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ENERGY STUDIES OF FREE-LIVING SEABIRDS: DO INJECTIONS OF DOUBLY-LABELED WATER AFFECT GENTOO PENGUIN BEHAVIOR?

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Abstract.—Fourteen Gentoo Penguins *Pygoscelis papua* breeding at Ardley Island, Antarctica, were fitted with data loggers which recorded swim speed, dive depth and bird heading at 10- or 15-s intervals. Ten of these birds were injected with 5 ml doubly labeled water in the pectoralis, seven individuals receiving the full dose in one site whereas in three individuals the dose was spread out over four sites. Four birds were not injected. None of the injected birds went to sea for longer than 4 min within 12 h of their treatment whereas all non-injected birds did so. Once-injected birds swam slower and dives were of shorter duration than non-injected or four-times-injected birds. In general, once-injected birds differed more in their foraging parameters (dive depths, dive angles and foraging ranges) from the non-injected birds than did the four-times-injected birds. It is suggested that the relatively large volume of liquid injected intramuscularly causes discomfort which lasts for at least 2 d, which dissuades birds from engaging in normal foraging behavior. This problem may be alleviated by multiple small intramuscular injections or intra-peritoneal injections.

ESTUDIO ENERGÉTICO DE AVES MARINAS LIBRES: ¿PUEDEN LAS INYECCIONES DE AGUA MARCADA AFECTAR LA CONDUCTA DE PINGÜINOS?

Sinopsis.—Catorce individuos del pingüino *Pygoscelis papua* de la población reproductive de la isla Ardley, fueron equipados con equipo de muestreo para poder tomar información sobre su velocidad al nadar, profundidad de sus sumergidas y la dirección de los individuos a intervalos de 10 15 segundos. Diez de los pingüinos fueron inyectados con 5 cc de agua marcada en el músculo pectoral. Siete de los individuos recibieron toda la dosis en una sola localidad y tres en cuatro partes diferentes del músculo. Cuatro aves no fueron inyectadas (control). Ninguna de las aves inyectadas fue al mar por más de cuatro minutos durante las primeras 12 horas de haber sido inyectadas, contrario a la conducta de las aves control. Las aves inyectadas en una sola localidad nadaron más lentamente, y las sumergidas fueron de menor duración que las aves que fueron inyectadas en cuatro localidades y las del grupo control. En general las aves que se inyectaron en una sola localidad se diferenciaron más en sus patrones de forrajeo que los otros dos grupos de aves. Se sugiere que el alto volumen de líquido inyectado a las aves en una sola localidad causó molestias, que duró al menos dos días, lo que disuadió a las aves de envolverse en conducta normal. El experimento tiende a indicar que el problema muy bien podría aliviarse inyectando pequeñas cantidades en diferentes localidades o de forma intraperitoneal.

Energy expenditure of free-living animals can be determined by using the doubly-labeled water (DLW) technique (Nagy 1987, 1989). DLW is injected into the study animal and concentrations of isotopically labeled hydrogen and oxygen are measured before and after field activity and the amount of water used in metabolic processes quantified (Lifson and McClintock 1966, Nagy 1980). Such measures provide an estimate of total energy expenditure over a specific period. By combining field metabolic rates, derived from DLW studies, with information on the normal activities of the study species, some researchers have attempted to define costs of specific activities (e.g., Chappel et al. 1993, Culik and Wilson 1992).

The activities of many species, particularly seabirds, were considered difficult to determine until recent technological advances made it possible to equip free-living animals with devices that transmit or store data on behavior (e.g., Kooyman et al. 1992). Considerable research has shown that results obtained from such units should, however, be interpreted with caution because foraging ecology of instrumented animals may be different from that of non-instrumented conspecifics (e.g., Croll et al. 1991, Wilson and Culik 1992).

Free-living, non-instrumented animals injected with DLW are generally considered to behave normally. To our knowledge, however, no one has examined possible deleterious effects of DLW on free-living study animals. Such knowledge is essential if measured field metabolic rates are to be considered realistic. It is also important to test studies that combine activity budgets determined using externally-attached devices with energy expenditure derived from DLW.

Recently, we conducted a study on the foraging ecology of Gentoo Penguins (*Pygoscelis papua*) at Ardley Island (62°13′S, 58°55′W), South Shetlands, Antarctica. Activity of all birds was determined by attached data loggers and some individuals were additionally injected with DLW. We were thus able to examine the effects that injection of DLW had on activity.

