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HYPERTHERMIA IN CAPTURED IMPALA (*AEPYCEROS MELAMPUS*): A FRIGHT NOT FLIGHT RESPONSE

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ABSTRACT: To investigate the patterns and mechanisms of capture-induced hyperthermia, we surgically implanted 26 impala (*Aepyceros melampus*) with miniature thermometric data loggers, which measured body temperatures continuously throughout capture procedures. Four groups of impala, which were habituated to varying levels of handling and boma-housing, were captured by net restraint or by chemical immobilization. The study took place between July 1999 and December 2005. Irrespective of whether impala were chemically captured, net-captured, or disturbed by exposure to a stressor, they developed a precipitous increase in body temperature. This increase in body temperature was not related to activity levels; animals that had low activity levels before immobilization had larger increases in body temperature compared to those that had high activity levels but were not immobilized ($t=3.6$, $P=0.001$, $n=5$). Similarly this increase in body temperature was not related to environmental heat load at the time of darting and immobilization ($r=-0.05$, $P=0.85$). Body temperature increase also did not depend on whether the animals were captured using drugs or not. However, we found that those animals that were habituated more to handling and boma-housing had smaller increases in body temperatures ($F=37$, $P<0.001$) and smaller stress responses, indicated by lower plasma cortisol concentrations ($F=5.5$, $P<0.05$), and less fractious behavior, compared to those animals that were habituated less or not at all. Therefore we believe that capture-induced hyperthermia in impala is caused predominantly by stress, which induces a rapid rise in body temperature.

Key words: Body temperature, capture, cortisol, habituation, opioid, stress.

INTRODUCTION

Wild animals typically develop an acute hyperthermia when captured (Hofmeyr et al., 1973; Gericke et al., 1978; Cheney and Hattingh, 1987; Kock et al., 1987a, b; Martucci et al., 1992; Montané et al., 2003, 2007). This hyperthermia is not accompanied by the rigor or other correlates of malignant hyperthermia or Porcine Stress Syndrome (Mitchell and Heffron, 1980, 1982), and currently there is no evidence for any peripheral metabolic derangement. Because it has been proposed that the hyperthermia may play a role in capture myopathy and acute death during capture (Gericke et al., 1978; Cheney and Hattingh, 1987; Antognini et al., 1996; Meltzer and Kock, 2006), measures usually are taken to decrease the incidence and severity of capture-induced hyperthermia. In the absence of a proper understanding of the mechanisms of capture-induced hyperthermia, these measures are based on anecdotal evidence, and on unproven assumptions that hot ambient temperatures and exercise during capture are the main causes of this hyperthermia.

Most records of capture-induced hyperthermia are based on single measurements of rectal temperature. To better understand the causes of capture-induced hyperthermia, it is essential to measure body temperatures continuously before, during, and after capture procedures. Continuous and accurate measurement of body temperature is possible through surgical implantation of miniature temperature-sensitive data loggers into the abdominal cavity. The loggers, which measure and record temperature at specified intervals, have been used successfully to examine thermoregulation in a variety of wild antelope species (Mitchell et al., 2002). Although capture-induced hyperthermia is evident in records of body temperature obtained from abdominal loggers in antelope (Jessen et al.,

1994; Fuller et al., 2005), no study has used the technology available for continuous measurements of body temperature to investigate systematically the cause or severity of capture-induced hyperthermia.

We used temperature-sensitive data loggers to measure continuously body temperature during different capture conditions. We hypothesized that the greater the reaction of an animal to the procedures leading to and including its capture, the greater would be the severity of hyperthermia it develops. We assumed that the reaction of the animal would be influenced by its activity level in attempting to escape capture, by the time between initial intervention and completion of capture, and by the familiarity of the animal with human activities. To vary the activity levels and capture times to test our hypothesis we primarily used chemical immobilization and employed immobilization regimens with increasing sedative efficacy, starting with a low dose of a conventional immobilizing opioid and ending with a higher dose of the same opioid plus a tranquilizing adjuvant. In a few animals we measured the consequences of capture without sedation. As we increased sedative efficacy of the immobilizing agents, we also increased the familiarity of the animals with human interference, by handling the animals and increasing the duration of boma-housing of the animals before our trials started. To investigate whether environmental temperatures play a role in capture-induced hyperthermia, we monitored the microclimate conditions during the trials. We chose impala (*Aepyceros melampus*) as our experimental animals because they are known to be highly excitable and reactive to capture and confinement (Knox and Hattingh, 1992). We found that, in impala at least, the major cause of acute capture-induced hyperthermia is related to stress rather than to the thermal effects of chemical immobilization, environmental heat load, or activity levels during capture.

MATERIALS AND METHODS

The results reported emanate from three studies conducted with the primary aim of investigating hyperthermia in captured impala, and the secondary aim of determining the effects of different capture drugs and catecholamine blockage on body temperature in chemically immobilized impala. The effects of the different capture drugs and catecholamine blockage on body temperature are not reported here, but where we report the effects of chemical immobilization on body temperature we focus on the immobilizing drug etorphine and the tranquilizer azaperone. The procedures were approved by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance numbers 1999/90/05, 2001/78/05, 2004/11/05). All studies took place between July 1999 and December 2005 at the Lichtenburg Game Breeding Centre of the South African National Zoological Gardens (26°07′S, 26°07′E), 220 km west of Johannesburg, South Africa.

