The researchers concluded that bolt tags were too short lived, while Rototags<sup>®</sup> and spaghetti tags were too small to allow identification at a practical distance. All of the tags caused tissue damage, and the authors concluded that all tags should be tested on new species prior to field attachment.

Tanaka et al. (1987) also evaluated methods of marking bottlenose dolphins, including button tags in a variety of colours and sizes. Polyurethane resin tags were attached to the dorsal fin using a stainless steel bolt and locking nut with a Teflon sleeve. A bolt hole was first bored in the fin before attaching the tag. The researchers found that red was the least visible tag colour, while yellow stood out more than orange at near distances. Neither yellow nor orange were visible from more than 120 m away. The larger tags (7.2 cm long, with 5-cm-high numerals) proved more useful than smaller tags, which were not readable at 70–80 m. Algal growth occurred on all tags after 1 month and made tags difficult to read after 3 months and unreadable from distances of 3–5 m.

The button tags were found to have poor durability, coming off the dorsal fin of bottlenose dolphins within 1 year (Tanaka et al. 1987). Similarly, plastic button tags attached to the dorsal fin of Amazon River dolphins (*Inia geoffrensis*) rarely lasted 12 months (Martin & da Silva 2003). Tanaka et al. (1987) reported pronounced backward migration of the bolt in the dorsal fin (4 cm within 81 days), owing to hydrodynamic drag, which caused significant tissue damage. Belting the tag to the frontal margin of the fin with a fibreglass strap did nothing to prevent bolt migration, and caused significant additional damage to the dorsal fin.

Although tagged dolphins in this study did not appear to behave unusually, oedema of part of the dorsal fin was noted 1 month after marking, and irritation of the skin in contact with

Tag on the dorsal fin of a rough-tooth porpoise (*Steno bredanensis*). From Norris & Pryor 1970. Reprinted with permission of ACG, a division of Allen Press, Inc.



the tag was observed (Tanaka et al. 1987). When the tags were attached to free-ranging dolphins, 9 of the 40 tagged animals (23%) died from entanglement in nets. Whether this was related to the attachment of tags is not known. In addition, three dolphins shed their tags and none of the numerals on the tags were readable upon re-sighting. The authors concluded that button tags are not an effective method for long-term marking of small cetaceans. There is also a distinct possibility that the tags caused an increase in mortality of the free-ranging dolphins.

Hector's dolphins were tagged at Cloudy Bay off New Zealand's South Island in 1978–79 by A.N. Baker (described in Cawthorn 1988). The dolphins were tagged on the dorsal fin with small circular Allflex sheep tags, which were colour and number coded. Re-sightings were made up to 5 years later, all within a few kilometres of the tagging location.

Tags attached to the dorsal fin of small cetaceans may be useful for short-term studies. However, there are inherent difficulties with the attachment of tags, including increases in hydrodynamic drag, changes in energy expenditure and behaviour, tissue trauma and premature mark loss. In addition, small tags, which may cause fewer problems than large ones, may be difficult to read at practical distances. Finally, the growth of algae can obscure tag numbers and other codes, even those that were easy to read upon application.

### **Harnesses**

Tanaka et al. (1987) evaluated several different harness designs for attaching satellite-linked radio-tags to bottlenose dolphins. They first evaluated hydrodynamic drag using a half-size dolphin model in a water tank, before applying the most successful design to a live dolphin. The harness design that created the least amount of drag had projections in front of the transmitter, which itself was placed in front of the dorsal fin. The harness was attached to the live, captive dolphin using a belly belt and bolt through the dorsal fin (a 0.8-cm diameter hole was drilled).

After attachment, the harnessed dolphin did not swim with the others and repeatedly tried to remove the equipment. The animal's swimming position changed to head above water, dorsal fin below it, with rotation about its long axis. This was probably due to the shift in weight caused by the apparatus. The dolphin returned to almost normal swimming after a few days, but changes to diving patterns persisted. After 1 month, the belly belt had discoloured the skin beneath it and serious injuries were evident on the anterior margin and posterior base of the dorsal fin. Bleeding occurred at part of the bore-hole, owing to drag on the harness (Tanaka et al. 1987).

Later experiments used a harness lined with neoprene to reduce skin irritation. This harness was dyed yellow, which probably weakened the material, as the harness broke and fell off within 2 months. Algal growth on the harness increased its weight and drag, and probably affected the efficiency of the surfacing switch and antenna of the radio transmitter as well (Tanaka et al. 1987).

Free-ranging dolphins harnessed with the same design all appeared to swim normally. However, the diving periods of dolphins with belly belts were significantly shorter than before harnessing. Plastic straps attached through a hole in the dorsal fin caused unusual swimming for days, and longer diving

periods for at least 2 months after application. The straps also caused serious injury to the anterior margin of the dorsal fin (Tanaka et al. 1987).

The longest period of radio transmission from the harnessed dolphins was 35 days, and the researchers could not be sure if the equipment was still attached to the animals at this point (Tanaka et al. 1987). Several of the radio-tags were found on beaches and were damaged. The harnesses probably slipped off because of the increased water pressure produced by forward swimming and/or the compression of the body caused by diving in deep water. The use of elastic belly belts did not increase retention of the harnesses, as the harness lifted, moved backwards and then broke owing to drag on the attached equipment. Harnesses need to be made smaller and attachment methods improved in order to decrease drag and the subsequent effects on physical health and behaviour.

Martin (2003) also reported the use of a harness instead of bolts to attach transmitters to beluga whales (*Delphinapterus leucas*), in an effort to reduce the invasiveness of attachment. However, the straps encircling the whales altered their behaviour, and the use of harnesses is not recommended for this species.

The marked effects on behaviour, as well as the significant injuries, caused by harnesses make them unsuitable for studying small cetaceans. Harnesses are now rarely used, and most equipment is now attached directly to the animal (see, *Telemetry and archival recorders*, pp. 94-99).

### **Telemetry and archival recorders**

Radio-telemetry is now the most common method for collecting information on the behaviour, habitat, physiology and demographic parameters of cetaceans. Radio signals can be tracked from ships, planes and from shore for species with small home ranges, while satellite reception is more useful and labour- and cost-effective for wide-ranging or remotely located species (Bryden & Harrison 1986).

Collecting data on cetaceans by radio- and satellite-telemetry is expensive. For example, Hobbs et al. (2003) reported that each beluga whale cost US\$1000-\$5000 to instrument with a satellite-linked radio-tag. Collection and analysis of the data cost an additional US\$1000-\$3000 per animal. Cost also depends on the type of information sought, and the equipment used. More than one transmitter or sensor can be attached to an animal, depending on the questions to be answered. Often a VHF transmitter is attached along with a satellite transmitter, to allow researchers to relocate a tagged whale using a directional antenna. In addition, the geographic location and behaviour of the tagged animal can affect the cost of telemetric equipment. For example, in deep-diving species, plexiglass housing designed to resist pressure must be used to protect the recorders (MacDonald 1978).

Sensors are often incorporated into transmitter packages. These sensors can measure parameters such as heart rate and respiration rates of free-living cetaceans (e.g. Ponganis & Kooyman 1999). Radio pills can also be placed in the stomachs of cetaceans to obtain internal temperatures (Schevill & Watkins 1966). Timed depth recorders (TDRs) can be manually retrieved (archival recorders) or linked to satellite receivers (Satellite-linked dive recorders or SLDRs) in order to send information on diving behaviour (Hanson 2003b). Large transmitter packages can accommodate more sensors and batteries and are,

therefore, longer lived and more powerful. Smaller packages provide less information, but have less impact on the animal. A salt-water switch can be included in the transmitter to restrict transmission to times when the animal is at the surface, thus saving battery power and extending tracking time.

### ***Large cetaceans***

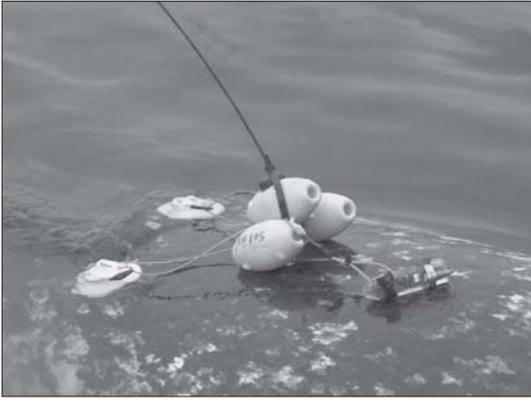
Not only the marking procedure, but also the capture and handling required, make identification of cetaceans difficult. Large cetaceans are generally marked while freely swimming at the surface, and telemetric equipment can be applied in deep or shallow water (e.g. Mate et al. 2000; Heide-Jorgensen et al. 2003). Attachment and retention of the necessary equipment is the greatest challenge in telemetric studies of marine mammals. Because of the suppurating nature of cetacean blubber, implanted tags are easily shed by this tissue. However, tags implanted in blubber can be extremely useful for tracking large cetaceans for short periods. Radio-tags implanted using a pointed projectile remained in the blubber of fin and humpback whales for 16-17 days (Watkins et al. 1981). Watkins (1981) reported that sperm, fin and humpback whales show little response to the application of radio-tags implanted in the blubber.

Schevill & Watkins (1966) were the first to try radio-tracking a large cetacean. They attached a transmitter to an adult northern right whale (*Eubalaena glacialis*) from an aircraft using small darts embedded in the blubber. Unfortunately, no tracks were obtained from the adult animal, probably because the darts did not hold.

Heide-Jorgensen et al. (2003) used two different methods to attach three types of satellite-tags to bowhead whales (*Balaena mysticetus*). Five whales, 12-15 m in length, were tagged in deep water (200 m) around Greenland. The tags were glued to a stainless steel base mounted to a titanium spear. Telonics® ST-15 tags were deployed using a pneumatic gun. Researchers have also used non-lethal firing of projectiles to attach tags to other species of large cetacean (e.g. Mate & Harvey 1983; Goodyear 1993; Baird 1994; Mate et al. 1998). In addition, modified crossbows have been used to apply radio-tags to whales (Mate et al. 2000).

Heide-Jorgensen et al. (2003) also deployed Telonics® ST-16 and SPOT1 (Wildlife Computers Inc.) satellite-linked tags with an 8-m fibreglass pole 4-5 m from the whale. The tag was mounted on the tip of the pole, and the titanium spear pushed through the skin into the blubber. All tags were positioned high on the whale's back, just below the dorsal line, about halfway along the whale's length. This position was chosen to ensure that the tags would be above water when the animal surfaced, so that transmissions could be received by the satellite. Two of the five tags functioned until their batteries were exhausted. The failure of the other three tags was related to poor implantation in the blubber, poor positioning on the back (e.g. too low) or equipment failure. Pole-deployed tags performed better than those applied using the pneumatic gun. This may be because tags fired at close range with the gun were damaged upon impact (Heide-Jorgensen et al. 2003).