METHODS

Between 4 and 19 Jan. 1992, 10 Gentoo Penguins (mean mass 5.63 kg, SD 0.52) tending small chicks were injected in the pectoralis muscle with 5 ml of isotope-enriched water (Nagy et al. 1984, cf. Chappel et al. 1993). In seven cases all 5 ml was injected in one spot (once-injected birds), whereas in three birds the 5 ml was injected in equal quantities in four different localities within the pectoralis (four-times-injected birds). This method was used because it was considered that such a large volume of liquid in one locality might cause some distension of surrounding muscle fibres resulting in pain. Birds were injected at the nest as they brooded chicks. They were then watched for 3 h before being removed from the nest and a blood sample taken from a vein in the foot. These birds, and four other individuals (to be used as controls) also brooding chicks, were then instrumented with multi-channel data loggers (mass 0.19 kg) (Driesen and Kern GmbH, Am Hasselt 25, D-24576 Bad Bramsted, Germany), which, among other things, recorded swim speed, dive depth and bird heading at intervals of 10 or 15 s (Wilson et al. 1993). The devices had been shaped following extensive tests in wind tunnels and a water flume so as to minimize drag (see Bannasch et al. 1994). Tests performed at the time of the field work with birds in a swim channel showed that increases in drag due to the device did not exceed 10% at normal swim speeds (Culik et al. 1994). All devices were attached by taping them (Wilson and Wilson 1989) to feathers in the dorsal mid-line near the tail. Following instrumentation and blood sampling, which took approximately 10 min, birds were replaced back at the nest where they all continued brooding their chicks. Nests containing study birds were then surveyed either continuously, using a remote-controlled video camera, or every 3 h by an observer, except at night where darkness and fog sometimes made it impossible to check the nest properly for up to 8 h. Additionally, when birds were absent, adjacent beaches were patrolled to ascertain whether the penguins were at sea or resting on land. The birds were recaptured after periods varying between 24 and 44 h when the birds were considered to have been to sea at least once to forage. Data loggers were removed and a second blood sample taken before the birds were released at the nest site again.

RESULTS

There was no apparent difference in colony attendance among the study groups, with generally one or two absences from the colony occurring every 24 h. No bird that had been injected with DLW went to sea for longer than 4 min within 12 h of injection, however, whereas all non-injected birds did so (Fig. 1).

Swim speeds of once-injected birds were considerably lower than those of either four-times-injected or control birds (Kruskal-Wallis test where individuals, not trips, were used as replicates; $H = 40.1$, df = 2, $P < 0.001$) (Table 1). Mean foraging ranges, deduced by vectorial analysis of swim speed, dive depth and swim direction (Wilson et al. 1991, 1993), were 2.8, 3.2 and 6.2 km for the once-, four-times- and uninjected birds, respectively (Fig. 2). Dive duration of once-injected birds was also significantly shorter than four-times-injected or control birds ($H = 21.0$, df = 2, $P < 0.001$) although the time spent at the deepest part of the dive (bottom time) did not differ significantly among groups ($P > 0.05$). Other measured foraging parameters (dive depths, dive angles and return-to-surface angles) were significantly different between groups ($P < 0.05$) (Table 1).

DISCUSSION

The effects of the injection are not manifest in the rhythm adhered to by the penguins when on land. When partners arrived to replace their injected mates, the replaced birds left the colony in normal fashion. Thus, conclusions based on observations made at the colonies alone are inadequate to document potential effects of DLW. The reluctance of Gentoo Penguins to swim after injection, and the reduced swim speeds, foraging range and dive duration in the once-injected birds compared to the other two groups indicate, however, that intra-muscular injection of DLW in one spot leads to serious alteration of normal behavior. If, on the basis of observations made on land, such birds are then considered to forage normally and the field metabolic rate interpreted accordingly, calculated activity-specific and daily metabolic rates (e.g., Culik and Wilson 1992) will be wrong. Nominally, activity-specific metabolic rate, calculated by regressing time engaged in a specific activity against energy expenditure (e.g., Birt-Friesen et al. 1989), should not be affected by injection-induced

