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IMPLANTED TRANSPONDERS IN PENGUINS: IMPLANTATION, RELIABILITY, AND LONG-TERM EFFECTS

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Abstract.—Subcutaneously implanted transponders have proved to be a reliable means of identifying individual penguins. We found survival of Adélie Penguins (*Pygoscelis adeliae*) carrying transponders over five seasons to be equal to or better than that of birds with flipper bands, although not statistically significant on an annual basis. There were, however, occasional problems associated with the use of implanted transponders. The transponder removed from one bird had developed a slimy biofilm harboring potentially pathogenic organisms incorporated at the time of implantation. If such contamination is common, it is possible that the long-term survival of groups of birds carrying implanted transponders may be lower than that of unmarked populations. Migration of transponders away from the injection site has also been demonstrated and may compromise survival in some individuals. These risk factors could limit the use of implanted identification devices in long-lived or endangered species. Introduction of bacteria can be minimized by careful injection techniques and cleansing of instruments and skin with iodine or alcohol. The choice of a suitable implantation site, such as midway down the back, from which transponders may migrate without impinging upon vital structures is also important. We believe that transponders, when used with care, provide a useful alternative to flipper bands in demographic studies of penguins.

TRANSPONDORES IMPLANTADOS EN PINGUINOS: IMPLANTACIÓN, CONFIABILIDAD, Y EFECTOS A LARGO PLAZO.

Sinopsis.—Transpondores implantados subcutaneamente han demostrado ser una forma confiable para identificar pinguinos individuales. Hallamos la supervivencia de *Pygoscelis adeliae* conteniendo transpondores más de cinco temporadas serigual o mejor que la de aves con bandas en las aletas, aunque no estadisticamente significativo en términos anuales. Sin embargo, hallamos problemas ocasionales asociados al uso de transpondores implantados. El transpondor removido de un ave habia desarrollado una biopelicula babosa que tenia organismos potencialmente patógenos incorporados al momento de la implantación. De esta contaminación ser común, es posible que la supervivencia a largo plazo de grupos de aves cargando transpondores implantados puede ser inferior a la de poblaciones sin marcar. La migración de transpondores lejos del lugar de inyección también se ha demostrado y puede comprometer la supervivencia de algunos individuos. Estos factores de riesgo podrian limitar el uso de arteiactos de identificación implantados en especies de larga vida o en peligro de extinción. La introducción de bacterias se puede minimizar con técnicas de inyección cuidadosa y de la limpieza de los instrumentos y de la piel con yodo o alcohol. La selección de un lugar apropiado para la implantación, tal como a mitad de la espalda, del cual los transpondores pueden migrar sin afectar estructuras vitales es igual de importante. Creemos que los transpondores son una alternativa converiente a las bandas de aletas en estudios demográficos de pinguinos si son utilizados con el cuidado apropiado.

Flipper bands have traditionally been used to mark penguins for long-term study, and manual observation methods employed to detect returning individuals. The detrimental effects of bands are well documented and include mortality during molt (Ainley et al. 1983), physical injury (Sallaberry and Valencia 1985), and increased energy expenditure during swimming (Culik and Wilson 1991). Band loss is also known to occur (Ainley and DeMaster 1980), and returns of some proportion of individuals can be expected to be missed annually (Ainley et al. 1983).

The disadvantages of flipper-banding have led to the search for alternative marking techniques for penguins and other seabirds. The use of implanted identification transponders has become popular in the livestock industry and in zoos over recent years (Behlert and Willms 1992; D. Spielman, pers. comm.). We have developed an automated weighing and identification system for use in our study of Adélie Penguins (*Pygoscelis adeliae*) that incorporates implanted transponders and a data-logging device to identify and record individual birds as they travel in and out of their colony (Kerry et al. 1993). Other researchers have also started to use such identification systems in colonies of Little (*Eudyptula minor*), King (*Aptenodytes patagonicus*), Royal (*Eudyptes schlegeli*), Gentoo (*P. papua*), and Adélie Penguins (A. Chiaradia, pers. comm.; Gendner et al. 1992; Hindell et al. 1996; McCormick et al. 1993; P. Wilson, pers. comm.).