Large cetaceans can also be instrumented by attaching a buoyant float, containing transmitter equipment, by a line to an anchor in the blubber. The buoy is trailed behind the animal, and sits on top of the water allowing transmission while the animal is submerged near the surface. One body length behind the whale



Suction cup system for short-term attachment of a Time-Depth Recorder.

PHOTO: M.P. HEIDE-JORGENSEN, NOAA.

(measured from the tail flukes) is considered the ideal distance for the buoy. The float must be compact and produce low drag, while being able to support the transmitter so that its antenna is held above the surface of the water. The transmitter and buoy must be able to survive submersion, as well as the rigours of rapid and/or extended dives. This type of attachment provides a high number of good quality location fixes when attached to slow moving whales with long surface intervals between dives, or to animals that live in shallow water (e.g. Rathbun & Marsh 1987; Mate et al. 1988).

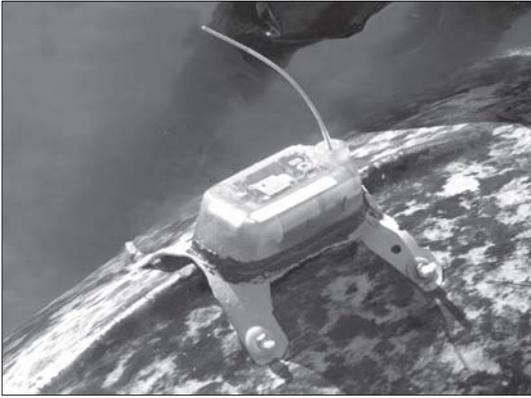
Because radio transmissions cannot penetrate sea water, some researchers use sonar-tags to study the underwater movements and behaviour of large cetaceans (e.g. Watkins & Goebel 1984; Goodyear 1993; Watkins et al. 1993). Sonar is a system for underwater detection of objects by the reflection of sound (echolocation). Acoustic tags can be applied to whales in the same way as radio-tags, and sounds emitted by the tag are received by an underwater hydrophone to give information on location (Priede 1992). Combined radio- and sonar-tags are commonly used, allowing underwater and surface data to be collected (Goodyear 1993). The attachment of sonar-tags raises the same issues and concerns as radio-tag application.

Because large cetaceans generally cannot be captured or restrained for marking, telemetric equipment is usually applied to them using a pole or projectile system. This precludes the use of well-anchored, long-lasting attachment devices, as does the suppurating nature of blubber tissue, into which most tags are implanted. Retention, therefore, is relatively short-lived, and large cetaceans can be tracked only for short periods. However, telemetric methods, especially satellite-linked radio-telemetry, have been invaluable in providing information on movement, behaviour and distribution of large cetaceans.

### ***Small cetaceans***

Smaller cetaceans such as dolphins, pilot whales (*Globicephala melaena*) and orcas, as well as juveniles of larger species, can often be captured and restrained for the duration of mark application (Hanson 2003a). This allows the application of more stable attachment devices to secure transmitter equipment to the animal (e.g. pins or bolts).

The dorsal fin is the most common location for the attachment of transmitter equipment to small cetaceans. There are three common tag configurations used: front mount, single-side mount and paired-side mount. The front-mounted tag is attached to the leading edge of the dorsal fin, with the transmitting antenna following the curve of the fin. The single-side-mounted tag is bolted to one side of the dorsal fin using four bolts, with the antenna trailing from the posterior edge of the fin. Hanson (2003a) reported variable retention of single-side-mounted tags, with a maximum duration of 150 days on small cetaceans. Paired-side-mounted tags, which are attached to both sides of the dorsal fin with bolts, have been applied to animals ranging in size from bottlenose dolphins to orcas. Hanson (2003a) reported variable retention of paired-side mounts, with all tags lost 8 months after application. With all designs, Hanson noted that there was posterior migration of the package and tissue damage related to the continuous force of water over the tag. The single-side mount showed greater



'Turtle tag' radio-transmitter attached to the dorsal ridge of female cetacean.

PHOTO: M.P. HEIDE-JORGENSEN, NOAA.

and faster migration than the paired-side mount, probably owing to the greater movement of the tag relative to the animal.

Evans (1974) radio-tracked two species of small odontocetes, the short-beaked common dolphin (*Delphinus delphis*) and the pilot whale. The animals were captured and tags attached through the dorsal fin by a corrodible bolt. The bolt was designed to release the package 30–60 days after application. Each time the animal surfaced, the transmitter turned on and emitted a continuous signal indicating the maximum depth of the last dive. The total

transmission time, if the antenna was exposed for 10% of the time, was predicted to be 40 days. The equipment and procedures were found to be quite reliable, with 9 of the 12 tagged animals yielding data. The radio-tags had to be relocated by aircraft after disengagement.

Schneider et al. (1998) attempted to attach combined TDR/radio-tags to bottlenose dolphins using a modified crossbow system. The tags were attached to rubber suction cups (8 cm in diameter) originally designed for car roof racks. The tags, weighing 340 g, were fired from 5–15 m away, and were aimed at the flank near the dorsal fin. Of 84 tagging attempts, only 29 hit the animal. Of these 'hits', only 6 were successfully attached, and only two of these tags yielded data.

Some species of small cetacean lack defined dorsal fins, making equipment attachment more challenging (Reeves & St Aubin 2001). Beluga whales have no dorsal fin, but the dorsal ridge is composed of blubber, through which pins can be threaded without damage to internal organs (Martin 2003). Richard et al. (2001) attached satellite-linked tags to 12 beluga whales. The tags were mounted onto saddles, which were attached using 2–3 plastic pins passed through the dorsal ridge. The authors observed no visible reactions to the surgical procedure. Of 21 tags attached, two failed in the first 2 days and the rest functioned properly for between 30 and 126 days (Richard et al. 2001).

Hobbs (2003) used several different methods to attach satellite-linked transmitters to beluga whales in Alaska. The first method used conveyor belt straps to attach a 900-g package containing four batteries to the dorsal ridge. This belt tag was secured using three or four pins (6 mm diameter), threaded through the dorsal ridge, and was typically retained for 2–3 months. The other designs ('spider tags') used nylon pins threaded from one side of the dorsal ridge to the other, with six Monel cables attached to the ends of the pins and tightened to get the package as close to the dorsal ridge as possible. The early spider tag designs used three pins (6 mm diameter), and typically lasted 3 months. Later spider tags used two rather than four batteries, reducing the package weight to 300 g, and were secured using larger pins (9.5 mm diameter). This tag type was retained for an average of 8 months. The apparatus was released when the pins pulled out of the dorsal ridge or broke through fatigue (Hobbs 2003).

Most equipment eventually migrates out of the dorsal fin or ridge, which can lead to premature loss of the transmitter package. Radio-tags bolted through the dorsal fin in bottlenose dolphins were found to cause tissue damage and were frequently lost prematurely (Irvine et al. 1982). However, the resulting pin or bolt holes appear to heal relatively well (Martin & da Silva 2003). Occasionally

the hole heals around the pin or bolt after the tag has gone, like a pierced ear healing around an earring. Scarring on the backs of previously tagged beluga whales was taken by Orr et al. (1998) to indicate that tagging had no lingering effect on the animal's health or behaviour.

Several research groups have studied the behavioural responses of small cetaceans to the application of telemetric equipment. The most important result of these studies is the amount of variability in response between species. Bottlenose dolphins were found to have strong and relatively long-lasting reactions to a crossbow application of rubber suction cup radio-tags (Schneider et al. 1998). In contrast, orcas showed only minor behavioural reactions to the same attachment method (Baird 1994). Some Dall's porpoises (*Phocoenoides dalli*) tagged with suction-cup tags returned to bow-wave riding immediately (54%), while others swam away at high speeds (31%) (Hanson & Baird 1998). One porpoise, tracked for 41 minutes, swam at high velocities for the first 8 minutes after attachment, suggesting a short-lived reaction to suction-cup tagging. Northern bottlenose whales (*Hyperoodon ampullatus*) generally exhibited low-level, short-lived responses to the projectile application of satellite-tags (Hooker et al. 2001). These results indicate that different species may cope differently with tag application, which may also apply to the subsequent wearing of the tag. They also highlight the need for species-specific research on the effects of marking.

Archival recorders collect and archive data for later recovery. The greatest disadvantage of archival recorders is that they must be manually recovered from the animal to obtain the data. In an attempt to circumvent this problem, Baird & Goodyear (1993) attached a buoyant TDR to orcas in British Columbia, Canada. The apparatus was attached with suction cups using a long pole or crossbow, thereby minimising interference with the animal. Each suction cup had a magnesium disc incorporated into its wall. When this disc corrodes in the sea water, the suction of the cup is broken, the package is released and floats to the surface. A radio transmitter can be included that will begin to transmit once the antenna is above the sea water, allowing recovery without further capture. The deployed recorder was recovered after 8 hours, revealing information on the diving behaviour of orcas (Baird & Goodyear 1993).

Westgate et al. (1995) attached buoyant TDR/VHF packages to three species of dolphins. The packages were attached using two bolts with magnesium nuts that corrode in salt water, releasing the package, which then floats to the surface. Seven of eight packages deployed were recovered. Although ingenious and non-invasive, such methods do not allow long-term deployment of archival recorders.

Early in 2004, a collaborative project involving the New Zealand Department of Conservation, the New England Aquarium, Wildlife Computers, Inc., the Danish Government, and several NZ universities, developed and deployed the smallest satellite-linked transmitter tag yet produced for cetaceans, in a trial on Hector's dolphins (see figure p. 104). The tags weighed 50 g and were designed especially for this species, to minimise hydrodynamic drag. The trial resulted in collection of data from three dolphins, over 5 months, and baseline health information was collected at the same time.<sup>6</sup>

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<sup>6</sup> Two preliminary reports on the results of these trials are available from the Marine Conservation Unit, Department of Conservation, Wellington, New Zealand.

Radio- and satellite-telemetry facilitate the vast majority of modern studies of cetaceans. Potential effects of the radio transmission itself on cetaceans have not been reported. The primary problem with telemetry for studying cetaceans is the attachment of transmitter packages to the animal. Any external attachment is likely to increase hydrodynamic drag, which can affect swimming and diving behaviour and energy expenditure. As well as the initial trauma of attachment, the continuous force of water over the attachment can lead to tissue injury. Tielmann (2003) noted that researchers have an obligation to reduce the impact of tags on the animal. This can be achieved by reducing drag with smaller, more hydrodynamic tags, by preventing pressure from the tag on the side of the dorsal fin, by altering the materials used (to aid healing and prevent pin migration), and by incorporating a release mechanism to be triggered when the batteries are exhausted, freeing the animal as soon as possible. Other improvements in telemetric methodology should involve the remote application of radio-tracking equipment to whales, and attachment to allow the greatest amount of antenna exposure and least hindrance to the animal's movement.

## PERMANENT MARKS

### **Freeze branding**

Freeze branding has been attempted only on small cetaceans, primarily because of the duration of application required to produce the mark. Capture and restraint of the animal is required, which is feasible only for small cetaceans.