Animals and surgery

Twenty-six adult female impala were used. The animals were caught from the wild and transported to bomas (holding pens with high wooden pole walls) no less than 2 wk before they underwent surgery. On the day of surgery they were herded into a game capture vehicle or crate where they received a tranquilizer (zuclopenthixol acetate, 50 mg, Clopixol-Acuphase, Lundbeck, Johannesburg, South Africa; or azaperone, 40 mg, Stresnil, Janssen Pharmaceutica, Johannesburg, South Africa; or haloperidol, 15 mg, Kyron Laboratories, Johannesburg, South Africa), intramuscularly (IM) via a pole syringe. Once tranquil, they were captured individually by hand and anesthetized with halothane (Fluothane, Astra Zeneca, Johannesburg, South Africa) in 100% oxygen via a face mask. Once the animal was anesthetized, a 200×100 mm midline area on the ventral abdomen was shaved and sterilized with a mixture of 5% chlorhexidine gluconate (Hibitane, Astra Zeneca, Johannesburg, South Africa) in 100% ethanol. Within this area, a 50 mm midline incision was made through the skin and *linea alba*, and a miniature temperature-sensitive data logger was placed into the abdominal cavity, where it could float freely. The *linea alba* and skin were sutured closed. The surgical wound was coated with a topical tick repellent (Tickgrease, Cypermethrin 0.025% m/m, Bayer Animal Health Pty, Isando, South Africa) and sprayed with a topical antiseptic spray (Necrospray, Centaur

Labs, Johannesburg, South Africa) to prevent infection, fly worry, and myiasis. Each of the impala received a long-acting penicillin-based antibiotic (4–5 ml IM, Peni LA Phenix, Virbac Animal Health, Johannesburg, South Africa), an analgesic and anti-inflammatory medication containing 140 mg/ml ramiphenazone, 70 mg/ml sodium phenylbutazone, and 0.5 mg/ml dexamethazone (4–5 ml IM, Dexa-Tomanol, Centaur Labs, Johannesburg, South Africa), and a long-acting parasiticide, doramectin (5 mg subcutaneously [SC], Dectomax, Pfizer Laboratories, Johannesburg, South Africa). Animals were marked with different colored plastic ear tags for identification.

At the end of the studies, the impala were recaught, and the data loggers were removed using a similar anesthetic and surgical procedure to that used for implantation. After surgery the impala were released back into a large camp where they ranged free.

Body temperature measurements

Temperature was measured at 10-min intervals in the abdominal cavity of the impala with miniature temperature-sensitive data loggers (StowAway XTI, Onset Computer Corporation, Pocasset, Massachusetts, USA). These data loggers had a measurement range of +34 C to +46 C, to a resolution of 0.04 C. The loggers had a mass of ~40 g, and dimensions of ~50×45×20 mm when covered in an inert wax (Sasol wax EXP986, Johannesburg, South Africa). Before implantation the loggers were calibrated individually, in an insulated water bath, against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) and were found to have an accuracy of better than 0.05 C.

Darting procedure

Impala were darted with either a Telinject air rifle (VARIO 3R, Telinject, California, USA) and Dan-Inject darts (S300 dart with 1.5×30 mm collared needle, Dan-Inject, Børkop, Denmark) or a Sabi 500 dart gun (SABI Werkswinkel t/a Magnum Arms, Nelspruit, South Africa) and a Pneu-Dart dart (Type P, 3 cc, 13 gauge, 25 mm long wire barbed needle, Pneu-Dart, Williamsport, Pennsylvania, USA). All the darts were positioned in the gluteus muscle group. Once the animal was recumbent it was blindfolded and cotton wool was placed in its ears to reduce external sensory stimuli, as is common practice in professional game capture procedures.

Naïve animals

Six nonhabituated (naïve) impala were used to examine body temperature responses to opioid immobilization and net capture (Table 1). After the impala had been implanted with the data loggers, they were released into a fenced 62-ha camp where they ranged freely. On two occasions, separated by two weeks, the impala were herded into a smaller enclosure (0.25 ha) within the 62-ha camp. After 24 hr, in a random order, the impala were darted with either 0.5 mg etorphine hydrochloride IM (M99, Novartis, Johannesburg, South Africa), or with 10–12 mg fentanyl citrate IM (Kyron Laboratories, Johannesburg, South Africa). Three animals were darted in short succession (<3 min in total). The other three animals in the group, which were not darted, remained in the group and were exposed to the presence of humans in the enclosure and to the darting of their companions. They were darted approximately 1 hr after the first three

TABLE 1. Summary of differences in immobilization regimens, activity levels, and habituation between groups.