We describe in this paper an assessment of the practical use of implanted transponders in Adélie Penguins and present data on mortality rates and transponder durability on the basis of six years of records from a combined tagging and banding study. We draw attention to the potentially serious effects of biofilms on implants that may harbor and release pathogenic bacteria into the bloodstream. We also document the problems of transponder migration and loss, and discuss possible long-term effects of implants on individuals and populations. We believe it is important that those intending to use implanted transponders as identification markers be alerted to potential problems with these devices.

METHODS

Our study commenced in the austral summer of 1991–1992 when 132 breeding adult Adélie Penguins at Béchervaise Island (67°35S, 62°49E) near Mawson base, eastern Antarctica were implanted with glass-encapsulated electronic transponders produced by TIRIS™ (Texas Instruments, USA). Sixty of these implanted birds were also fitted with stainless steel flipper bands. A further 85 and 116 penguins received transponders in the 1992–1993 and 1993–1994 seasons respectively (Table 1); 40 and 49 of these were also given bands. (Not included in Table 1 are an additional 165 birds that received transponders in a season different to that in which they were first flipper-banded.)

TABLE 1. Multi-year return rates of Adelie Penguins carrying flipper bands and implanted transponders compared to those carrying transponders only. All birds were first banded/implanted as adults and are grouped according to the year in which the band and/or transponder were first applied. Annual differences were not statistically significant. ($\chi^2 > 3.84$, df = 1, $P < 0.05$).

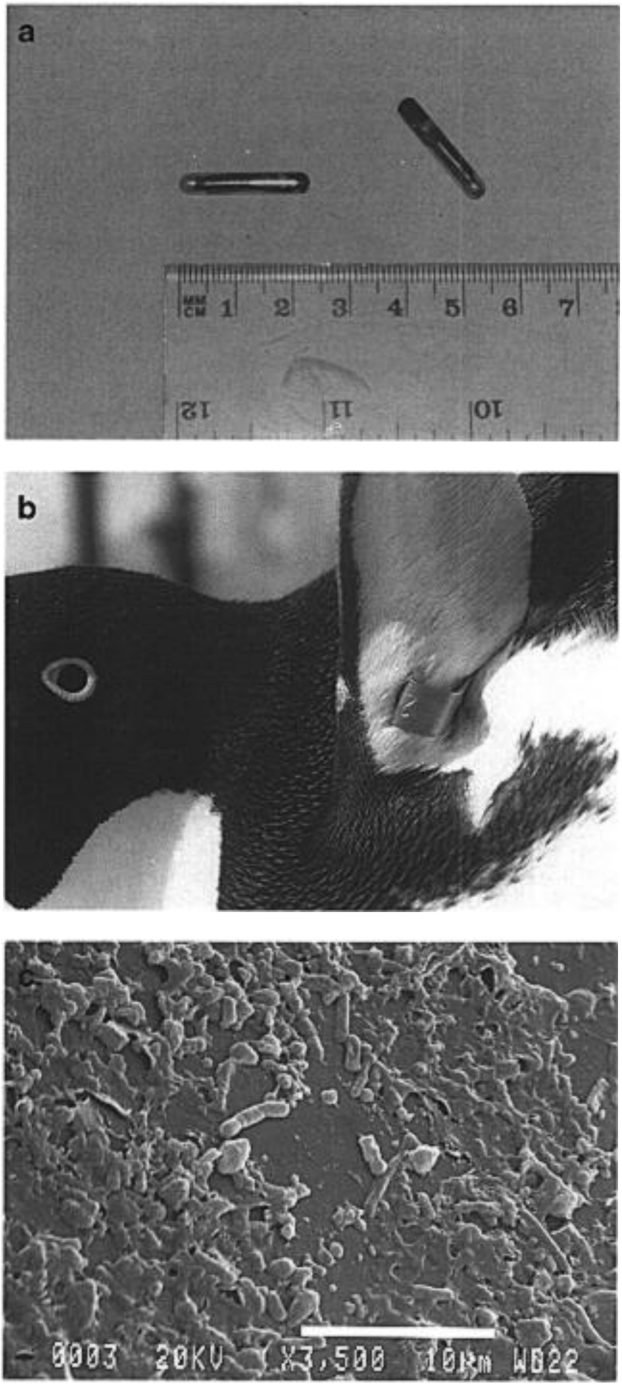
Birds carrying bands and transponders			Birds carrying transponders only			
	N	%		N	%	Difference
Banded and tagged 91–92	60[a]		Tagged 91–92	72*		
return 92–93	45	75%	return 92–93	56	78%	3%
return 93–94	36	80%	return 93–94	49	88%	8%
return 94–95	31	86%	return 94–95	41	84%	−2%
return 95–96	21	68%	return 95–96	32	78%	10%
return 96–97	19	90%	return 96–97	29	91%	0%
Banded and tagged 92–93	40		Tagged 92–93	45		
return 93–94	33	83%	return 93–94	39	87%	4%
return 94–95	27	82%	return 94–95	35	90%	8%
return 95–96	17	63%	return 95–96	31	89%	26%
return 96–97	14	82%	return 96–97	27	87%	5%
Banded and tagged 93–94	49		Tagged 93–94	67		
return 94–95	41	84%	return 94–95	61	91%	7%
return 95–96	27	66%	return 95–96	49	80%	14%
return 96–97	24	89%	return 96–97	44	90%	1%