Freeze branding small odontocetes was found to produce a distinctive white scar, easily visible at sea (Tomilin 1960). These brands could be made as large as the dorsal fin, using numbers or symbols, and generally remained visible as long as the porpoise skin was free of algae. Dohl (pers. comm. in Tomilin 1960) reported freeze branding a captive *Stenella*, and that the mark persisted for at least 4 years.

Tomilin et al. (1982) designed a special clamp to hold a stencil on either side of the dorsal fin of small cetaceans. This clamped stencil caused the epithelium underneath the pressurised area to be exfoliated and replaced by de-pigmented skin. The resulting marks were visible for at least 2 years. However, the stencil must remain on the animal for 4 days to produce a lasting mark, which limits use of this method in the field. In addition, the animal would probably experience significant discomfort due to the pressure of the stencil.

Bottlenose dolphins were freeze branded with good results using dry ice and alcohol, or liquid nitrogen (Irvine et al. 1982). The authors found that freeze brands were the most readable marks at a distance of 30 m, and were more durable than all tags evaluated, lasting an average of almost 5 years. In addition, freeze brands create no hydrodynamic drag, which is often responsible for changes in behaviour and energy expenditure in marine mammals. The authors concluded that, overall, freeze branding was less harmful than all other marking methods evaluated in the study.

Bottlenose dolphins have also been freeze branded using Freon 12 gas sprayed through neoprene moulds onto the skin around the dorsal fin (Tanaka et al.

1987). The Freon was sprayed from about 15 cm above the skin, with application times of 15–90 seconds. After application, the skin turned black, and a few days later the longer applications (30–90 seconds) had caused the skin to become sunken, with white circumferences. All applications were made on the same dolphin, which subsequently died of blood poisoning. Another dolphin was freeze branded for 15, 20 and 25 seconds with Freon gas. After 20 seconds the skin became whitish, but after 15 or 25 seconds the skin turned black. The authors concluded that although freeze brands were more durable than button tags, the most suitable method for branding with Freon 12 gas could not be determined. The researchers had difficulty in obtaining constant freezing conditions, with the brand quality affected by distance, duration and pressure of the gas spray (Tanaka et al. 1987).

The persistence of marks created by freeze branding is extremely variable, even within the same species. Freeze branding using liquid nitrogen was found to be the most useful method for marking Amazon River dolphins (Martin & da Silva 2003). However, although some brands lasted the lifetime of the animal, others faded after 1 year. Freeze brands may disappear as the white scar tissue is covered by pigmented skin. In addition, algal growth on the skin may obscure freeze brands (Wright et al. 1998). Therefore, although useful for short-term identification, freeze brands generally cannot be considered a permanent marking method for cetaceans.

### **Vital stains**

Vital stains are useful for obtaining age-related information about cetaceans. Vital stain marking involves injection of a marker compound and often requires the animal be killed for information recovery (Schevill 1974). Best (1976) used tetracycline to mark growth layers in the teeth of dusky dolphins (*Lagenorhynchus obscurus*). The earplugs of some whale species have conspicuous annual laminations that can be stained and used as a point of reference. In the past, staining the earplug with quinacrine was the most common method of ageing whales (Mitchell & Kozicki 1975). However, as many whale species are protected today, kill methods of age determination are generally considered to be unacceptable. Alternative methods include tooth extraction from odontocete cetaceans, genetic tagging and longitudinal studies of individuals using markings for identification.

### **Natural marking identification**

#### ***Large cetaceans***

Owing to their anatomy and habitat, and the lack of suitable alternatives for creating long-lasting low-impact marks, cetaceans, in particular, have been identified using their natural markings. This is especially true of large cetaceans, which generally cannot be captured and restrained to securely attach equipment. In the past, the use of natural marking identification has been largely limited to short-term behaviour studies of specific groups of cetaceans, as recognition was usually confined to a single group of researchers. However, for species of cetaceans that range widely, the development of international catalogues of photo-identifiable individuals is recommended. Such catalogues are in international use for humpbacks, southern right whales and other cetacean species. Examples

include the Antarctic Humpback Whale Catalogue (<http://www.coa.edu/antarctic>), Allied Whale North Atlantic Humpback Catalogue (<http://www.coa.edu/alliedwhale/nahump.htm>) and Ocean Alliance Southern Right Whale Catalogue ([http://www.oceanalliance.org/wci/wci\\_rwresearch.html](http://www.oceanalliance.org/wci/wci_rwresearch.html)).

Morphometrics such as callosities and bonnets (right whales), flipper and baleen colourations (minke whales) and baleen plate shape (Bryde's whales) have been used to identify individuals of large cetacean species (Schevill 1974) (see figure p. 103). Kemper et al. (1997) used head callosity patterns and other body markings to identify individual southern right whales. Initially, photographs of the whales were taken from planes about 150 m above the whale. However, it was found that the animals often submerged in response to the planes, especially cows with calves. Therefore, heights of 210–240 m were maintained in later seasons. Photo-identification was also used to estimate the size of the population of southern right whales wintering in the Auckland Islands (Patenaude & Baker 2001). The researchers used callosity ridges, lip ridges and unusual skin pigmentations for identification.

Individual North Pacific humpback whales were identified using the black and white pigments and irregularities of the trailing edge of their tail flukes (Katona et al. 1979). The investigators were convinced that the patterns on the tail flukes were stable, at least over the period of the study. This technique is applicable only to adult whales, as the fluke patterns of calves and yearlings are not yet fully developed (Katona et al. 1979). Tail fluke patterns were also used to estimate the size of the whole North Atlantic population of humpback whales (Read 1995).

Stevick et al. (2001) used a double marking system to evaluate the reliability of photo-identification for individual humpback whales. Using micro-satellite genetic markers for identification, the authors confirmed that natural markings are a reliable means of identifying individual whales on a large scale. They also noted that the error rate for individual identification rose as the quality of the photographic images decreased, highlighting the need for high quality equipment and images.

### ***Small cetaceans***

Small cetaceans are commonly identified by their natural markings, especially isolated populations, or those species that do not range widely. Pigmentation patterns, rake marks, scarring and dorsal fin shape, size and colouration are commonly used to identify individuals (Green & Corkeron 1991) (see figure p. 104).

Variations in the trailing edge of the dorsal fin have been used to identify individual bottlenose dolphins (Wursig & Wursig 1977). Because this edge is very thin and readily tatters during an animal's lifetime, prominent nicks and scars develop. As the tissue apparently does not regenerate, these patterns are preserved and can be used for identification purposes. Wursig & Wursig (1977) were able to identify 53 animals by variations in their dorsal fins. Pigment spots and bite marks made by conspecifics were also used, but these marks are less lasting. A small, isolated population of bottlenose dolphins in Moray Firth in Scotland was also monitored using nicks on the dorsal fin, combined with pigmentation patterns of the skin (Thompson & Hammond 1992). The left and right sides were sampled separately, as they exhibited different markings.

Scar patterns have often been used to identify dolphins (Green & Corkeron 1991) (see figure p. 103). Individual bottlenose dolphins in Moreton Bay, Australia, were identified using scars on the dorsal surface, especially those on the caudal edge of the dorsal fin (Corkeron 1997). At least 37% of the dolphins in this bay were reported to have sustained shark bites (Corkeron et al. 1987). However, wounds heal extremely quickly in dolphins, and major scars have been observed to fade to obscurity within 9 months (Orams & Deakin 1997). Therefore, scar patterns may not be a reliable method of permanently identifying individual cetaceans.

Natural marking identification is especially useful for small, distinct populations that do not mix with other groups, for example Hector's dolphins in New Zealand. Most of the recent studies of Hector's dolphins have concentrated on building a catalogue of photo-identification profiles for each population using natural markings (e.g. Slooten et al. 1992; Stone 1992; Brager 1998; Brager et al. 2002). However, this method cannot be used to recognise immature or unmarked individuals in the population (Brager 1998). In addition, Slooten et al. (1993) noted that only a relatively low proportion of Hector's dolphins could be identified reliably. Brager et al. (2002) noted that re-sightings should be reported only from photographs, and that the photographs must be taken at ranges of less than 7 m to provide reliable identification of individuals. Although there are limitations, natural marking identification is appropriate for such vulnerable populations, as it is non-invasive and is likely to have fewer effects on behaviour and survivability than artificial marking methods.

A photo-catalogue of individual short-beaked common dolphins in northeast New Zealand has been compiled (Neumann et al. 2002). Orcas in New Zealand waters have also been identified using the shape of their eye patches, along with other natural markings (Visser & Makelainen 2000). In addition, a number of international databases of individually recognisable orcas are in use, e.g. the Antarctic Killer Whale Identification Catalogue (<http://www.akwic.org>).

Identification of individual cetaceans from photographs has several advantages. The animals are not generally captured or handled, and identification is non-invasive and does not alter hydrodynamic drag, energy expenditure or behaviour, although some effects on behaviour may result from the proximity of boats carrying researchers during identification. Therefore, it is likely to have less impact on the 'marked' animal than other methods. These characteristics make natural marking identification the most suitable method for long-term studies, research which requires repeated identification, or studies of vulnerable populations. Unfortunately, natural markings cannot be used to identify all species of cetaceans. Many species lack distinguishing marks and immature cetaceans are often unmarked. For studies of such species, artificial marks must be used.

## SUMMARY

Cetaceans are difficult to mark because of their anatomy, marine habitat and wide-ranging lifestyles. External visual markers, such as streamers and tags, are not particularly effective and often cause significant tissue damage in smaller species. The growing trend in cetacean identification is to recognise individuals by their natural markings. It is important that international databases of recognisable individuals be established, as cetaceans cross many study regions during their migrations. Satellite-telemetry has increased the potential for tracking the movements and behaviours of cetaceans. However, the effects of transmitter packages on hydrodynamic drag, behaviour and energy expenditure should be systematically evaluated and carefully considered for each population so marked, in order to balance the use of the method against the value of the information obtained.

An example of callosity patterns which can be used to identify individual southern right whales (*Eubalaena australis*), Te Wae Wae Bay, Southland, July 1990. PHOTO: ANDY COX, DOC.



Scars used to identify Hector's dolphins (*Cephalorhynchus hectori*). From Dawson & Sooten 1996. PHOTOS: © STEVE DAWSON, OTAGO UNIVERSITY.



An example of natural markings that could be used for individual identification of an Andrew's beaked whale (*Mesoplodon bowdoini*). Such scars are probably from shark bites and intraspecific fighting.

Owahanga, Wairarapa,  
November 1994.

PHOTO: BRUCE DIX, DOC.



Satellite-linked tag (SPOT-3) specifically designed for Hector's dolphin (*Cephalorhynchus hectori*), attached to dorsal fin, March 2004.

PHOTO: ROB SUISTED.



# Marine mammals—pinnipeds

There are two sub-groups of pinnipeds found in New Zealand: the phocids (earless seals and elephant seals) and otariids (fur seals and sea lions). New Zealand has five phocid pinnipeds, one of which—the southern elephant seal (*Mirounga leonina*)—is listed as Nationally Critical (Hitchmough 2002). Phocids have no external ears, little or no under-fur and short coarse hair. Otariid pinnipeds found in New Zealand are the New Zealand, Antarctic and Subantarctic fur seals (*Arctocephalus forsteri*, *A. gazella* and *A. tropicalis*, respectively) and the New Zealand or Hooker's sea lion (*Phocarctos hookeri*).