Group	Opioid	Dose (mg)	Tranquilizer	Dose (mg)	Activity levels[a]	Habituation	
						Boma-housing (mo)	Handling
Naïve $n=6$	Etorphine	0.5	None		+++	None	None
Four month $n=5$	Etorphine	1.0	Azaperone	40	++	4	4×hand caught +tranquilized[b]
Two month $n=9$	Etorphine	1.5	Azaperone	40	+	2	1×immobilization[c]
Five month $n=6$	Etorphine	1.5	Azaperone	40	+	5	2×immobilization[c]

[a] Activity levels from darting up until recumbency. +++ = high-intensity activity; ++ = medium-intensity activity; + = low-intensity activity.

[b] Each time these animals were handled, they were tranquilized with the long-acting tranquilizer haloperidol.

[c] The animals were immobilized with etorphine to be weighed.

animals had been darted. Once an animal was recumbent a 10 ml venous blood sample was drawn. The animals remained recumbent for 20 min, in a quiet part of the enclosure, after which we reversed the immobilizing effects of the opioids using 1 mg diprenorphine hydrochloride intravenously (IV) (M5050, Novartis) for the animals that received etorphine, and 20 mg nalorphine hydrochloride IV (Kyron Laboratories) for those that received fentanyl. Once fully conscious the first three impala were released back into the enclosure to join their companions, and only after the second three impala were reversed were all the animals released back into the 62-ha camp. The time from darting to the opioid reversal, and release of the animals, was approximately 35 min. On one occasion, two of these impala were chased by a vehicle into capture nets, where they were restrained, blindfolded, and cotton wool was placed in their ears for 15 min before they were released back into the 62-ha camp.

Four-month habituated animals

Five impala were used in a study with a secondary aim of investigating the effects of catecholamine blockade after chemical capture (results reported from control animals only; see below). The impala had been housed in a 30×50 m boma for 4 mo before the experiments. During this 4-mo period, the impala became accustomed and adapted (habituated) to the presence of humans, who provided fodder (lucerne) and cleaned the bomas. On four occasions (Table 1) during the 4-mo period, these animals had been herded into a game capture vehicle, where they were handled, physically restrained, and then injected with the long-acting tranquilizer haloperidol (5–20 mg, according to mass, Kyron Laboratories), and transported to test for the effects of transport on body temperatures (see Wimberger, 2005).

On our experimental days, we herded the group of impala into an adjacent 10×20 m boma, to ensure reliable darting from a closer range. Each impala in the group was darted on two occasions, separated by 2 wk, with 1 mg etorphine and 40 mg azaperone. Once an impala had been immobilized, it was removed from the group and placed in an adjacent boma where it received a bolus IV injection of a catecholamine blocker or a control (2 ml dimethyl sulphoxide, Merck Chemicals, Gauteng, South Africa). After 20 min, we reversed the effects of etorphine using 2 mg diprenorphine hydrochloride and returned the impala to the rest of the group. The time from darting to reversal, and release of the animals, was approximately 30 min. The human activity in the boma, from the time of darting to removal of the immobilized animal, caused agitation and attempted escape in the other impala, even though, at the time, there was no direct interference with them. We did not collect blood samples from these animals because they had received catecholamine blockers, which we expected would alter the normal plasma cortisol changes (Liu et al., 1991).

Two- and 5-mo habituated animals

Fifteen impala were used at different times over a 2-yr period. The animals were housed, in 5×10 m bomas, with a maximum of three animals per boma. They were fed lucerne every second day, and the bomas were cleaned regularly. Nine animals were exempt from our experimental interventions for 2 mo, except for a single occasion on which they were immobilized to be weighed (Table 1). The other six animals were exempt from interventions for 5 mo, except for two occasions on which they were immobilized to be weighed (Table 1). After their exempt period, each animal was darted IM on four occasions, fortnightly in a random order, with four different combinations of opioid plus adjuvant, namely, 1.5 mg etorphine+40 mg azaperone, 1.5 mg etorphine+2 mg medetomidine hydrochloride (Domitor, Novartis, Johannesburg, South Africa) 1.2 mg thiofentanyl oxalate (A3080, Wildlife Pharmaceuticals, Karino, South Africa) +40 mg azaperone, and 1.2 mg thiofentanyl+2 mg medetomidine. We darted three animals shortly after each other, within 3 min of the first dart, with about 20 min between the groups of three. Once each animal was recumbent, we drew a 10 ml venous blood sample. After 30 min we reversed the effects of the drugs, using 3 mg diprenorphine IV for etorphine, 12 mg naltrexone hydrochloride IV (Trexonil, Wildlife Pharmaceuticals) for thiofentanyl, and 10 mg atipamezole hydrochloride IM (Antisedan, Novartis) for medetomidine. There is no recognized reversing agent for azaperone, and 40 mg of azaperone alone did not immobilize or sedate the animals.

Plasma cortisol

Blood samples were drawn from either the cephalic or jugular veins, into a lithium heparin tube (BD Vacutainer Systems, Plymouth, UK). In the field, the samples were kept on ice until they could be centrifuged to separate the plasma. The plasma was stored in a −70 C freezer, until thawed for cortisol concentrations to be determined by radioim-

munoassay (Coat-A-Count Cortisol Kit, Diagnostic Products, Los Angeles, California, USA).