[a] These numbers do not include birds tagged in a season different to that in which they were banded, and thus differ from those published in Clarke and Kerry (1994).

The transponders implanted in the first two seasons were 30-mm long and 3-mm diameter; after 1992–1993 a shorter version 23-mm long became available (Fig. 1a). Use of transponders without banding has continued from 1994–1995 onwards at Béchervaise Island as well as at nearby Verner Island and at Edmonson Point (74°20S, 165°09E) in the Ross Sea. Chicks have received transponders (but not bands) in all seasons and locations at a rate of up to 300/yr (except in 1994–1995 at Mawson when starvation caused the whole cohort for that year to die; Kerry et al. 1995). Overall, 1142 adults and 1929 chicks received transponders by the end of the 1996–1997 breeding season.

TIRIS™ transponders were chosen because they can be detected from a distance of 0.7 m compared to those of other manufacturers which, although smaller, have a shorter range. Unique identification numbers are transmitted passively from each transponder in response to electromagnetic interrogation from a reader. The TIRIS™ system enables automatic detection of birds passing in and out of a fenced colony through a 1-m wide passage using an antenna connected to a data-logging system (Kerry et al. 1993).

Returns of implanted birds were detected annually by our Automated Penguin Monitoring System (APMS), which ran continuously throughout each breeding season. In addition, the colony was manually checked using binoculars during the incubation period each season for banded birds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that had moved to sub-colonies other than those serviced by the APMS. The majority of birds carrying both transponders and bands came from marked nests; these were checked manually with a hand-held transponder detector to pick up any banded birds whose transponders had failed.

Transponder implantation.—Transponders were implanted subcutaneously at the back of the neck or between the shoulder blades. These locations were chosen for ease of implantation as they have the most loose skin. Transponders were injected using an applicator provided by Texas Instruments. This device takes cartridges of ten sterile transponders individually surrounded by iodine gel and allows injection via a large bore needle without direct handling of the implant. The needle was cleaned with iodine solution (Betadine™) between each application to minimize the opportunities for infection.

Transponders were injected with the needle pointing away from the head and the neck extended. Transponders were massaged well under the skin to prevent them being lost from the injection wound before it healed. After injection the head was allowed to move freely and the wound rechecked to ensure that the transponder had remained properly under the skin. A cyanoacrylate skin glue (Vetbond™) was used as a wound sealant on approximately 90 penguins during 1995 and 1996.

The site of transponder implantation was changed in 1996–1997 to midway down the back subsequent to radiographic investigation of the extent of transponder migration in 20 individuals implanted in previous seasons. Dorso-ventral and lateral radiographs of the neck and thorax of these birds were taken using a portable X-ray machine, and the distance and direction of transponder migration measured.