Currently, the New Zealand sea lion is fully protected under the Marine Mammals Protection Act 1978. The southern elephant seal, leopard seal (*Hydrurga leptonyx*), crabeater seal (*Lobodon carcinophagus*), Weddell seal (*Leptonychotes weddelli*), Ross seal (*Ommatophoca rossi*) and all fur seals (*Arctocephalus* spp.) found in the Antarctic area are fully protected under the Convention for the Conservation of Antarctic Seals (1972). The New Zealand fur seal has been protected since 1894 and is now fully protected under the Marine Mammal Protection Act 1978.

Pinnipeds are difficult to mark and study because they spend much of their time in the water. However, they are easier to study than cetaceans because they are amphibious and must return to land to breed, moult and pup. The use of small, remote breeding grounds creates high-density areas, making marking efforts highly efficient (Norris 1966). Pinnipeds are usually marked as pups, because adults are more difficult and dangerous to handle. However, pup marking is inefficient overall, as only a moderate percentage of animals are expected to survive to breeding age, e.g. 50% in grey seals (*Halichoerus grypus*) (Erickson et al. 1993).

Pinnipeds are marked only when they venture onto land. Haul-out habitats vary widely, from pack ice to sandy beaches, and habitat features will affect the choice of marking method and retention of marks. Due to increased abrasion, the rate of mark loss may be higher in species that haul-out on rocks rather than on smooth beaches or pack ice (Summers & Witthames 1978). The risk of infection is higher in animals that stay on land after marking, and in temperate as opposed to polar environments (Summers & Witthames 1978).

It is important to consider the amount of restraint required when selecting a marking method for pinnipeds. Certain large species can put researchers in significant danger. For reasons of safety and efficiency, physical and/or chemical immobilisation is often required in pinniped research (Laws 1993). Although physical restraint can be more dangerous to the researcher, it is usually inexpensive. Physical restraint alone has been used successfully for many pinniped species, including leopard, Ross and fur seals (Laws 1993). Rand (1950) used nets or bags to catch South African fur seals (*Arctocephalus pusillus pusillus*), Australian sea lions (*Neophoca cinerea*) and southern elephant seals weighing up to 500 kg. Stirling (1966) demonstrated that simply covering the head quickly subdues most seals, making it possible for one person to restrain an adult seal while a second completes the marking process.



Live seal being held by a single researcher. From Stirling 1966. Reprinted with permission of ACG, a division of Allen Press, Inc.

Chemical immobilisation of pinnipeds is costly and time-consuming, and can have unpredictable results (Stirling 1966). The efficacy of immobilising drugs may vary according to species, age, sex, time of year and weather conditions (Laws 1993). Chemicals used include succinylcholine chloride, phencyclidine, isofluorane and halothane. Isofluorane inhaled under a hood was found to be the most stable anaesthetic, with a shorter recovery time, shorter time to release and no lingering effects on behaviour (Summers & Witthames 1978). Chemical immobilisation of pinnipeds has been reviewed thoroughly by Erickson & Bester (1993).

Researchers must carefully consider the time of year marking is to be undertaken. In pinnipeds, moulting is accompanied by a period of anorexia. This appears to be a time of considerable stress and vulnerability for the animal (Sweeney 1974). Moulting begins with swelling of patches of skin, followed by lesion formation. The skin and hair then slough off to make way for re-growth. Continuous weight loss is observed, along with eye opacity, lethargy and irritable behaviour. Viewing, handling and disruptions related to marking should, therefore, be kept to a minimum during moulting (Sweeney 1974; Baker & Johanos 2002), and marks or attached devices should not interfere with the moulting process.

Marine mammals, including pinnipeds, exhibit one other unique feature which must also be considered when selecting a marking method. Body mass, and the tension placed on the skin due to the underlying blubber layer, fluctuate over the year and throughout the animal's lifetime. Around the time of weaning, the skin is stretched tightly around the animal, following a time of intensive growth and blubber storage. During moulting, young pinnipeds lose a great deal of body mass, causing the skin to slacken. Then, as they embark on their first foraging trips, they gradually regain mass over a longer period (Gales n.d.). These marked fluctuations in body mass and skin tension must be considered when attaching external devices such as neck collars, or when applying marks such as brands to the skin.

## TEMPORARY MARKS

### Painting

Painting is usually used as an adjunct to a more permanent method, to aid in remote identification or for short-term studies of pinnipeds. Often restraint is not necessary to apply paint marks. One disadvantage is the short duration of the marks; they are usually retained only until the next moult (Erickson et al. 1993). In addition, the application of paint to thickly furred animals can cause clumping and matting of the fur and can lead to fur loss or problems in the underlying skin (Taber 1956).

Several different kinds of paint have been used, including marine paints, rubber-based highway paint, quick-drying cellulose paint, aerosol spray paint and general house paint. Smearing often occurs, unless a quick-drying paint is used, and it can be difficult to achieve a legible mark when the animals are wet.

Quick-drying cellulose paint was used to mark southern elephant seals, which have relatively sparse hair (Laws 1956). The paint was applied with brushes attached to bamboo poles, allowing researchers to maintain a relatively safe distance from the animals. The cows were marked with an identifying number, while bulls were marked with a spot on the bare skin of the proboscis. The marks on the bulls generally persisted for 8 weeks, while cows had to be repainted every 2 weeks.

Northern elephant seals (*Mirounga angustirostris*) were paint-marked by filling plastic bags with marine paint and throwing them at the seals (Le Boeuf 1971). Individuals were identified by the location and colour of the paint marks, which were conspicuous and lasted for several months. However, only a small number of animals could be individually identified with such a system. Le Boeuf also marked seals using a paint roller on a long stick, and a carbon dioxide colour-marking pistol. This gun fired capsules of non-toxic paint to leave bright 5–10 cm marks, and was accurate from distances of 15–20 m. However, the capsules often did not break when fired at a seal, except at point blank range, and the small mark produced was inadequate for reliable identification (Le Boeuf 1971).

Griben et al. (1984) used fluorescent paint and paste to mark northern fur seals (*Callorhinus ursinus*). The paint was applied to the mid-dorsum using pressurised air and the paste with a squeeze bottle, to create symbols 20 cm high. Yellow paste was found to be most useful as it dried much more quickly than paint, and was highly visible on un-sheared pelage. About 84% of paste-marked animals were re-sighted 3 months later. Paste marks were visible for up to 2 years, and animals could be identified from up to 300 m away using binoculars. Less than 20 animals out of 2500 so marked had indistinct symbols.

Marks made with fluorescent plastic-resin paint remained visible for 2–12 months (Erickson et al. 1993). Even when the paint had disappeared, the pattern of the marks frequently remained where the guard hairs had been matted by the paint and had broken off (Griben et al. 1984; Erickson et al. 1993). Griben et al. (1984) concluded that fluorescent paste was easy and quick to apply, inexpensive and portable. In addition, no adverse behavioural effects or tissue abnormalities were observed. However, the authors noted that marks applied to wet animals were rapidly lost.

In general, painting is a suitable method for the short-term identification of pinnipeds. Often capture and restraint are not necessary for the application of paint, and the resulting marks can be highly visible from a distance. There are no reports to indicate that painting has negative effects on inter- or intra-specific interactions or other behaviours of pinnipeds. Researchers should apply the minimum amount of paint required for successful identification, to avoid damage to hair and underlying skin.

## **Dyeing**

Pelage dyes, human hair dyes and lighteners containing peroxide bleaches have been used successfully for short-term identification of pinnipeds (e.g. Johnson et al. 1981; Gentry & Holt 1982). However, dye marking can be difficult, as the animals must be dry and remain out of the water for some time after application. Dye solutions tend to be thin and spread easily, making small, discrete marks difficult to achieve. In order to dye darkly furred seals, pigment-

removing dyes must be used (e.g. bleach, peroxide), and these can take significantly longer to work than darkening dyes. The chemicals in dyes and bleaches may be toxic and can irritate or burn the skin and eyes of animals.

Boyd & Campbell (1971) dyed grey seal pups green, pink, and purple, while Pitcher (1979) used Red Woolite® liquid to mark harbour seals (*Phoca vitulina*). Black Nyanzol D dye persisted for at least 3 months on California (*Zalophus californianus*) and Stellar (*Eumetopias jubatus*) sea lions (Gentry 1979). The addition of absolute alcohol to Nyanzol D dissolved the fur oils and resulted in a more distinct dye mark, as well as preventing the solution from freezing in cold weather (Gentry 1979). Picric acid can be applied to wet and dry pups and brightens with exposure to sun. Beck (pers. comm. in Pitcher 1979) reported that picric acid marks on grey seals lasted through the pup moult and showed up on adult pelage as well. However, picric acid is difficult and dangerous to use.

Lady Clairol Ultra Blue® hair bleach produced a white or cream-coloured mark on dark pinnipeds, e.g. northern fur seals (Gentry 1979). The thick consistency of the compound allowed researchers to write clear, thin lines on immobilised animals. Less precise applications only took a few minutes and could be applied to unrestrained, sleeping animals (Le Boeuf, pers. comm. in Gentry 1979). Some marks were found to be visible on northern fur seals two seasons after application. However, it is imperative that the animals remain out of the water for about 20 minutes after application.

Dyes are useful for short-term identification of pinnipeds, and do not clump or mat the hair as paints can. However, the chemicals used for dyeing the hair can be irritating to the skin, and animals often need to be restrained to create discernible marks. For successful use of most dyes, the animals must be dry and remain out of the water for some time after application.

## **Hair removal**

Distinctive patches or identifying codes can be created by clipping or singeing the pelage of pinnipeds. Hair clipping is particularly useful on neonate seals too young to survive branding or tagging (Gentry 1979). Both hair clipping and singeing are painless and relatively easy to perform, and can be used in dense colonies to avoid counting errors. These techniques are most useful if the under-fur is a different colour from the guard hairs, e.g. in some otariid pinnipeds. Guard hairs can also be clipped away, and the under-fur dyed or painted a contrasting colour.

Scheffer (1950) and Payne (1977) used hair clipping to mark seals. Sheared patches on the heads of northern fur seals revealed the contrasting colour of the under-fur, facilitating distant identification of animals also marked by less visible permanent methods (Griben et al. 1984). Burning the tips off hair has also been used to mark fur seals, and produces a sharp, highly visible mark (Gentry 1979). This method requires a series of irons and a heat source, so hair burning may be less practical than clipping or shearing in the field.

Hair clipping and singeing may offer a relatively easy, painless method for marking pinnipeds until the next moult. However, the animals must be restrained or immobilised for longer periods than for paint or dye application, and the marks may be less visible from a distance.