Climatic data

Climatic data at the study site were obtained from a nearby (<1 km) open area with a Hobo portable weather station (Onset Computer Corporation). For the study of the "1–5-mo habituated animals," dry-bulb and black globe temperatures were measured in the bomas, at 2-min intervals, with a Hobo Data Logger (H08-007-02, Onset Computer Corporation) connected to a black globe thermometer. Black globe temperature incorporates air temperature, solar radiation, and wind speed and therefore provides the best single index of environmental heat load.

Data analysis

Results are reported as mean ±SD, and a $P<0.05$ was considered statistically significant. Reported results were derived only from animals that received etorphine or etorphine +azaperone. Changes in body temperature were calculated as the difference between maximum body temperature attained after darting and the mean body temperature over the 30 min before darting. A Pearson's correlation was used to determine the relationship between the body temperature changes and the environmental temperature and between the body temperature changes and times to recumbency across the groups. An unpaired Student's *t*-test was used to test for differences in body temperature changes between animals that were darted and those that were not darted in the "4-mo habituated animals," and body temperature changes in animals that were not darted between the "naïve" and "4-mo habituated group." A one-way analysis of variance (ANOVA) followed by a Student Neuman Keuls (SNK) post-hoc test was used to test for differences between body temperature changes, plasma cortisol concentrations, and times to recumbency between the different groups of animals.

RESULTS

Naïve animals

In the free-ranging impala, body temperature followed a nychthemeral rhythm (Fig. 1, days 1–6), with a mean amplitude of 1.1 C, minimum temperatures in the morning (~06:30 AM) and peak temperatures, of ~39.5 C, in the late afternoon to early evening (~6:00 PM). In response to darting and immobilization, by an opioid alone, the impala became hyperthermic, with body temperatures increasing within minutes and reaching levels as high as 43.5 C (Figs. 1 and 2). Severe hyperthermia was evident not only in the animals that were immobilized (temperature change 3.2±0.6 C) but also in animals in the same group that were not immobilized (temperature change 2.9±0.5 C; Fig. 2). The profile of the body temperature changes was identical, on each day, in all six animals (Fig. 2), despite the animals being darted at different times and with two different opioids. The hyperthermia, related to darting and immobilization, peaked after the reversal of the opioid drugs and then resolved gradually (Fig. 2).

Two free-ranging impala captured after being chased into a capture net had similar hyperthermic responses (Fig. 3, solid arrow) to those of the animals captured by chemical immobilization (Figs. 1 and 2). However, high-intensity exercise, induced by pursuit in a vehicle, in two earlier unsuccessful attempts to capture the impala in the net caused a rise in body temperature (temperature change ~0.7 C; Fig. 3) much smaller than the rise evident when the animals were captured in the net and handled (temperature change ~3 C to ~4 C). The duration of

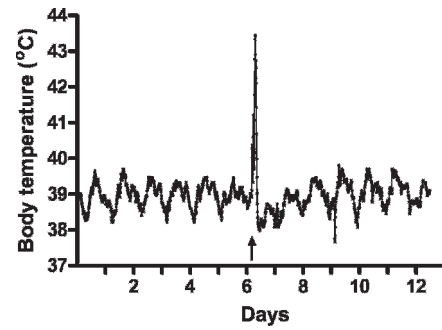

FIGURE 1. Body temperature, measured at 10-min intervals in one, naïve (nonhabituated) female impala over 13 days. The arrow indicates when the impala was immobilized, in this case with etorphine (0.5 mg).

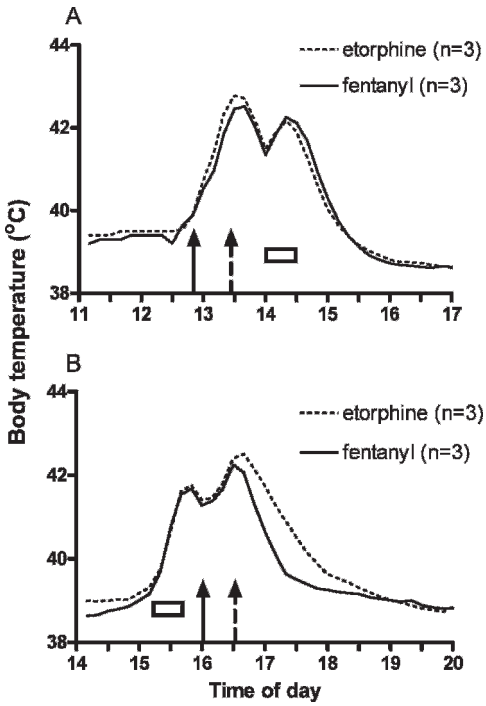

FIGURE 2. Mean body temperatures, measured at 10-min intervals, of six naïve impala before, during, and after immobilization by darting with etorphine (0.5 mg) and fentanyl (10–12 mg), in random order, on two separate nonconsecutive days (A and B). The solid arrows indicate the time at which three impala, in the group of six, were darted and immobilized with etorphine and the dashed arrow the time at which the effects of the etorphine were reversed with diprenorphine in those impala (20 min after darting). The open bars indicate the time at which the other three impala were immobilized with fentanyl. The three impala darted first were in the company of the other three impala in the group, which were to be darted later, until the darted animals became recumbent.

the unsuccessful pursuits (8 and 10 min), and the running speed of the impala, was similar to that of the successful pursuit (10 min).

