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First successful hand-rearing and release to the wild of two orphan brown bear cubs in Greece

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Η πρώτη επιτυχής περίθαλψη και επανένταξη δύο νεαρών ορφανών καφέ αρκούδων (Ursus arctos) στην Ελλάδα

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Ημερομηνία αρχικής υποβολής: 29 Αυγούστου 2014 Ημερομηνία αναθεωρημένης υποβολής: 30 Σεπτεμβρίου 2014 Ημερομηνία αποδοχής: 2 Οκτωβρίου 2014 **ABSTRACT.** The rehabilitation and release of orphan brown bears *(Ursus arctos)* to the wild is of increasing importance in the conservation and management of the species. In April 2011 two orphan male brown bears were found and rehabilitated for the first time at a dedicated Bear Rehabilitation Centre in Greece. In this case report we describe in detail the veterinary procedures and the feeding regime followed during the 9-month rehabilitation process. For the release on the 23rd of January 2012 the two bears were anaesthetized with a combination of xylazine and ketamine, and satellite collars were placed on them for post-release monitoring. Eight hours later the bears were anaesthetized with the same mixture for a second time, transported and placed into an artificial den in the wild; recovery from anaesthesia took place without complications. Three months later the bears left the den and started moving in the wider area of northwestern Greece. We conclude that rehabilitation and release of orphan bears is an important tool in the management and conservation of this endangered species in Greece.

Keywords: conservation, endangered species, Greece, management, rehabilitation, Ursus arctos

ΠΕΡΙΛΗΨΗ. Η επανένταξη και απελευθέρωση ορφανών νεαρών αρκούδων στο φυσικό τους περιβάλλον, είναι μεγάλης σημασίας για τη διατήρηση αυτών των ειδών που απειλούνται με εξαφάνιση. Τον Απρίλιο του 2011, δύο αρσενικές καφέ αρκούδες (Ursus arctos) ηλικίας 2 και 2,5 μηνών, βρέθηκαν ορφανές στην ΒΔ Μακεδονία και μεταφέρθηκαν στο Κέντρο Περίθαλψης και Επανένταξης της ΜΚΟ «ΑΡΚΤΟΥΡΟΣ», όπου παρέμειναν εκεί για 9 μήνες. Τον Ιανουάριο του 2012 οι αρκούδες αναισθητοποιήθηκαν με συνδυασμό ξυλαζίνης και κεταμίνης im, που χορηγήθηκαν με αναισθητικό όπλο. Ακολούθησε λεπτομερής κλινική εξέταση, αιμοληψία για αιματολογικό και βιοχημικό έλεγχο και τοποθετήθηκαν κολάρα δορυφορικού εντοπισμού. Στη συνέχεια μεταφέρθηκαν στο βουνό, όπου 8 περίπου ώρες αργότερα αναισθητοποιήθηκαν για δεύτερη φορά με το ίδιο αναισθητικό σχήμα και τοποθετήθηκαν σε τεχνητή φωλιά στο δάσος. Με τη βοήθεια κάμερας που είχε εγκατασταθεί στη φωλιά, διαπιστώθηκε ότι οι αρκούδες ανένηψαν φυσιολογικά και μερικές ώρες αργότερα έπεσαν σε «χειμέριο ύπνο». Τον Μάρτιο του 2012 οι αρκούδες «ξύπνησαν», εγκατέλειψαν τη φωλιά τους και άρχισαν να κινούνται στην ευρύτερη περιοχή της ΒΔ Μακεδονίας.

Λέζεις-κλειδιά: αρκούδα, επανένταζη, περίθαλψη, Ελλάδα

INTRODUCTION

rphan brown bears (Ursus arctos L.) are becoming more common in Greece due to increasing interactions with humans. Several options for dealing with orphan bear cubs exist, but hand-rearing them in captivity and then releasing them back to the wild is one of the most attractive (Beecham et al., 2015). Rehabilitation and release efforts provide managers the opportunity to support the welfare of disabled animals, attract public attention to conservation efforts and collect important biological information (Moore et al., 2007). Brown bears are considered to be endangered in Greece; in recent years however the population has made a significant recovery and it is estimated that >450 individuals currently live in the country (Karamanlidis et al., 2015). At the same time negative human – bear interactions have also been increasing (Karamanlidis et al., 2011) and therefore informed,

science-based management and conservation measures are urgently required to safeguard the recovery and survival of this endangered brown bear population. In this study we present and evaluate the first rehabilitation and release attempt of brown bears in Greece.