## SEMI-PERMANENT MARKS

### Tags

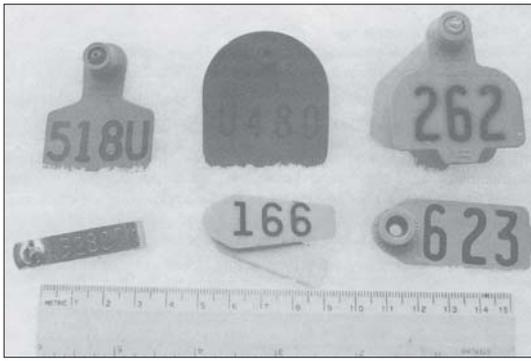
Tagging is the most common marking method in studies of pinnipeds. Tags are not a permanent method; tag loss occurs in all such studies. Thus, any calculations of population or demographic parameters from mark-recapture data must account for tag loss (Wilkinson & Bester 1997), which can be quantified by simultaneously applying a permanent mark such as a hot brand or tattoo, or by double tagging the animals (Erickson et al. 1993). Double tagging may not be as accurate as a brand or tattoo application, as loss may not be independent for the two tags. Diefenbach & Alt (1998) found that tag loss was influenced by individual behavioural characteristics (such as male dominance), which also affected whether an additional tag would be lost as well.

Sivertsen (1941) applied rudimentary tags, consisting of paired discs joined by wires, across the tail vertebrae in harp (*Phoca groenlandica*) and hooded (*Cystophora cristata*) seals. In the late 1950s, pinniped researchers began experimenting with tags as an alternative identification method to hot branding. They found that tags were easier to apply and considered them to be more humane than branding (Erickson et al. 1993). Cattle ear tags of the strap variety were used on fur seals, attached to the hind margin of the fore flipper, or to the hind flipper proximal to the claws (Scheffer 1950). Metal tags composed of silver, Monel or stainless steel, to avoid corrosion in sea water, were also used (Scheffer 1950). Hewer (1955) designed an unsuccessful flipper ring, which was frequently lost because the ends often did not engage.

In the past, most Antarctic seals were tagged with Monel metal tags from the National Band and Tag Company (Kentucky, USA). However, in one study, only 17 recoveries were made out of 15000 southern elephant seals tagged with Monel tags (Dickinson 1967). In another study of the same species (Condy 1977), 8% of Monel tags were lost in the first 6 months after tagging, with losses rising to 25% after 2 years. High losses of metal tags have also been reported in Weddell seals (Stirling 1979; Siniff & Demaster 1979) and otariid species (Payne 1977; Roppel 1979). Likewise, 15–28% of metal tags were lost from Australian sea lions in the 2 years after tagging (Ling, pers. comm. in Erickson et al. 1993).

Plastic tags made by Allflex Incorporated and the Dalton Group Ltd. are now the most commonly used tags for pinnipeds. These plastic tags consist of two discs joined by a round post that passes through the tissue and is secured by interlocking male and female components (see figure p. 124). Special pliers are used to apply the tags, which are most easily inserted if a small hole is made first. However, there are reports that punching a hole prior to tag application may significantly increase tag loss (e.g. Stobo & Horne 1994).

Plastic tags are generally retained longer than metal tags. Only 2% of Dalton Rototags® were lost from Weddell seal pups and none were lost from adults one year after application (Testa & Rothery 1992). Likewise, less than 1% of Rototags® were lost 1 year after marking southern elephant seals at Marion Island, Antarctica (Wilkinson 1991). Although the annual loss rate rose to 4% by 5 years, it then fell back to less than 2% at 6 years after tagging (Wilkinson 1991). One hooded seal marked with Rototags® in the Denmark Strait was recaptured 17 years later with its tags still intact (Kapel 1996).



Tags commonly used to mark seals. From Testa & Rothery 1992.

In contrast, Siniff & Demaster (1979) reported 5–10% losses of Allflex tags over several years, whereas 15% of Dalton tags were lost from seals in Antarctica. Testa & Rothery (1992) reported 19% loss of new Allflex tags from adult Weddell seals and 28% loss from pups in the first year after application. Some plastic tag designs will be better retained than others in certain species and environments. However, in general, plastic tags are retained better than metal tags.

As well as their longer retention times, plastic tags tend to be more visible than metal tags, facilitating identification of marked animals from farther away. The re-sighting rates of marked elephant seals were greater with plastic than with metal tags, reported as 3.3% compared to 1.7% (Le Boeuf et al. 1974) and 8% versus 4% (Burton 1985). The superior visibility of plastic tags is due to their larger size and bright colouration. However, they have little resistance to abrasion and often have low stability in UV light. Inscriptions can wear away, colours can fade and the strength of plastic decreases over time. Generally, the more pigment in the tag, the more likely it is to become brittle and break (Hoek 1979; Pitcher 1979). Stobo & Horne (1994) found that red tags were lost two to five times more often than any other colour. Some colours fade quickly and are unrecognisable within 2 years of marking, e.g. blue. Fading and discolouration can cause confusion between colours—for example, between pale blue and green, yellow and white, and red and orange (Testa & Rothery 1992). However, UV-stable colours are now available.

Coloured and numbered plastic tags glued to the heads of grey seals were found to be highly visible, and more legible than flipper tags (Vincent et al. 2002) (see figure p. 124). The head tags were retained for only a few months, but resulted in a recapture rate of 61% within that time. Colour-coded or numbered streamers can be attached to smaller, less visible tags to temporarily enhance the probability of detection. Nylon streamers reinforced with vinyl often lasted a year or more (Erickson et al. 1993). Flagging strips were found to increase the visibility of marked seals for up to 2 years, and their use was recommended (Stobo & Horne 1994). However, such devices do not increase the distance at which tag numbers can be identified.

Tag loss rates can vary markedly between species, even when the same type of tag is used. This may be due to differences in habitat use or behaviour between species. Cawthorn (pers. comm. in Erickson et al. 1993) reported annual loss rates of 5–10% for Allflex tags on New Zealand sea lions. However, Ling (pers. comm. in Erickson et al. 1993) found that the same tags were lost at much higher rates from Australian sea lions: between 30% and 60% of sea lions tagged in 1976/77 lost their tags. Plastic sheep tags lasted 3 years when attached to Weddell seals in New Zealand (Stirling 1979). In contrast, the same tags attached to New Zealand fur seals lasted only 1 year. The major difference in retention was related to the difference in habitat, with the fur seals hauling out on dirty, rocky substrates that were more damaging to the tags.

Within a species, tag loss rates may differ between groups of animals. Differences in habitat use, activity level, seasonal (e.g. mating) and other behaviours between age or gender groups may contribute to non-uniform rates of tag loss within a species. Negligible losses of plastic tags were reported in the

first 6 months after tagging South African fur seals; however, losses increased to 13% at 8 months of age (Best & Rand 1975). Reiter (1984) found 11% losses at 2 years after applying Dalton Junior Rototags® to northern elephant seal pups, but only 6% per year thereafter. Extensive studies on grey seals have shown substantial increases in annual tag loss rates after 1 year of age (Stobo & Horne 1994). Tag loss remained constant at 0.3% up to 5 months of age, then increased to reach a maximum (44%) at 6–8 years of age, and remained high in animals up to 13 years old. In contrast, studies of Weddell seals showed that loss rates were highest among animals in their first year (Testa & Rothery 1992).

In other studies, changes in tag loss rates over time have not been evident. Bowen & Sergeant (1983) conducted a double-tagging experiment (Rototags®) in harp seals and found that the estimated rate of tag loss was 0.5% over the first 3 months, with no evidence of loss rates increasing over time. Wilkinson & Bester (1997) reported some of the lowest loss rates recorded for southern elephant seals tagged with Jumbo Rototags®, and observed no age- or sex-related differences in tag loss.

As well as differences in behaviour and habitat use, changes in body size may also contribute to increasing rates of tag loss with age. When first attached to the flippers, the length of the tag post exceeds the thickness of the interdigital webbing. As the animal grows, this thickness increases until it exceeds the post length. The tag will then cut into the flesh and cause a wound that may enlarge and allow the tag to fall through (Wilkinson 1991). Whatever the reason for differences in tag loss rates within a species, researchers should evaluate loss rates separately for each cohort of animals, and incorporate that specific rate into subsequent calculations of population estimates.

Operator proficiency has also been shown to have a significant effect on tag retention. In a study of South African fur seals, single tag loss rates varied between 7% and 34%, depending on who tagged the animals (Shaughnessy 1993). David (pers. comm. in Erickson et al. 1993) reported tag loss rates of between 3% and 15%, and concluded that loss rates were clearly influenced by the skill of individual taggers.

Correct placement of flipper tags can aid in reducing tag loss. Tag location is particularly important in temperate regions owing to the increased potential for infection (Testa & Rothery 1992). In the hind flipper, tags should be inserted into the centre of the inner two sections of the interdigital webbing, so that approximately one-third of the length of the tag protrudes from the edge of the webbing (Wilkinson & Bester 1997). If the tag is placed too proximally, it will cut the tissue when the flipper is closed, causing necrosis and tag loss (Testa & Rothery 1992). If the tag is placed too distally on the flipper, it will pull through the hole because of drag. One solution is to make the tags smaller to reduce drag; another is to make tags bigger so they will not pull through the holes so easily.

Very few studies have explicitly examined the effects of tag application or wearing on pinnipeds. This is probably because of logistical and financial constraints, and the difficulty in finding and tracking appropriate controls. However, Baker & Johanos (2002) designed an experiment to specifically test the effects of handling and tagging on the probability of re-sighting and migration, and on the health and body condition of the endangered Hawaiian monk seal (*Monachus schauinslandi*), a species known to be particularly

sensitive to human disturbance. Weaned pups were tagged on each rear flipper with Temple® tags measuring 4.9 cm × 1.7 cm. The pups were captured and physically restrained for an average of 3.3 minutes to facilitate tag application. Controls were matched for sex, approximate age and location, and were identified either by tags applied in previous years, or by distinctive scars. The authors found no deleterious effects on survival (as indicated by re-sighting 1 year later), migration or body condition, associated with tagging or handling. Likewise, Henderson & Johanos (1988) found no indication that tagging pups of the same species resulted in any observable detrimental effects.

Tagging is, and is likely to remain, the most commonly used method for identifying pinnipeds. However, very few direct reports on the effects of tagging on the health or behaviour of marked pinnipeds have been found. Pinnipeds must be captured and restrained for tag application. Most animals are tagged as pups, making the process much less difficult and dangerous for the researcher. The major disadvantage of tagging pinnipeds is the high rate of mark loss. In general, plastic tags are better retained and more visible than metal tags. Larger tags are more visible, but tend to be lost more easily than small tags. Research is still required to develop improved tags for pinniped species, including more durable materials and better attachment methods. Tags specifically designed for the species under study would be valuable, owing to the significant interspecific differences in habitat and behaviour. In addition, direct research into the effects of tagging on specific pinniped species or populations should be undertaken.