Four-month habituated animals

Impala that were habituated for 4 mo also developed a hyperthermia when they were immobilized chemically (Fig. 4). Body temperature increases in these impala were significantly smaller than in the "naïve impala" (1.0±0.2 C vs. 3.6±0.6 C; $F=37$, $P<0.001$; Fig. 5A).

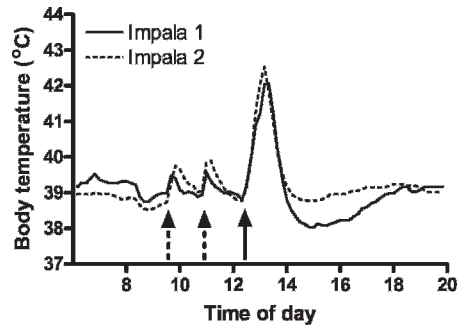

FIGURE 3. Body temperatures, measured at 10-min intervals, of two naïve (nonhabituated) impala. The dashed arrows indicate when both of the animals were chased by a vehicle, which caused full-speed flight and successful escape, followed by a chase, at about the same speed, into a capture net (solid arrow). After the animals became entangled in the net, they were restrained for 15 min by handlers, without chemical immobilization, after which they were released into a 62-ha camp, without further interference.

When an individual in the "4-mo habituated animals" was darted, the other animals in the group, which were actively running, also showed a transient increase

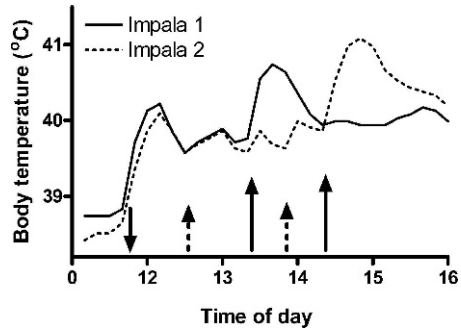

FIGURE 4. Body temperatures, measured at 10-min intervals, of two impala immobilized successively, about 30 min apart, in a group of five animals that were habituated for four months. The downward solid arrow indicates when the group of animals was herded into a novel 10×20 m boma. The following two upward dashed arrows indicate when other impala in the group were immobilized. The upward solid arrows indicate the times at which the two impala were darted (impala 1 depicted by the solid line was darted first). When an impala was immobilized, all the impala stayed together as a group, until the darted animal became recumbent. The immobilized impala was removed from the group to an adjacent boma, while the rest of the impala remained in the original boma where darting took place.

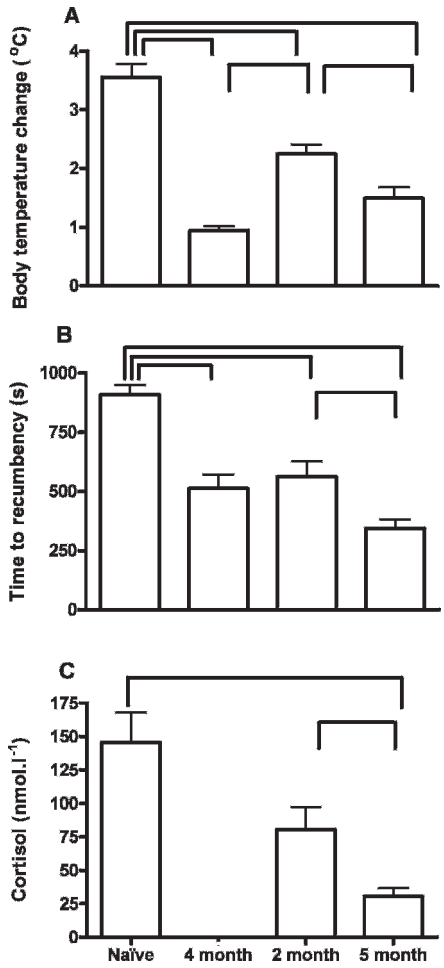

FIGURE 5. Change in body temperature (A), time to recumbency after darting (B), and cortisol concentration (C) in four groups of immobilized impala. "Naïve animals" ($n=6$) were free-ranging animals that had not been handled or habituated to boma-housing before the darting trials commenced; they were immobilized with etorphine (0.5 mg) only. "Four-month animals" ($n=5$) had been kept in a large (30×50 m) boma for 4 mo, handled four times, and were immobilized with etorphine (1 mg) plus azaperone (40 mg). "Two-month animals" ($n=9$) had been kept in small (5×10 m) bomas for 2 mo and had been handled once in that time, and were immobilized with etorphine (1.5 mg) and azaperone (40 mg). "Five-month animals" ($n=6$) were managed in the same way as the "2-mo animals," except for the longer captivity (5 mo), and were handled twice before been immobilized with etorphine (1.5 mg) plus azaperone (40 mg). Brackets above the bars indicate significant differences between the groups ($P<0.05$).

in body temperature (an example of this effect is depicted by the dashed arrows in Fig. 4). These increases in body temperature were significantly smaller than the increases in the darted animals, which also ran but were less active than nondarted animals (temperature change 0.4±0.3 C vs. 1.0±0.2 C, $t=3.6$, $P=0.001$; $n=5$; Fig. 4).