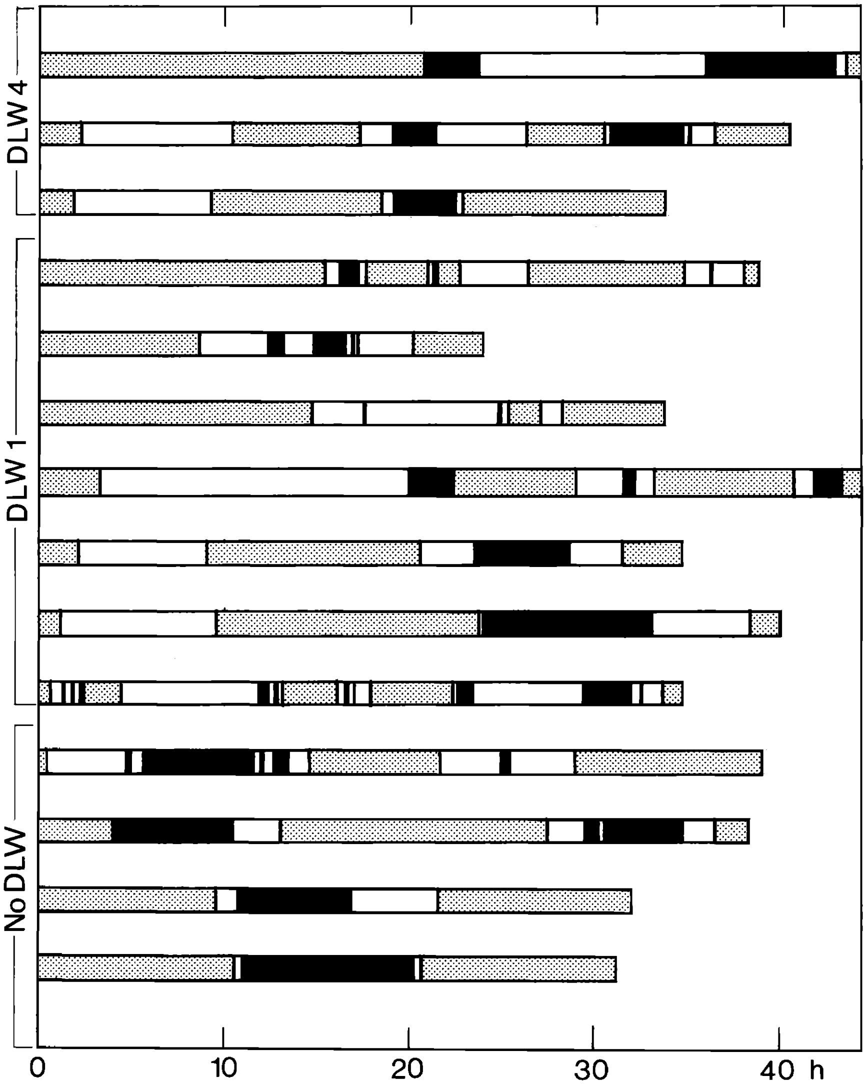

FIGURE 1. Activity of breeding Gentoo Penguins wearing loggers in relation to presence and pattern of doubly-labeled water injection. Each horizontal bar shows the activity of an individual bird as a function of time since being fitted with a logger. Black sections indicate when birds were at sea and associated stippled bars indicate periods when birds were visible in the colonies. Empty bars indicate periods when birds were not in the water and not visible in the colonies. The NO DLW section indicates birds that were uninjected while DLW1 and DLW4 indicate birds that were injected with doubly-labeled water once and four times, respectively.

TABLE 1. Changes in foraging parameters of Gentoo Penguins according to whether the birds were injected with doubly-labeled water in one spot (DLW1) or four spots (DLW4) in the pectoralis and compared to non-injected birds (NO DLW). Variables are presented as percent per category. Sample sizes (*n*) are numbers of birds in each category.

	Treatment		
Swim speed (m/s)	NO DLW (n = 4)	DLW1 (n = 7)	DLW4 (n = 3)
0–1	11.6	6.2	0.3
1–2	25.3	54.8	9.5
2–3	60.5	12.9	71.7
3–4	2.0	14.6	17.4
4–5	0.6	11.5	1.1
No. events	679	470	337
Dive duration (s)			
0–40	6.3	3.3	0.0
40–80	21.5	33.1	14.4
80–120	28.2	38.4	18.2
120–160	40.4	20.5	19.5
>160	3.6	4.7	47.9
No. events	731	301	312
Bottom duration (s)			
0–20	21.5	34.9	29.5
20–40	18.5	26.6	9.6
40–60	27.4	21.6	18.9
60–80	31.3	12.9	34.6
>80	1.3	4.0	7.4
No. events	731	301	312
Maximum depth (m)			
0–20	19.7	25.6	16.3
20–40	40.5	35.8	18.5
40–60	20.8	27.2	38.7
60–80	6.3	3.6	15.3
>80	12.7	7.8	11.2
No. events	699	301	312
Dive angle (°)			
0–10	21.5	30.4	46.3
10–20	8.5	10.1	15.7
20–30	26.7	25.7	19.3
30–40	28.5	13.1	10.0
>40	14.8	20.7	8.7
No. events	632	237	311
Surface angle (°)			
0–10	29.8	37.0	65.3
10–20	31.1	32.7	18.3
20–30	24.2	14.0	11.2
30–40	11.3	8.2	5.1
>40	3.6	8.1	0.1
No. events	603	257	311