### **Neck collars, bands and harnesses**

Rubber neck collars have been applied to northern fur seals (Scheffer 1950), and bracelets—fashioned by inserting nylon-reinforced rubber straps (1.5 cm wide) into surgical rubber tubing—have also been used for seals (Bengston 1993). Each bracelet, with telemetric equipment bolted to it, was fastened around the ankle of one rear flipper. The link holding the band together was designed to corrode, eventually releasing the bracelet from the animal. The fit of the bracelet was a major issue: when too tight, the underlying skin became abraded and inflamed; when too loose, the equipment was often lost. When correctly fitted, the researcher could fit an index finger between the bracelet and the skin.

Time Depth Recorders (TDRs) were attached to California sea lions using nylon webbing harnesses (Feldkamp 1985). The harness crossed the seal's back and belly and was connected with steel rivets, which eventually rusted away, freeing the animal and allowing recovery of the recorder. The author expressed concern about increased hydrodynamic drag, which can affect a seal's swimming performance. Costa & Gentry (1986) measured a consistent 20% increase in metabolic rate when lactating female northern fur seals wore harnesses.

Harnesses are not considered suitable for pinnipeds, due to increases in hydrodynamic drag and subsequent changes in energy expenditure and behaviour. Bands and neck collars may be acceptable for short-term studies, but proper fit is important to reduce the likelihood of injury to adjacent tissues. It is also important to note that the natural fluctuations in mass that pinnipeds undergo throughout the year may make collars, in particular, inappropriate for long-term studies.

## Telemetry

Historically, most pinniped studies have been performed at breeding sites, so less is known about pinniped behaviour outside the pupping season. Ultrasonic-telemetry has been used for studying fish, but is not suitable for marine mammals because they are sensitive to high frequency sounds (Troy et al. 1997). Aerial or ship-based radio-tracking is more useful, but is expensive and time-consuming to perform, and ground-based stations have limited ranges (5–20 km at sea level) (Hammond et al. 1992). Automatic recording stations can be used to detect the location of a number of tagged animals at one time, and may be extremely useful for intensive monitoring of small, local or seasonally sedentary pinniped populations (Hammond et al. 1992). The use of satellite-linked transmitters allows remote monitoring of pinnipeds without the need to stay in continuous radio-contact with the animals.

Most telemetric systems for pinnipeds have been designed to relay information such as geographical location, time and haul-out. Haul-out can be detected because the reception of radio signals is blocked when the animal is submerged in salt water. In addition, sensors in Time Depth Recorders (TDRs) use increasing measures of pressure to extrapolate diving depths and durations. Physiological variables such as the heart's rate and electrical activity (as displayed in an electrocardiogram, ECG), and body temperature can also be sensed and the data transmitted (e.g. Kooyman et al. 1983; Ponganis et al. 1991; Folkow & Blix 1995). Tags placed inside the stomachs of harbour seals recorded body temperature for 2–20 days (Bjorge et al. 1995). In addition, the distances travelled by individuals can be determined by attaching a turbine odometer on top of the transmitter package (McConnell et al. 2002).

No matter which method is used to collect data—an archival recorder or radio- or satellite-telemetry—the transmitter must be attached to the animal, and packages must be kept to a size and design appropriate for the species. Pinnipeds are often anaesthetised to attach transmitter equipment (e.g. Lowry et al. 2001; Baker & Johanos 2002; McConnell et al. 2002), but the necessity for anaesthesia depends on the species and age group involved. Equipment used to track pinnipeds must be waterproof and protected from the high pressures experienced during dives. Siniff et al. (1971) tried to attach transmitters using sutures and tail mounts, but these methods were not satisfactory. Today, transmitters are attached by three main methods: harnesses, bracelets, and glues or epoxy resins. As harnesses and bracelets have been discussed above (see, *Neck collars, bands and harnesses*, p. 112), only adhesive attachment will be discussed here.

Glues and epoxy resins are now the most common method for attaching transmitters to pinnipeds (see figure p. 124) (e.g. Loughlin et al. 1987; Gjertz et al. 2001; Lowry et al. 2001; McConnell et al. 2002). Superglues are quick to set, but degrade in water over time. In contrast, epoxy resins are more durable in water, but most take longer to set upon application (often the animal must be kept out of the water for 5–20 minutes to allow the epoxy to set). However, fast-drying epoxy resins are available. Alternatively, a combination of the two systems can be used. Superglue will hold the transmitter in place while the epoxy cures, allowing the animal to be released much sooner. Some epoxy compounds can be applied in only 1 cm-thick layers at a time, as the heat produced during curing can burn the seal's skin.

Transmitter packages attached with adhesive are lost during the annual moult, if not before. Therefore, telemetry cannot usually be used to track animals throughout the entire summer period or from year to year. This means that equipment must be reattached for continued monitoring. However, it also means that the animals do not have to be recaptured to remove transmitting equipment (Folkow & Blix 1995).

The position of the tag on the animal's body is critical for maximising the number of uplinks to the satellite or contact with a VHF receiver (Stewart et al. 1989). When tracking seals on shore, low profile antennae are applied to lie flat on the seal's body. In contrast, when animals are being tracked at sea, an upright antenna is required, to transmit a signal whenever the seal comes to the surface to breathe (Bengston 1993). As phocid pinnipeds, in particular, perform series of many dives with only short periods at the surface between each dive, maximising the chance of a successful uplink is critical to achieve at-sea location fixes. The optimum location is the top of the head for larger species, or between the scapulae for smaller species.

Some concessions to the natural streamlining of the animal must be made when considering the placement of transmitter equipment. Many researchers place the package at the top of the neck behind the head, to allow the antenna to emerge as the seal surfaces, while attempting to minimise the increase in hydrodynamic drag during swimming (e.g. McConnell et al. 2002). When attaching transmitter packages to the head they must be properly centred or they will quickly fall off, owing to unbalanced water pressure (Fedak et al. 1982).

The retention and operational lifespan of transmitters attached to pinnipeds vary widely. Transmissions from adult harbour seals were detected for 7-313 days (Gjertz et al. 2001). Southern elephant seals have been tracked for 2-179 days, with an average of 77 days of transmission in one study (McConnell et al. 2002) and an average of 76 days in another (Slip 1993). Transmitters were retained for about 90 days on Antarctic fur seals and approximately 300 days on Weddell and crabeater seals (Fedak et al. 1982).

As well as equipment characteristics (e.g. transmission schedule and power, inclusion of a salt-water switch), the species and time of year can affect the retention and operational lifespan of the transmitter. Changes in habitat use (hauled-out versus at-sea) and behaviours such as mating, fighting or rubbing may result in decreased transmitter retention and lifespan. For example, individual characteristics of tagged southern elephant seal pups (e.g. body weight) were found to affect the chance of damage to the antenna, and larger pups were tracked for shorter periods, suggesting that a difference in behaviour might have affected the operational lifespan of the transmitter equipment (McConnell et al. 2002).

Seal behaviour differs near shore and offshore, and can therefore alter the probability of transmission detection or uplinks to the satellite. For example, location fixes are less likely to be obtained during offshore activities such as active diving. This kind of bias is particularly problematic with satellite tracking, and can contribute to an unrealistic representation of the animal's activity budget and patterns in time and space. In addition, archival data loggers such as TDRs have been shown to provide more accurate and detailed records of dive behaviour than satellite-linked sensors. Dives recorded by TDRs were

found to be twice as long as those indicated by satellite records for the same animal (Stewart et al. 1989). Therefore, data from satellite-telemetry may not accurately reflect the true range in behavioural, physiological or environmental variables at sea. In order to compensate for some of the bias, researchers filter the raw data obtained from satellite tracking. A common algorithm used for filtering is based on the rejection of locations that would require unrealistic rates of travel (e.g. McConnell et al. 1999).

Very few studies have deliberately evaluated the effects of telemetric instrumentation on pinnipeds. However, Baker & Johanos (2002) specifically investigated the effects of instrumentation on the survivorship, migration and health of Hawaiian monk seals. Animals were captured, physically restrained and sedated, and a variety of instruments were attached to the dorsal pelage using epoxy glue. The average restraint time for instrumentation was 26 minutes. The devices, ranging in mass from 0.2 kg to 2.4 kg, included TDRs, satellite-linked TDRs, CritterCam<sup>®</sup> video recorders<sup>7</sup> and Global Positioning System (GPS) data loggers. The largest devices (CritterCam<sup>®</sup> and GPS logger) were removed within 31 days, while the other devices were removed within 119 days, or were left to drop off at the next moult. The authors found no observable deleterious effects of instrumentation with any of the devices.

Harcourt & Davis (1997) compared the haul-out behaviour of female fur seals with and without transmitters. Two female fur seals were fitted with satellite-linked transmitters weighing about 0.7% of their body weight. Although it was impossible to determine the effect of instrumentation on their behaviour at sea, female attendance at haul-out was compared. The authors found no difference in the number of days at sea, or on land, between females with TDRs and tagged or bleached animals (Harcourt & Davis 1997).

Stewart et al. (1989) found no difference in the pre- and post-tagging behaviour of a captive ringed seal (*Phoca hispida*) in terms of surface and submersion durations. A satellite-linked transmitter package, weighing 700 g, was glued to the animal's back, and haul-out prevented for 76 hours, to simulate at-sea conditions. The amount of time spent at the surface and submerged was measured for 24 hours before and after instrumentation. Based on this short and somewhat limited evaluation of a captive ringed seal, the authors inferred that instrumentation with a satellite-linked transmitter, TDR and VHF transmitter did not change the behaviour of a free-swimming harbour seal.

In contrast, several studies have shown that wearing telemetric equipment may affect the bearer. Webb et al. (1998) found that experimentally altering the buoyancy of northern elephant seals by attaching Styrofoam<sup>™</sup> or lead weights to them, affected their diving behaviour. This implies that the addition of telemetric instruments or data loggers could potentially affect the very parameters being measured. Walker & Boveng (1995) found that female Antarctic fur seals carrying TDRs and radio transmitters behaved differently from females carrying radio transmitters alone. The seals instrumented with both devices spent longer on foraging trips and nursing visits than those carrying only one device. The authors took this as evidence that results obtained from instrumented animals may not be representative of the natural (non-instrumented) population. Studies on other groups of marine mammals

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<sup>7</sup> CritterCam<sup>®</sup> recorders are attached to wild animals and provide images for *National Geographic*.

(e.g. penguins and turtles) have also revealed that the attachment and wearing of telemetric instruments can affect swimming and diving behaviour, energy expenditure and even breeding success (e.g. Wilson et al. 1986; Bannasch et al. 1994; Watson & Granger 1998).

Radio- and satellite-telemetry and archival data recorders provide invaluable information on the behaviour, movement and physiology of pinnipeds, both on land and at sea. However, researchers often find high individual variability in behaviour within populations of tracked pinnipeds, and Hammond et al. (1992) have questioned the assumption that small samples are representative of the population at large. Therefore, researchers must use caution when extrapolating from a small number of tagged animals to the general population or a larger subpopulation.