Two- and 5-mo habituated animals

When impala that were habituated for 2–5 mo were immobilized with different drug combinations they exhibited an increase in body temperature, with temperature profiles similar to the other immobilized animals described above. When the same amount of etorphine and azaperone were used to immobilize the two groups of impala, the body temperatures in the animals that had been habituated for 5 mo rose significantly less than those that had been habituated for 2 mo (temperature change 1.4±0.4 C vs. 2.1±0.4 C; $F=37$, $P<0.01$; Fig. 5A). Even though the doses, per kg body mass, of etorphine and azaperone were similar ($F=1.51$, $P=0.26$), the animals that had been habituated for 5 mo became recumbent in a significantly shorter time compared to those that had been habituated for 2 mo ($F=11.7$, $P<0.05$; Fig. 5B).

Comparison between groups

The body temperature increases during immobilization were greatest in the "naïve impala" and were generally significantly less, in a descending order, as the impala became more habituated to human handling (Fig. 5A, $F=37$, $P<0.001$). Although this trend in body temperature changes between the groups was similar to the trend in the times to recumbency, those animals that were habituated for 4 mo did not become recumbent in a significantly shorter time compared to the "2-mo habituated animals" ($F=37$, $P>0.05$; Fig. 5B), and there was no relationship, across the groups, between mean body temperature changes and mean times to

recumbency (r=0.87, P=0.14). The level of activity (see summary in Table 1), from dart placement to recumbency, also was not related to body temperature changes (Fig. 5A).

Plasma cortisol concentrations, after immobilization, were higher in "naïve impala" that received only etorphine, compared to impala that were handled twice, habituated for 5 mo, and received etorphine and azaperone (Fig. 5C, F=5.5, P<0.05). When impala received the same amounts of etorphine and azaperone, those animals that were handled twice and habituated for 5 mo had lower plasma cortisol concentrations compared to those that were handled once and habituated for 2 mo (Fig. 5C, F=5.5, P<0.05).

The body temperature changes that occurred in animals that were not immobilized (these animals were not under the influence of any drugs but with the exception of the actual darting were exposed to the same capture-related stresses) were significantly less in impala that were habituated for 4 mo as compared to "naïve impala" (t=8.05, P<0.0001; Fig. 6).

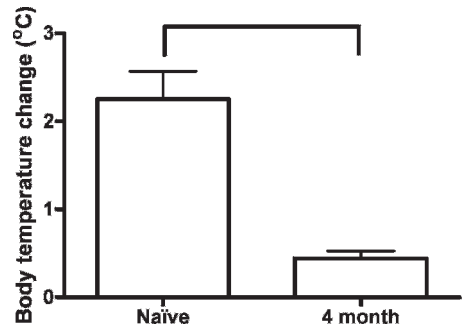

FIGURE 6. Change in body temperature in six "naïve impala" and five "4-mo habituated impala" in response to disturbance when other animals in the group were darted and chemically immobilized. The human activity in the enclosure or boma caused agitation, and attempted escape, in the disturbed animals, even though, at the time, there was no direct interference with the animals. Once the darted animals were immobilized they were either moved to an adjacent boma or were placed in a quiet area of the enclosure, so as to reduce the disturbance of the other members of the group that were not immobilized. Brackets above the bars indicate significant differences between the groups (P<0.05).

Observations

"Naïve impala" appeared to be extremely fractious when approached, and once darted they engaged in high-intensity activity before they became recumbent. Impala that were habituated were less fractious than "naïve impala." Those impala that were habituated for 4 mo, but were darted in the bigger 10×20 m boma, were more active after darting compared to those impala that were habituated for 2–5 mo but darted in the 5×10 m bomas (Table 1). There did not appear to be any differences in activity levels, postdarting, between the impala that had been habituated for 2 mo compared to those that had been habituated for 5 mo, but those impala that had been habituated for the longer time appeared to be less fractious when approached.

Climatic data

Although there were day-to-day and seasonal variations in climatic conditions, and the trials took place throughout the calendar year, the weather conditions did not differ significantly between the trials. Mean dry bulb temperature was 16±8 C and mean black globe temperature was 20±12 C over the study periods. In the "2–5-mo habituated animal study," in which local microclimates were recorded continuously in the boma, the animal's body temperature changes during immobilization were not correlated to dry bulb temperatures (r=−0.19, P=0.5; Fig. 7A) or black globe temperatures (r=−0.05, P=0.85; Fig. 7B).