RESULTS AND DISCUSSION

Types of transponders.—A range of identification transponders is now available from different manufacturers. These vary in size, surface coating, and distance over which they may be read. Our experience as reported here is restricted to the TIRIS™ transponders manufactured by Texas Instruments, USA. These transponders are larger than most, however their size enables them to be read over correspondingly greater distances. Identical transponders are being used in studies of Little Penguins (the smallest of all penguin species) and can be read from outside shallow

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FIGURE 1. a) Electronic identification transponders produced by TIRIS™ (Texas Instruments, USA). These transponders (23 mm long and 3 mm in diameter) were implanted subcutaneously at the back of the neck or between the shoulder blades. Longer (30 mm) transponders were used during the first two years of our study. b) Injury due to a flipper band opening and penetrating the radio-carpal joint of an adult Adélie penguin (after 1 year of wear). The band was subsequently removed and, although the joint remained swollen for several months, the bird returned to the colony to breed in the following two seasons. c) Electron micrograph showing bacteria in the slimy biofilm coating the glass surface of an encapsulated transponder removed from a fledgling.

burrows in sand and through the wood of artificial nest boxes (A. Chiaradia, pers. comm.).

We considered the possibility that the glass encapsulating the electronics of the transponder could implode under the pressure of diving and harm the bird. Adélie Penguins can dive to 175 m (Whitehead 1989) and Emperor Penguins (*A. forsteri*) have been recorded to depths of 534 m (Kooyman and Kooyman 1995). A number of TIRIS™ transponders from different batches were tested to the equivalent of 1000 m depth and showed no damage. We consider these transponders suitable for all penguin species. Other brands of transponders should be pressure tested to such depths before use on deep-diving species.

Transponder loss and long-term function.—Transponder loss in the first 3 wk after injection proved to be a major problem. During the first 2 yr of our study we lost up to 30% of all implants. At that stage we were injecting transponders with the needle pointing towards the head as it was easier to part the feathers in that direction. We discovered that transponders were falling out, assisted by gravity, before the wound healed properly. The problem was exacerbated in thin dehydrated birds during the incubation period (when most of our tagging was carried out). Regular nest checks enabled us to detect transponder losses and we re-tagged all such individuals before the end of each breeding season. We found that if a transponder was retained for 3 wk after tagging then in almost all cases it continued to function into the future.

We now inject transponders with the needle pointing away from the head and take care to massage the transponder well under the skin. We re-check the hole after the bird has retracted its neck to make sure the transponder is not being expelled. Use of 23-mm long transponders instead of the 30 mm ones used in the first two years has also improved the retention rate. However, the receiving antenna on the APMS needed to be more finely tuned to detect reliably these smaller transponders. We now experience a transponder loss rate of only 3–5% and are finding that the use of skin glues lowers this rate further. Only one transponder was lost from the 90 Adélie Penguins on which Vetbond™ skin glue was used during 1995 and 1996, and the same glue has been used successfully as a sealant for injection wounds in Little Penguins (A. Chiaradia, pers. comm.).

Once a transponder has been successfully implanted and retained for 3 wk, it is generally highly reliable. However, we have found some evidence of long-term transponder failure over the years that birds have been carrying these implants to date. Three hundred and fourteen individuals have now been carrying bands and transponders for 3–5 yr. From these individuals, five transponders have failed (after 1–4 winters) and six bands have been lost (after 2–5 yr of wear). Two of the birds whose transponders had failed were radiographed and in neither was the implant visible in the neck or thoracic region. We assume that in these cases the transponders have migrated right out of the bird, especially since our radiographic survey showed that tag migration over distances up to 5 cm was a common occurrence.

Numerous flipper bands have been observed to be partially open after one or more seasons of wear and we believe it likely that the penguins are opening bands with their bills, thus contributing to band loss and increasing the danger of injuries such as that depicted in Figure 1b where the end of the band had penetrated 5 mm into the tissue. We have removed several bands because they had opened to such a degree that injury was present or imminent. Four of the six missing bands were lost during the fifth year of wear, suggesting that the rate of band loss will increase over time as partially opened bands fall off.

Return rates of tagged versus banded adults.—The return rates of tagged and banded adults over the first two seasons of our study were reported in Clarke and Kerry (1994). At that stage we could not demonstrate any differences in survival between the two groups over the short period. Hindell et al. (1996) also found no difference in the survival rates of banded and tagged Royal Penguins over a single winter. We now have six seasons of data and have analysed the annual survival rates over each winter of birds banded and tagged in the first three seasons of our study.