Specific research into the effects of telemetric equipment on the bearer is lacking. For each package design and population to be instrumented, a systematic evaluation of the effects should be undertaken, in order to balance such effects against the value of the information obtained. Continuing research should concentrate on minimising the effects of transmitter packages on hydrodynamic drag and behaviour of the bearer. This will improve the chances that the behaviour of telemetered animals represents that of the natural population. In addition, improvements to the frequency and quality of satellite uplinks will make satellite-telemetry even more valuable as a tool to study free-ranging pinnipeds.

## PERMANENT MARKS

### **Hot branding**

Hot branding was first used on northern fur seals in 1912 (Osgood et al. 1915) and has since been used extensively on a range of phocid and otariid species. Because of the need for bulky apparatus, the use of hot branding is largely confined to colonial or at least aggregated pinnipeds (Erickson et al. 1993).

Homstead et al. (1972) experimented with an explosive branding device for marking pinnipeds. An explosive charge was loaded into a modified spearfishing gun, with a rubber template. The length of the gun's shaft allowed the operator to maintain a safe distance from the unrestrained seal. The authors reported that after branding, newborn pups showed no distress, became quiet and resumed normal behaviours such as suckling. However, suckling is often interpreted as a way for young mammals to reduce distress (e.g. Gunnar et al. 1988). Twelve months after marking, northern elephant seals displayed clearly legible brands. The use and transport of explosive materials poses a real danger to people and animals, and the injection of fine lead particles from the fuse into the skin of the marked animal may also affect its health. The long-term effects and durability of explosive marks were not assessed and this practice was not continued.

Forges are generally the heat source of choice, but furnaces fired by propane burners are also used for pinniped branding. The branding irons are heated to approximately 700°C. Application duration may vary between species, based on the amount of hair and condition of the animal (e.g. blubber thickness), and the appropriate duration should be determined before large-scale branding

programmes are undertaken. Some researchers choose to remove the hair first, particularly on heavily furred animals. This can be achieved by clipping or by lightly singeing the hair with a hot iron (Gentry & Holt 1982). Troy et al. (1997) first singed the fur from New Zealand fur seals, then applied steel brands for 4 seconds. A 2-second brand application on dry pelt was found to be appropriate in grey seals and Arctic phocids (Hoek 1979). Erickson et al. (1993) branded Antarctic seals for about 3 seconds on the first brand, and 4 seconds for the other side of the body, to account for iron cooling (the iron was not reheated between the two applications).

Pinnipeds are usually branded on both sides in order to ensure positive identification; often a letter or numeral that is unreadable on one side will be well preserved on the other. Brands are positioned on the mid dorso-lateral flank area (see figure p. 124) and are applied two characters at a time. A rod with a round cross-section is preferred for branding because it contacts the skin evenly over its surface; otherwise, the skin will be burnt only at the edges of the iron (Erickson et al. 1993). Wet animals should not be branded, as the iron will lose heat rapidly, resulting in a scald instead of a burn. Scalds cause slow healing scars, which are difficult to read (Battaglia 2001). Most workers do not administer wound dressings or antibiotics after branding, but Anderson (1985) applied burn ointment to alleviate pain and minimise infection.

One problem relating to branding, which is unique to marine mammals, is the tension placed on the skin owing to the underlying blubber layer, and its fluctuations over the year and throughout the animal's lifetime. Branding of pinnipeds usually occurs around weaning, following a time of intensive growth and blubber storage. This means that the skin is stretched tightly around the animal. During moulting and healing, the young pinnipeds lose a great deal of body mass, causing the skin to slacken. Then, as they embark on their first foraging trips, they gradually regain mass over a longer period. These fluctuations in skin tension can interfere with wound contraction and scar formation at the branding sites. The result can be distorted brands, and decreased readability (Gales n.d.).

Variation in factors such as application duration, environmental conditions, dermal and blubber thickness and operator proficiency leads to a wide range of healing outcomes. Brand wounds that are too shallow will not produce a hairless scar, and the brand may be obscured by new hair and pigment (Montagna & Harrison 1957). If the brand wound is too deep, and penetrates the poorly perfused hypodermis, the burnt fat cells can become necrotic and chronically infected. This occurs because blubber has inadequate inflammatory responses to deal with this type of injury. These types of burns can lead to excess scarring, cracked, open wounds, ulcerative lesions and suppurating or bleeding fistulae. After such extensive tissue damage, scarring can spread laterally from the burn site, leading to misshapen, unreadable scars (Gales n.d.).

About 14 000 weaned southern elephant seal pups at Macquarie Island were hot branded between 1993 and 1999, under the jurisdiction of the Australian Antarctic Division (AAD), and the guidance of the Antarctic Animal Ethics Committee. In 1999, in response to public concern over the large proportion of unhealed wounds on branded animals, the Australian Federal Environmental Minister and the Tasmanian Minister for the Environment called for an

immediate moratorium on hot branding of Macquarie Island elephant seals. The AAD commissioned an inquiry into hot branding of the seals, specifically looking at the degree and nature of healing and brand readability. The resulting report did not offer any definitive conclusions on the appropriateness of hot iron branding as a method for marking elephant seals at Macquarie Island. However, the high proportion of unhealed brand wounds, the large number of animals that could not be identified by their brands, and the error rates in brand transcription were noted as causes of particular concern (Gales n.d.). About 14% of all the Macquarie Island elephant seals observed had unreadable brands, and only 10% of the brands could be read quickly and easily (Gales n.d.).

Gales considered that the day-to-day behaviour of elephant seals was not likely to be affected by the types of open wounds observed. However, the very protracted nature of healing would carry significant energetic and physiological costs and, in combination with pain-induced behavioural changes, could become significant in terms of animal welfare. In addition, any animal that cannot be recognised by its brand will have experienced some degree of welfare compromise, without any compensating benefit, in terms of knowledge gained.

In 2000, over 400 New Zealand sea lions were hot branded in the Auckland Islands. The New Zealand Department of Conservation's Animal Ethics Committee approved the 1-year trial provided that several conditions were met: a supervising veterinarian was to be present during marking; sea lions were to be sedated during restraint; and animals were to be anaesthetised during branding.<sup>8</sup> Hot branding was selected to mark the sea lions for several reasons: the relative permanence of the marks, which reduced sample sizes and errors related to mark loss; the ability to identify animals from a distance, which reduced the risks to researchers; and to avoid repeated disturbance of the animals for the purposes of identification and re-marking.<sup>8</sup> However, in April 2000 the identification trials were halted by the Minister for Conservation. In addition, South Island residents who had encountered branded animals expressed concern about the marks later that year (Beston 2000).

The population of New Zealand sea lions was monitored after branding, and showed no evidence of acute phase or chronic inflammatory response. There were no significant differences in inflammatory responses between branded, flipper tagged or unmarked pups (P.J. Duignan, I.S. Wilkinson & P. Clark, Massey University, New Zealand, pers. comm. 2004). Three months after hot branding, 30% of the sea lion pups were fully healed, and 91% of the pups had fully healed brands after 12 months. However, only 63% of branded adults had fully healed 12 months after marking. The adults' use of freshwater and mud wallows may have delayed their healing. No significant relationship between body mass at 12 weeks and marking method were found for either sex. In addition, all of the brands were found to be fully legible at 12 months post marking. Troy et al. (1997) also found that all hot branded New Zealand fur seals had clear marks 10-14 months after application, when moulting was complete. P.J. Duignan, I.S. Wilkinson, and P. Clark (pers. comm. 2004) concluded that hot iron branding did not appear to have adverse impacts on the growth or potential survivorship of New Zealand sea lion pups, and that the healing observed was as expected for the type of tissue damage inflicted.

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<sup>8</sup> DOC Animal Ethics Committee Approval AEC51 made at committee meeting of 21 June 1999.

Pinnipeds with clear brands can usually be identified without subsequent capture. Adult grey seals, branded as pups with 10-cm-high numbers, had healed brands that were usually discernible at distances of 2–5 m (Schwarz & Stobo 2000). In addition, successful hot brands are usually permanent and can be used to identify the animal for the rest of its life. Kemper et al. (1997) found one female elephant seal, branded as a pup, still showing a clear mark 23 years later. She was still exhibiting reproductive success, as evidenced by a newborn pup. Scheffer (1950) also reported northern fur seals having readable brands 20 years after marking.

Invasive marking techniques such as hot branding have often been criticised as being inhumane. However, some researchers believe that causing an injury to an animal once in its lifetime is more humane than repeatedly capturing it in order to refresh temporary marks (Erickson et al. 1993). Brands that heal quickly to produce lasting, legible marks are unlikely to negatively affect the bearer after healing has occurred. In addition, clear brands usually allow re-identification (on land) without recapture. The primary concern with branding is that there is unpredictable variation in the healing and legibility of the resulting marks. In addition, operator proficiency and experience can greatly affect the success of branding.

### **Freeze branding**

Freeze branding leaves a non-pigmented mark on the pelage, and has been used in pinnipeds with mixed success (Farrell 1979; Erickson et al. 1993). The quality of the marks resulting from freeze branding is primarily dependent on the duration of brand application.

Macpherson & Penner (1967b) freeze branded eight seals in the laboratory using liquid nitrogen and alcohol (xylo). Juvenile seals were branded using a 5-second application, and mature seals, a 7-second application. The seals were kept out of the water for 6 hours after marking, which might be impractical in the field. Six weeks after marking, all the young seals had clear brands; however, no colour change was observed in the pelage of the adults. The application times may have been inadequate to affect the thick hides of the adult seals. Harbour seals freeze branded for less than 17 seconds exhibited unclear brands, as fur grew back into the branded area (Harkonen et al. 1999). However, application times of 20–30 seconds resulted in clear brands, visible up to 500 m away (via a spotting scope), which have remained legible for at least 13 years.

Male New Zealand fur seals were marked using liquid nitrogen applied for 30 seconds, or alcohol and dry ice applied for 50 seconds (Troy et al. 1997). All but two males had clear brands after the next breeding season and moult. However, freeze branding did not always produce an identifying mark in the first year. Therefore, if identification was needed in the first 12 months after freeze branding, the authors recommended tagging the animals as well (Troy et al. 1997).

Erickson et al. (1993) reported that freeze-branded phocid seals exhibited dark brands that allowed identification for 1 year and were visible (but not distinct) for 3 years. Freeze branding California sea lions produced pink to dark-coloured brands readable for 18 months and discernible for 4 years. Freeze-branded walrus (*Odobenus rosmarus*) exhibited pink brands that were readable for many years (Erickson et al. 1993). The use of alcohol on the brand site was found to improve the contact between the branding iron and the skin (Warneke

1979). Using this method to mark Australian sea lions, Warneke found that freeze brands on the flippers were legible for 7 years and those on the flanks were discernible for 4 years. Freon 12 and Freon 22 have also been used to freeze brand northern fur seals (Keyes & Farrell 1979).