CASE HISTORY

In April 2011, two orphan male, unrelated, brown bear cubs, aged 2 and 2.5 months respectively, were found in separate locations in northwestern Greece. Following unsuccessful attempts to re-unite them with their mothers, the two cubs (named Little John and Nikitas) were transferred to the Bear Rehabilitation Centre of the non-governmental organization ARCTUROS. All research activities were carried out under the research permit 119628/1442 of the Hellenic Ministry of



Figure 1. Various images of the first two brown bears successfully rehabilitated and released in Greece. a. The cubs in their small, fenced area (at the age of 2-3 months); b. The cubs climbing on trees in their forest enclosure (at the age of 6-7 months); c. The cubs at their den (at the age of 10-11 months) in the rehabilitation centre; d. Image from the infrared camera from the inside of the winter den, where the two cubs were released, right after recovery from anaesthesia (© M. Avgerinou, A.A. Karamanlidis/ARCTUROS).

Environment, Energy and Climate Change.

Upon arrival the cubs were placed in a quarantined area (2.5 x 1.5 x 1.5m), and a detailed physical examination was performed. Both cubs were in a moderate body condition (i.e., Little John: 4kg; Nikitas: 3kg) and mildly dehydrated. Auscultation findings, respiratory rate and pulse rate were normal. Mucopurulent discharge was noticed in the eyes of Little John and was treated for 7 days with an antibiotic eye ointment (Fucidin® 1% eye drops, LEO Pharmaceutical Products Hellas Ltd). Mucus diarrhoea was observed in both cubs and was treated with nutritional measurements and administration of Diarsanyl Plus ® oral paste (Ceva Animal Health Inc., KS USA) and Pro-Enteric Triplex[®] and Enterochronic[®] paste (Bioberica Veterinaria, Spain) in order to balance the intestinal flora. Abdominal palpation did not reveal anything abnormal, and faecal parasitological examinations were negative; however, as bear cubs of this age are very susceptible to parasitic infestations (Beecham 2006), a prophylactic treatment for endoparasites with Banmith paste® (Pfizer Inc., U.S.A) was administered and repeated 3 weeks later. The parasitic treatment continued again once every two months, with Drontal plus® (Bayer HealthCare LLC, U.S.A) (Papageorgiou et al., 2002). No other clinical signs of disease or any injuries were observed.

Rehydration with liquid-electrolytes, Almora plus® (oral pd, ELPEN Co. Hellas) and bottle milk feeding was initiated. In addition, vitamins and supplements, such as Vetamix Osteoform® (Lloyd, U.S.A), VetStar Vital® (Provet Hellas) and Calo Pet® (Vetoquinol, France) were also administered to the cubs as part of their regular diet. No vaccines release area (Beecham, 2006). As both cubs showed natural avoidance behaviour towards people, they were considered good candidates for re-introduction into the wild and were entered into an intensive rehabilitation program for nine months. During the initial stabilization period (i.e., 10 days), both animals were housed together in a small enclosure and had minimal contact with humans; only two caretakers and the supervising veterinarian had access to them. After the first ten days both cubs were transferred to a small fenced area (80 m²) where they remained for two months (acclimatization period) (Figure 1a). This area had two artificial dens, a pool and several stimulating objects (i.e., tree trunks, rocks) that were used to reduce the possibility of developing stereotypic behaviours (Criswell and Galbreath 2005). Contact with humans remained minimal, with only two caretakers entering the enclosure to feed the animals. Then, at the age of approximately 4.5 - 5 months the cubs were transferred to an enclosed one-hectare forest of beech (Fagus sp.) and oak (Quercus sp.) where they remained for approximately seven months (pre-release period) (Figure 1b, 1c); restricted contact with humans was continued until their release on 23 January 2012.