The return rates of birds given transponders alone were greater (78–91%) than those of birds given bands in addition to transponders (63–90%) for almost all seasons following the marking (Table 1). The differences are, however, statistically insignificant due to small sample sizes. We assume that all birds that fail to return are dead because breeders rarely emigrate to other colonies or miss a season, our detection rate of tagged birds using the APMS is extremely high, we manually check all unfenced sub-colonies rigorously, and our tag loss rate is less than 3%.

The apparent mortality rate of birds with bands was particularly high over the 1995 winter for all three groups (Table 1). This winter followed a summer of severe food shortage (Kerry et al. 1995), and our results suggest that, when prey is scarce, the extra energy required to swim with a flipper band (quantified by Culik and Wilson, 1991) may actually compromise the survival of such birds. There is so far no evidence that implanted transponders increase mortality compared to bands; in fact the long-term survival of tagged birds may overall be better.

Chick survival and return rates.—Five hundred and thirty-nine chicks were implanted with transponders between 1992 and 1994. Forty-two percent of these have subsequently returned to the colony, the majority first appearing as 3-yr-olds. The percentages of each cohort known to have survived their first two winters ranged from 38–45%, and 32–40% of each cohort are known to have reached at least 3 yr of age (Table 2a). One hundred and seventy-two 3–5-yr-olds returned to the colony in 1996–1997, and APMS records indicated that some of these were attempting to breed. The survivorship of the three cohorts of chicks to 2, 3 and 4 yr of age is greater than that determined by Ainley et al. (1983) using banded populations of fledglings. In fact it is also greater than the rate these authors predicted for unbanded fledglings.

TABLE 2. Survival and age-specific return rates of chicks carrying implanted transponders. a) Percentages of each cohort known to survive to minimum ages of 2–5 yrs. b) Return rates of pooled cohorts of 2-, 3- and 4-yr-olds.

a) Chick survival			1992	1993	1994
Number of chicks tagged:			34	244	261
% chicks surviving to age:	2 yr		44%	38%	45%
	3 yr		32%	37%	40%
	4 yr		21%	25%	
	5 yr		12%		
b) Chick return rates		**N**	**%**		
2-yr-olds		48			
Return as 3-yr-olds		30	63%	(3 cohorts pooled)	
3-yr-olds		76			
Return as 4-yr-olds		44	58%	(2 cohorts pooled)	
4-yr-olds		5			
Return as 5-yr-olds		2	40%	(1 cohort)	

The age-specific annual return rates of 2–4-yr-olds have ranged from 40–63% (Table 2b), averaging 59%. These figures are lower than those of banded and/or tagged adults (Table 1), and it will be of interest to see if return rates improve once the birds are fully mature. The rate of transponder loss in chicks is unknown; however, there is no reason to expect it to be different to that in adults.

Overall, our results indicate that the use of transponders is a good alternative to banding for short to medium term demographic studies at least, providing that visual observation is not necessary for the detection of tagged individuals.

Transponder migration.—A major problem with implanted transponders is the potential for migration away from the original injection site. Implant migration may or may not cause problems depending on where the device ends up. Larger transponders and those coated with synthetic plastics have been shown to be more likely to migrate than small glass implants (Behlert and Willms 1992).

The transponders that we use are fairly large (in order to be detected from a distance) but are encapsulated in glass. In contrast to Behlert and Willms (1992) suggestion that glass implants are unlikely to move, we have noticed that some proportion of transponders retrieved from chicks killed by skuas have migrated away from the original site of injection. In most cases the transponders have only migrated slightly, usually to one side or the other of the neck. However, in two chicks transponders were found alongside the trachea and esophagus.

Transponder migration over distances of 1–5 cm was demonstrated in 13 of 20 previously implanted adults examined radiographically in 1996–1997. Five birds showed no transponder movement, and in two individuals the transponder no longer existed. Most transponders had moved either laterally or caudoventrally, several into the thoracic cavity. While it is possible that such transponders may never cause damage to surrounding organs, there is some danger that they may obstruct blood flow or damage nerves in the region.