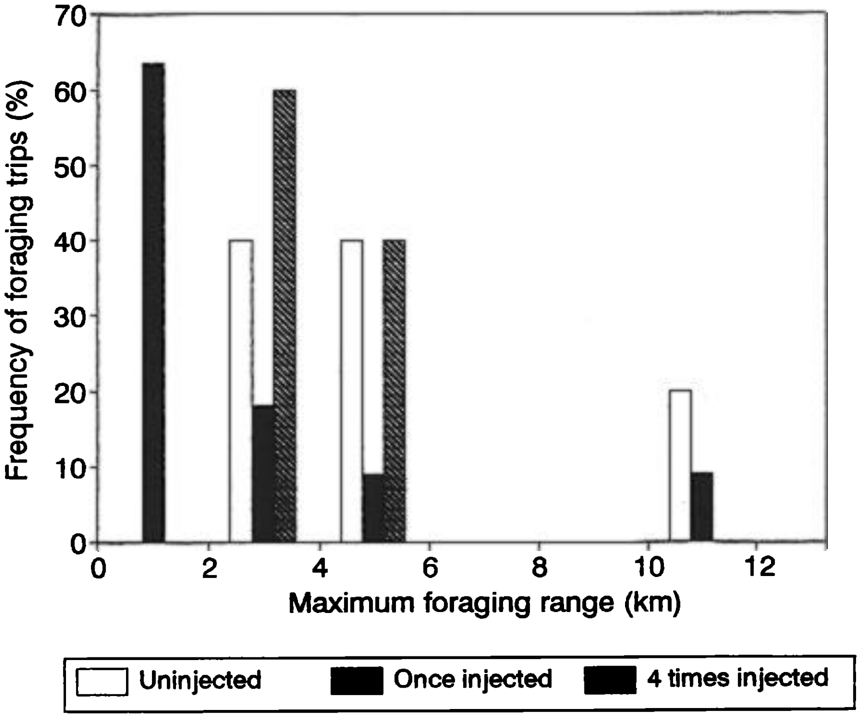

FIGURE 2. Frequency of maximum foraging ranges per foraging trip for Gentoo Penguins breeding at Ardley Island, Antarctica. The data for the once-injected birds are derived from seven individuals and 11 foraging trips, data for four-times-injected birds derived from three individuals over five foraging trips and data from non-injected birds derived from four individuals over five foraging trips.

behavioral anomalies. This analysis assumes, however, that injected animals physically locomote in exactly the same way as non-injected animals. This is clearly not the case in Gentoo Penguins, as evidenced by the differences in swim speeds between injected and control groups. Here, calculation of activity specific metabolic rate will be correct for the study birds, but will not be applicable to normal penguins.

The difference in foraging behavior between the once-injected birds and the four-times-injected birds suggests that disturbance results directly from the quantity of liquid injected per locality in the muscle. Discussions with various medics at the University Hospital in Kiel has revealed that, in humans at least, intra-muscular injections can be painful, even when quantities as little as 3 ml are injected. Intra-muscular doses of more than 5 ml are rarely, if ever, given to human adults, and babies (<5 kg, and therefore comparable in mass to Gentoo Penguins) are rarely given more than 1 ml. Where larger doses are unavoidable, products are usually given in the form of an intravenous drip. The discomfort experienced during intra-muscular injections is apparently attributable to the volume of liquid, which pushes the muscle fibres apart. When this is extreme, cell damage can occur which leads to the release of potassium, which produces pain. In such cases, the pain may continue for many hours, even after the injected product has been removed from the site, being particularly apparent when the muscle is contracted. For humans, an intramuscular injection of 5 ml is unacceptably large for a 5-kg baby and the same thing is likely to be true for Gentoo Penguins. In view of this it is little surprizing that birds injected in the pectoralis did little swimming and diving.

Our observations on Gentoo Penguins were carried out over approximately 2 d and as time progressed more normal behavior became apparent (e.g., Fig. 1). There is presumably some point at which no more muscle pain occurs when birds might be expected to behave normally. If this occurs while concentrations of DLW in the body are still high enough for metabolic studies to be carried out, researchers could wait for a period after the initial injection before attempting to calculate field metabolic rates. This solution is, however, far from ideal because it is still associated with pain in the initial stages. Where intra-muscular injection is unavoidable, we suggest injecting small amounts of DLW a large number of times, and preferably in a muscle that is not critical for locomotion. If practical, intra-peritoneal (e.g., Davis et al. 1989) or intravenous injection should be used, although both are not as easy to carry out as intra-muscular injections. Intra-peritoneal injections require that the researcher correctly locate the peritoneal cavity, whereas intra-venous injections can be problematic in penguins because these birds can reduce blood flow to extremities which leads to vein collapse.

The DLW technique has proved invaluable for the quantification of field metabolic rates, but in cases where labeled animals have been absent it now appears necessary to determine whether these animals are behaving in a similar manner to non-injected conspecifics. Tests using telemetric and logging units carried by the animals now make this determination possible.

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