During the initial stabilization period the cubs were bottle-fed with canine milk formula (Ormilak®ORSCO - Laboratoire Vétérinaire, France) and honey (1-2 teaspoons); meals were provided around the clock, every three hours. The quantity of food provided was slowly tapered off and 10 days later bottle feeding was discontinued. From then on and for 2 months (i.e., during the acclimatization period) the bear cubs were fed 10-20% of their body mass daily; they received 4 - 5 bowls of milk (Veta-Lac Milk Replacer®, Lloyd, U.S.A.), rice baby cream (Nounou baby rice cream, Royal Friesland Campina, The Netherlands), honey, yogurt, fruits, Quaker (Quaker, Pepsiko, U.S.A),

muesli, corn flakes, eggs and canned dog food (Royal Canin puppy, Royal Canin, U.S.A) in four meals. When moved to the forest enclosure in July (i.e., at the age of 4.5-5 months) the two bear cubs were fed twice daily. By mid-December feeding was gradually reduced to once every other day and then discontinued one month later. During this phase of rehabilitation (i.e., during the pre-release period), the bear cubs were fed milk, cereal, nuts, dry dog food, and several fruits and vegetables collected from the nearby forest. Solid foods were presented to bears in various ways to encourage them to search for their food as they do in the wild (Lintzenich et al., 2006).

The release of the two rehabilitated bear cubs was carried out on the 23rd of January 2012, when Little John and Nikitas weighed 50 and 45 kg respectively. At this time both bears had entered their hibernation phase and were slightly lethargic (inside the den in the enclosure). The two cubs were anaesthetized with a dart gun using a combination of xylazine (2mg/kg b.w.) and ketamine (3mg/ kg b.w.) intramuscularly. Fifteen minutes later the drugs went into effect and the bears went down, showing no response to auditory stimuli and no head-lifting reaction to tactile stimuli (Caulkett and Cattet 2002). Handling was initiated and the eyes of both bear cubs were covered with a piece of cloth to protect the corneas from direct sunlight and to reduce optic stimuli. Weight and body measurements were taken and a detailed clinical examination was performed. Rectal temperature was taken with a standard digital thermometer. Their body temperatures ranged between 36.5-37.2°C. Monitoring of anaesthesia was carried out by checking the palpebral reflex, the positioning of the eye globe, the presence of nystagmus, the respiratory and heart rate, as well as by examination of the colour of the mucus membrane, the capillary refill time, the peripheral pulse and the body temperature. To evaluate the health status, blood samples were taken from the jugular vein for haematologi-

	Haematological exa	Biochemical exam			
	Little John	Nikitas		Little John	Nikitas
RBC	8.69 M/µl	8.44 M/µl	ALB	4.6 g/dL	4.6 g/dL
HGB	19.0 gr/dL	18.4 gr/dL	UREA	10 mg/dL	12 mg/dL
НСТ	55.0%	52.3%	CREA	1.4 mg/dL	1.2 mg/dL
MCV	63.3 fl	61.9 fl	CHOL	374 mg/dL	359 mg/dL
МСН	21.8 pg	21.8 pg	TRIGL	252 mg/dL	221 mg/dL
MCHC	34.5 gr/dL	35.1 gr/dL	TBIL	1.4 mg/dL	1.9 mg/dL
RDW-CV	16.0%	16.0%	ALP	33 U/L	39 U/L
WBC	5.3 K/µL	5.6 K/µL	SGPT	17 U/L	14 U/L
NEUT	3.6 K/µL	3.8 K/µL	SGOT	72 U/L	51 U/L
LYM	1.1 K/µL	1.3 K/µL	γ - GT	4 U/L	4 U/L
MONO	0.3 K/µL	0.2 K/µL	СРК	977 U/L	252 U/L
EOS	0.29 K/µL	0.27 K/µL	LDH	1201 U/L	1146 U/L
BASO	0.01 K/µL	0.01 K/µL	Р	4.4 mg/dL	3.9 mg/dL
LUC%	0.02 K/µL	0.01 K/µL	Ca	6.8 mg/dL	6.7 mg/dL
PLT	349 K/µl	307 K/µl	K	5.0 mEq/L	4.7 mEq/L
MPV	7.9 fl	7.5 fl			
TS	6.8 gr/dL	7.4 gr/dL			

 Table 1: Haematological and biochemical values of two rehabilitated brown bear cubs.

cal and biochemical analyses (Table 1) and faeces were collected for parasitological examination. 3-D GPS-GSM (global positioning system, global system for mobile communication) collars (GPS Plus, Vectronic Aerospace GmbH, Germany) with cotton spacers were placed on both animals for post-release monitoring.