Transponder migration is a cause for concern, despite the fact that the return rates of implanted birds compare favorably to those of banded individuals, and that the birds in which migration has occurred appear healthy. Implantation of transponders farther down the back may reduce the chances of migration into the throat and thoracic cavity. The transponders we implanted into adults and chicks during 1996–1997 were injected midway down the back. Subsequent radiography of four individuals showed only slight lateral migration after two months, and no tendency of transponders to move into body cavities.

Wound infection and long-term effects of implants.—Localized wound infection was not a problem in the days following transponder implantation in either adults or chicks. However, it is possible that minor discharge contributed to the early loss of transponders in some birds during the first fortnight post-implantation. The use of Betadine™ to clean the injector needle and the presence of iodine gel surrounding each transponder help minimize bacterial contamination.

Significant bacterial growth can occur, however, within the biofilms surrounding implants in humans and animals over the long term (Costerton and Lappin-Scott 1989, Deighton and Balkau 1990, Vaudaux et al. 1994). Most implant-related infections described in the medical field are associated with devices made from metal and plastic, the surfaces of which tend to erode and facilitate bacterial growth (Deighton and Balkau 1990). Glass is immunologically neutral and less subject to pitting, but may chemically interact with surrounding tissues to form a stabilising capsule (Behlert and Willms 1992).

Bacteria associated with implants tend to remain localized, either attached to the implant or encapsulated with it. Occasionally, however, they become detached and disseminate via the bloodstream. The species of bacteria found in biofilms tend to be those normally present on the skin (commonly *Staphylococcus epidermidis* and less frequently *S. aureus*) which, although non-pathogenic in their normal environments, can become virulent when attached to surfaces of implanted materials (Deighton and Borland 1993, Vaudaux et al. 1994).

We have not seen any evidence of chronic localized or systemic infection attributable directly to transponder implants. However, such affected birds may be more likely to die at sea than in the colony. Our annual survivorship rates also give us no cause for concern. Although we have not killed any adult birds to check the status of their implants, we have dissected a number of tagged fledglings killed by skuas to investigate the tissue reaction around their transponders. In most cases we have observed very little tissue reaction ranging from no macroscopic change to encapsulation in thin fibrous collagen layers. These changes correlate well with those reported by, for example, Behlert and Willms (1992) for zoo animals carrying implanted glass transponders.

A notable exception was one implanted transponder removed from a nearly fledged chick which died of causes unrelated to implantation. The transponder was found to be encapsulated and surrounded by a thick layer of purulent material. A heavy biofilm had developed on the glass surface of the transponder (Fig. 1c). This material contained both cocci and rods, the latter of which appeared to be actively dividing. Culture of the purulent contents of the lesion under both aerobic and anaerobic conditions showed the presence of *Clostridium perfringens, C. sordellii* and some unidentified *Bacillus* species. No coccoid organisms could be cultured; presumably the long frozen-storage time between death of the bird and culture of the lesion had allowed only spore-forming species to survive.

We do not know yet what long-term effects may result from transponder implantation. Our population of Adélie Penguins could normally be expected to live up to 20 yr (Ainley et al. 1983). If biofilm formation and persistent bacterial infections are common then we may find that we are actually shortening the life expectancy of our birds to some degree. The level of such an effect may be difficult to determine, especially as we have only banded birds available for comparison and these are also likely to be adversely affected by their identification markers.

Further research is required to quantify and reduce the incidence and effects of both biofilm development and transponder migration, especially as such risk factors may limit the use of implanted transponders in endangered or long-lived species. Although transponder migration may be difficult to prevent, introduction of bacteria into the implantation site can be minimised. Cleansing of the skin at the injection site with 70% alcohol will help prevent incorporation of micro-organisms into the capsule around the implant, as will disinfection of the implantation device between individuals. In the case of the fledgling described earlier, the transponder had been unintentionally implanted within muscle tissue, a factor which may have exacerbated the tissue reaction. Glass-coated implants are likely to be safer than those with plastic surfaces, and care should be taker to ensure that each transponder is positioned subcutaneously in a location from which it is unlikely to migrate towards vital organs.

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