Morbidity and mortality after immobilization

Three of the "naïve impala" died after experimental trials. All three of these animals had body temperatures that exceeded 43 C during one of the two immobilizations. Macroscopic post-mortem indicated that these animals had lesions indicative of capture myopathy

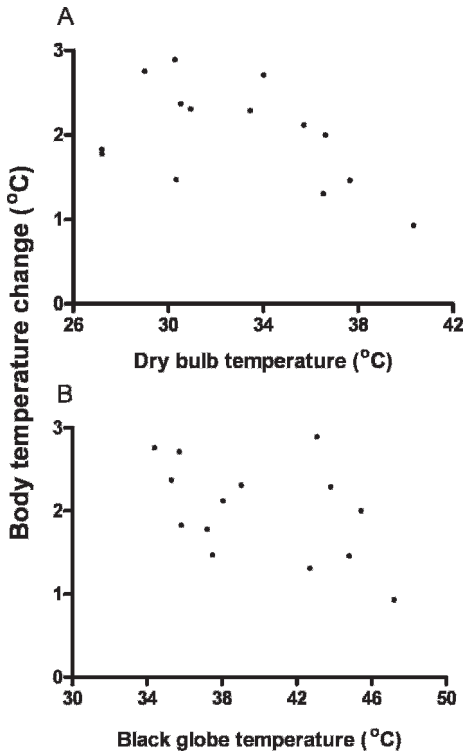

FIGURE 7. Change in body temperature, in response to darting and chemical immobilization, of 15 impala that were habituated for 2 and 5 mo and then darted with etorphine (1.5 mg) and azaperone (40 mg), versus the mean dry bulb temperature (A) and mean black globe temperature (B) measured at 2-min intervals in the bomas during the 30 min of darting and immobilization.

(Young, 1971; Gericke et al., 1978); both their cardiac and skeletal muscles, particularly the semimembranosus and semitendinosus muscles, had extensive areas of dull white necrotic tissue. There also was severe pulmonary and moderate generalized body congestion.

DISCUSSION

By obtaining the first continuous and accurate records of body temperature during a study on capture-induced hyperthermia, we have shown that irrespective of whether impala are chemically captured, net-captured, or disturbed by exposure to a stressor, they developed a precipitous increase in body temperature. This increase in body temperature was well above both the normal 24-hr body temperature patterns (Fig. 1) and the body temperature increase experienced during high-intensity exercise not associated with capture (Fig. 3). The magnitude of the body temperature increase during capture procedures was not related to activity level; animals that had low activity levels during capture had large increases in body temperature compared to those that had high activity levels but were not captured (Figs. 3 and 4). Also, the magnitude of the body temperature rise during capture was not related to environmental heat loads (Fig. 7) and did not depend on whether the animals were captured using drugs or not. Irrespective of whether animals were chemically captured, net-captured, or disturbed by exposure to a stressor, their body temperatures rose in a similar way (compare animals darted to those not darted in Figs. 2 and 3).

The main factor that appeared to influence the magnitude of the body temperature increase was the level of the stress response to capture. Those animals that were habituated more, predominantly by handling procedures and less so by boma-housing, had smaller changes in body temperatures and smaller stress responses, indicated by lower plasma cortisol concentrations and less fractious behavior, compared to those animals that were habituated less or those that were not habituated (Fig. 5). Similarly, those animals that were better habituated were less fractious and had smaller changes in body temperature when they were exposed to capture-related events without themselves being chemically captured (Fig. 6). Therefore capture-induced hyperthermia in impala was not related to the effects of drugs, environmental conditions, or activity, but rather appeared to be strongly related to the level of stress in response to capture.

Hyperthermia is a common sequel when wild animals are captured (Burroughs and McKenzie, 1993). A large

magnitude or prolonged duration of hyperthermia may result in mortality, or may compound capture-related pathologies like capture myopathy (Gericke et al., 1978; Antognini et al., 1996). In our study employing naïve impala, three animals whose temperatures were greater than 43 C died subsequently with macroscopic lesions indicative of capture myopathy. Similarly, two tsessebe (*Damaliscus lunatus*) that had been captured by manual restraint in nets and had body temperatures greater than 43.7 C died acutely without any abnormal macroscopic postmortem findings (Wimberger, 2005). Because of the morbidity and mortality associated with hyperthermia, it is recommended that capture operations take place during only the cooler times of the day (Murray et al., 1981) when air temperatures are below 25 C (Meltzer and Kock, 2006). This recommendation ignores the influence of radiant heat load on animals and does not consider that body temperatures of antelope, following a 24-hr rhythm, are greatest in the late afternoon to early evening when air temperatures are low. Also, there is little evidence that capture-induced hyperthermia is related to environmental heat load, and, to the best of our knowledge, only one study has shown that ambient temperature may influence body temperature during capture (Cheney and Hattingh, 1987). In that study impala were immobilized for 90-min, and ambient heat was most likely to have influenced body temperature due to the prolonged effects of the immobilizing agents, which would have caused the animals to become thermally labile over that time. Our animals were immobilized for a shorter duration (20–30 min), so it was unlikely that ambient heat load would have significantly influenced body temperature.