Harkonen et al. (1999) freeze branded harbour seals using brass irons cooled for 30 minutes in ethanol and dry ice. The irons were attached to a handle with a spring calibrated to deliver a standard force of 4.5 kg. The fur was washed with sea water and ethanol, and the irons applied for 17–30 seconds. Individual number codes 7.5 cm high were applied to both shoulders. Brands were not visible until 3–4 weeks after application. Before the first moult, the branded skin was purple, and thereafter turned brown. Freeze branding grey seal pups using the same method did not produce brands at all. However, when the branding irons were cooled in liquid nitrogen and applied for 25 seconds, legible brands were produced. This highlights the differences between species in the physiological response to freeze branding, and emphasises the importance of determining the appropriate cooling system and application duration for each pinniped species.

The major advantage of freeze branding is that it is presumed to be less painful and, therefore, more humane than hot branding. Physiological results imply that hot branding causes more severe acute reactions, as well as more prolonged responses, than freeze branding in cattle (Leblanc et al. 1980; Lay et al. 1992; Schwartzkopf-Genswein, Stookey, Janzen et al. 1997; Schwartzkopf-Genswein et al. 1998). Although hot branding causes a more acute response, there appear to be no long-term effects on animal fitness or production measures in domestic mammals (Schwartzkopf-Genswein, Stookey, Janzen et al. 1997).

Harkonen et al. (1999) noted that freeze branding, when successful, is one of the few methods that allow pinnipeds to be studied during the entire summer period (moulting), and from year to year without reapplying the marks. Other benefits of freeze branding were that sedation was not necessary, the method did not lead to open wounds and that the animals were handled only once in their lives (Harkonen et al. 1999). Troy et al. (1997) also noted that freeze branding may be safer for the animal, owing to the risk of excessive tissue damage and infection associated with hot branding.

The primary disadvantage of freeze branding is the fact that the quality of the brand cannot be determined at the time of application. In some pinnipeds, brands can be obscured by re-pigmentation of the skin within 2 years (Cornell et al. 1979). For long-term studies, capture and freeze branding may have to be repeated. Additionally, the hair must be removed before the brand is applied, whereas hot brands can burn through hair. Finally, there are logistical problems associated with the use of dry ice or liquid nitrogen in the field (Keyes & Johnson 1971).

Successful freeze branding will provide clear, long-lasting marks in some pinniped species. The risk of excessive tissue damage and infection is lower for freeze than hot branding. However, the inability to determine the success of freeze branding at the time of marking, and the high variation in mark durability, makes this method less useful for wild populations. In addition, freeze branding may not be permanent in pinnipeds, and re-marking may be necessary in long-term studies.

## **Tattooing**

Tattooing is not often used in pinniped research because it requires close examination of the animal for identification. Identification would, therefore, require capture and restraint, and (sometimes) chemical immobilisation, with all the associated risks to animal and researcher. However, tattoos placed on the inner upper lip offer a good way to permanently mark pinnipeds. Tattooing may be used for auxiliary identification in the event of lost marks, e.g. tags. Indeed, tattooing may be used to evaluate the reliability of other marking methods (Erickson et al. 1993).

## **Passive Integrated Transponders**

PIT tags have been used to identify southern elephant seals (Galimberti et al. 2000). Implantation into weanlings, without restraint or warning, took 1–2 seconds, as opposed to 6–120 seconds when animals were restrained. Less than 1% of implantations failed and most failures were due to the implant falling out of the needle. All successfully implanted transponders were checked and found to be readable, and no tissue reactions were observed. The failure rate 1 year later was 2.2% (Galimberti et al. 2000).

Trovan<sup>®</sup> microchips were implanted into New Zealand sea lions in the Auckland Islands (P.J. Duignan, I.S. Wilkinson & P. Clark, Massey University, New Zealand, pers. comm. 2004). The advantages of using PIT tags in this species included the ease of application and minimum impact on the animals. The authors did not consider PIT tags to be a replacement for plastic tags, which are cheaper and can facilitate identification from a greater distance. However, PIT tags can provide permanent identification for the lifetime of the animal, and reduce the problems associated with external tag loss. Galimberti et al. (2000) found that when properly positioned, PIT tags in southern elephant seals remained in place, without migrating into deeper tissues. However, 4% to 10% of PITs implanted into New Zealand sea lions were lost due to migration from the application site (P.J. Duignan, I.S. Wilkinson & P. Clark, pers. comm. 2004).

Disadvantages of PIT use in pinnipeds include the relatively close proximity required to recognise marked animals (the greatest scanning distance of the Trovan<sup>®</sup> reader is 18–20 cm). However, Galimberti et al. (2000) found that most resting southern elephant seals could easily be approached and scanned from 5 cm. Gales (n.d.) reported that it was not necessary to approach the animals any closer than is required to read hot brands. Scanning can also be achieved at greater distances, by mounting the transponder reader onto a pole. In addition, non-readability and observer error, which are significant problems with other methods (e.g. branding), are avoided (Gales n.d.).

The lack of external marks after PIT implantation is desirable in terms of positive public perception. However, it means that unless PIT-tagged animals are also marked with an externally visible mark (e.g. paint), all members of the pinniped colony would have to be scanned to identify the marked individuals. In addition, marked animals cannot be recognised at other locations (e.g. distant haul-outs) where researchers might not have PIT readers.

The effects of PIT tags on pinnipeds have not been directly assessed. This is most likely because of the very small size of the implants, relative to the animal. Intraperitoneal implants in medium to large mammals appear to have little

effect on their survival or behaviour (e.g. Green et al. 1985; Van Vuren 1989; Hernandez-Divers et al. 2001). PITs are generally implanted subcutaneously in pinnipeds, and no reports of adverse effects of such implants on health, behaviour or survival have been found.

PIT tags may be useful for longitudinal studies that require identification of individual pinnipeds over their lifetimes. PIT tag loss is low, relative to other marking methods, readability is not reduced over time and there are no aesthetic problems when the study area is also used for leisure or tourism. However, animals tagged with PITs are not identifiable without readers, and reading distances are relatively short.

### **Tissue removal**

Punch marks and other tissue removals are easy to perform and require very little equipment. Scheffer (1950) produced coded marks in the flipper webs of northern fur seals, by varying the position and number of punched holes. The method proved to be unreliable because the holes quickly became occluded and difficult to see. Flipper notching in Antarctic fur seals was also found to be unreliable owing to tissue re-growth (Bonner 1968). Bonner punched 6-mm-wide holes that subsequently healed to 1–2 mm widths and were difficult to visualise. This method would be expected to be even less effective when applied to the hair-covered flippers of phocid pinnipeds.

Roppel (1979) used ear clips to mark cohorts of otariid seals. However, this method was discontinued because of concerns that removing part of the ear pinnae may affect deep diving abilities. The external ears of otariids are thought to play a role in pressure regulation during deep dives (Scheffer 1950).

In general, mammalian toes are large and fleshy and bleed profusely with even the cleanest of cuts (Stoddardt 1970). The risk of bleeding, infection and behavioural effects make toe clipping less appropriate for pinnipeds than other groups (Johnson 1971). Tissue removals are not often used to mark pinnipeds, as they are largely unsuccessful, and capture would usually be required for re-identification. Other methods are more appropriate for marking pinnipeds.

### **Vital stains**

Vital stains have been used to mark hard tissues such as bone and teeth, and were previously used to calibrate age criteria in pinniped studies. Examples of vital stains used in pinniped research include tetracyclines, alizarine red and lead acetate (Erickson et al. 1993). This type of marking is not useful for large-scale or individual marking, and is of limited use in pinniped field studies, as the equipment and procedures required to perform readings are fairly sophisticated. Vital staining for age determination of pinnipeds is no longer common, as researchers have validated the use of other aging methods (e.g. Dietz et al. 1991; Oosthuizen & Bester 1997).

### **Natural marking identification**

Identification of pinnipeds by their natural markings is generally limited to small numbers of animals and is only applicable to colonial species. Forcada & Aguilar (2000) found that the distinctiveness of an individual's markings, as well as the quality of the photographs used to record them, predicted the certainty with which individuals could be identified. Phocid pinnipeds can sometimes be identified by

pelage patterns (e.g. Le Boeuf 1972; Hiby & Lovell 1990; Yochem et al. 1990). However, in general, only adult phocids have pelage patterns distinctive enough to allow individual identification (Hiby 1994; Forcada & Aguilar 2000).

Features such as flipper damage, body and facial scars and lower canine tooth size were used to identify individual New Zealand sea lions in a 3-year study on the Otago Peninsula (MacConkey 1999). Colour photographs taken from 1–5 m away facilitated individual identification in 82% of re-sightings. Other otariid species have also been identified by body and facial scars (e.g. Peterson & Bartholomew 1967; Beentjes 1989). Stellar and California sea lions have also been identified using natural markings (Gentry 1979). MacConkey (1999) noted that although this method is not intrusive, it is labour-intensive.

In general, a combination of distinguishing characteristics should be used for natural identification, to safeguard against the loss of certain features. Researchers must use caution when employing scars and other potentially temporary features for identification, as the time that scars remain distinctive can vary greatly. In contrast, damage to flippers is permanent, and cannot be altered except by the addition of another, larger mark. Of 48 New Zealand sea lions identifiable by natural markings, almost 80% could be recognised by flipper features alone (MacConkey 1999). Overall, the natural markings used in this study did not change enough to affect accurate identification of individuals over 3 years. Likewise, changes in the markings of Mediterranean monk seals (*Monachus monachus*) over 3 years were not sufficient to affect the accuracy of identification (Forcada & Aguilar 2000).

Identification using natural markings is less invasive than other marking techniques. However, this method is applicable only to those pinnipeds species with distinctive pelage patterns, or other distinguishing marks. Combinations of marks such as scars and flipper features may be useful for identification in small populations of pinnipeds.

## S U M M A R Y

Pinnipeds are marked most often by tagging, painting, dyeing or hot branding. Paint and dye marks are visible from considerable distances and are often durable enough for short-term studies. Tag loss is a serious issue in pinniped studies, especially in those estimating population size, and loss rates should be calculated for every population under study. Concerns about hot branding have not yet been resolved, although several authors advise that carefully executed hot branding is not likely to have significant effects on the daily life of the animals. Hot branding is often less satisfactory than other marking methods owing to the variability in wound healing and the legibility of the resulting marks. Satellite- and radio-telemetry can provide important information on pinniped behaviour that could not otherwise be obtained. However, the effects of transmitter packages on hydrodynamic drag, behaviour and energy expenditure should be systematically evaluated and carefully considered for each population so marked, in order to balance the use of the method against the value of the information obtained.

New Zealand sea lion (*Phocarctos hookeri*) pups marked with PVC hats glued to the fur for mark/recapture estimates, January 1996.  
 PHOTO: BRUCE DIX, DOC.



Weanling New Zealand fur seal (*Arctocephalus forsteri*) wearing a flipper tag.  
 Taumaka, Open Bay Islands. PHOTO: MALCOLM HADDON, VICTORIA UNIVERSITY.



Brand on adult sea lion.  
 Adult female NZ sea lion (*Phocarctos hookeri*) with satellite transmitter (shoulder), time-depth recorder (mid-back) and VHF transmitter (hip) temporarily glued to the fur. She also has plastic flipper tags as does her pup. PHOTOS: PADRAIG DUIGNAN, MASSEY UNIVERSITY.



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