Although there is evidence that opioid drugs, including etorphine, cause a change in body temperature that is dose-dependent and profoundly influenced by environmental temperature in small animals (Rosow et al., 1980; Clark and Lipton, 1985), it has been suggested that etorphine, when used to immobilize wildlife, causes hyperthermia not through any action on the opioid receptors but through its adrenergic activity (Meltzer and Kock, 2006). The adrenergic activity of etorphine (Roquebert and Delgoulet, 1988) may well have contributed to the hyperthermia that occurred during chemical immobilization, but it cannot account for a similar magnitude of hyperthermia in the animals during net capture, or the hyperthermia that occurred in animals that were not immobilized but disturbed by the presence of humans in a confined area when other members of their group were immobilized. It also has been suggested that there is an increase in muscle activity, and thus an increase in heat production, in animals experiencing the excitement phase of chemical immobilization before they become recumbent (Burroughs and McKenzie, 1993; Meltzer and Kock, 2006). This physical activity again may contribute to the overall hyperthermia, but the contribution to the overall change in body temperature during capture is likely to be small (Bakken et al., 1999).

The major factor contributing to the body temperature elevation appears to be closely associated with the level of the stress response in the animals. Chemical immobilization of wild animals clearly evokes a stress response (Cheney and Hattingh, 1987; Morton et al., 1995; Meltzer and Kock, 2006), and in chemically immobilized impala there is a distinct elevation of stress response variables in plasma (Cheney and Hattingh, 1987) from the "normal" values obtained from brain-shot animals (Hattingh et al., 1988). Our impala had comparable increases in plasma cortisol concentrations compared to captured impala in a previous study (Cheney and Hattingh, 1987). Although there is no objective measure of stress levels in animals, biochemical variables can be used to indicate the level of response to a stressor (Hattingh, 1988;

Hattingh and Petty, 1992). Morton et al. (1995) showed that plasma cortisol concentrations provided a relatively good indication of the levels of the stress response in captured impala and other antelope species. They also found that when antelope were housed in bomas for a protracted time (35 days) they became habituated, and their response to a stressor, indicated by plasma cortisol concentrations, decreased. On the contrary, Knox et al. (1990) found that boma-housed impala that were physically restrained in nets every week for 8 wk did not show a statistically significant decrease in stress hormone response over that time. Plasma cortisol and catecholamine concentrations may have been lower if the duration of their study had been longer and less stressful procedures, such as chemical immobilization, had been used (Hattingh and Petty, 1992). Although we did not measure plasma cortisol concentrations when we initially caught our impala from the wild, and therefore cannot compare the differences in plasma cortisol concentrations in individual animals over time, we believe that habituation occurred in our animals because plasma cortisol concentrations were lower in animals that were handled more, and boma-housed for extended periods of time, compared to "naïve animals." The effects of habituation also were evident in the animals' behavior; when animals were approached they were less fractious if they were habituated more compared to if they were habituated less. However, irrespective of whether the impala were habituated or not, the animals displayed a stress response to being chemically immobilized; all the animals had elevated body temperatures and plasma cortisol concentrations compared to normal values.

The procedure of chemical immobilization probably induced a stress response because of the fear induced by the presence of humans, the fright that occurs with darting, and the anxiety accompanying an inability to escape the "perceived danger" of the capture procedure. Stress-induced hyperthermia is a reaction to a stressor, or is caused by anxiety, and is common to many mammalian species (Bouwknecht et al., 2007) and occurs irrespective of changes in ambient temperatures (Oka et al., 2001) or activity (Moe and Bakken, 1997; Bakken et al., 1999; Montané et al., 2003). The precise mechanisms underlying stress-induced hyperthermia are not known, but the hyperthermia does not arise solely as the result of the metabolic and vascular effects of catecholamine release (Mitchell and Heffron, 1980; Nakamori et al., 1993; Oka et al., 2001). Oka et al. (2001) propose that this hyperthermia results from a centrally regulated rise in body temperature. But whether this hyperthermia indeed is centrally regulated or is consequent of a derangement in metabolism, as are malignant hyperthermia and Porcine Stress Syndrome (Mitchell and Heffron, 1980; Mitchell and Heffron, 1982), remains to be elucidated. What is clear from our data, and from studies carried out in laboratory animals (Olivier et al., 2003; Veening et al., 2004), is that the magnitude of the hyperthermia developed during a stressful event is directly related to the stress response of the animal, and that the magnitude of the stress-induced hyperthermia exceeds that of exercise-induced, and probably all other, hyperthermias.

In summary, we have shown that capture-induced hyperthermia in impala is caused predominantly by stress rather than the effects of physical activity that occur before recumbency, environmental conditions, or the effects of immobilizing drugs. Although we believe that stress is the major cause of capture-induced hyperthermia, we do not suggest that physical activity will not alter body temperature. On the contrary, we propose that excessive physical activity superimposed on stress-induced hyperthermia will compound capture-induced hyperthermia, especially if an animal has peripheral vasoconstriction from increased sympa-

thetic activity. Similarly environmental heat conditions, and the effects of the capture drugs on thermoregulation, would compound capture-induced hyperthermia. To reduce capture-induced hyperthermia, and its associated morbidity and mortality, capture techniques that invoke lower stress responses should be used whenever possible; the period of time that an animal is exposed to a stressor should be kept to a minimum, long high-intensity chases should be avoided, and immobilizing drug cocktails should be chosen so as to restrict times and distances to recumbency. During extreme environmental temperatures, prolonged immobilization and post-capture confinement in hot or cold vehicles should be avoided, but short capture procedures that induce minimal stress can be considered if animals are quickly released into stress-free environments.

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