After the end of this procedure Little John appeared cyanotic and therefore supplementary oxygen was given through a nasal catheter for 10 min. In order to reverse xylazine and accelerate recovery, atipamezol (0.20.1 mg/kg b.w. intramuscularly) was administered, and Little John recovered from anaesthesia 10 min later without any further complications. Nikitas recovered from anaesthesia one hour later without additional intervention. One hour after full recovery both bear cubs were transported to a forested area in Mount Vitsi in northwestern Greece (altitude 1,400 m above sea level) where an artificial den had been constructed in order to increase the cub's chances of survival in the wild. Approximately 8 hours after the first anaesthetic episode, the two cubs were anaesthetized again using xylazine (2 mg/kg b.w.) and ketamine (3 mg/ kg b.w.) intramuscularly. It was necessary to administer supplemental doses of xylazine (1 mg/kg b.w.) and ketamine (6 mg/kg b.w.) intramuscularly to Little John; this was most likely necessary due to the injection of the drugs into the fat during the first injection or due to a residual action of atipamezole. Both bear cubs were placed in the den and the entrance was partially sealed off with snow. The interior of the den was monitored with an infrared camera. Monitoring of anaesthesia was achieved solely through the infrared camera. Full recovery from anaesthesia was completed uneventfully 90-100 min after the administration of the drugs (Figure 1d). Following a three-month denning period, both bear cubs emerged from their den in the wild in March 2012, and started moving in the wider area of northwestern Greece.

DISCUSSION

Raising orphan wildlife in captive-rearing facilities for release back to the wild is expanding globally (Kelly et al., 2010) and has been performed with success with orphan birds, marine mammals and primates (Agoramoorthy and Hsu, 1999; Golightly et al., 2002; Moore et al., 2007). For more than 30 years bear conservationists and managers around the world have been experimenting with the rehabilitation and release of bears; in north America the rehabilitation and release of American black bears (Ursus americanus) and brown bears has proved to be a very effective and valuable conservation tool (Beecham et al., 2015). In contrast, in Europe, where several small and endangered bear populations still exist (Zedrosser et al., 2001), there has been little experience with bear rehabilitation (Bereczky, 2010). In this study we provide information and evaluate the usefulness of the rehabilitation and release of orphan brown bears as a management tool for the species in Greece.

New-born bear cubs are totally dependent on their mothers to receive adequate nutrition for rapid growth in the early stages of their life. Depending on species, bear cubs may nurse for up to 28 months and the bear milk they feed on has a very high caloric value (Jenness et al., 1972). Young bears in rehabilitation need to be fed 15-25% of their body mass daily (Lintzenich et al., 2006) of a substitute that has the same effect of rapid growth as the mother's milk. It has been suggested that the formula should be high in calories, protein and fat and low in carbohydrates; in order to achieve the desired rapid growth curves bears in their initial stages of their rehabilitation should be fed around the clock every 2-3 hours (Huber et al., 1993). In our cases the formula used was tolerated well and the cubs gained body mass rapidly. Based on our experience we recommend that during these initial phases of feeding (but also throughout the entire rehabilitation process) rehabilitated animals should be closely monitored for bloating, inappetence, discomfort and constipation. Gradually, the number of feedings per day and the quantity of food can be tapered off to 10-20% of their body mass. It has been suggested that at weaning (i.e., approximately at the age of 5-6 months) the diet of bears in rehabilitation should consist primarily of fruits, dry dog food (for nutritional balance), hard mast, some vegetables, and occasionally fish or the carcasses of wild animals (Lintzenich et al., 2006). In our cases the feeding regime followed (i.e., Ouaker, muesli, corn flakes, eggs, as well as several fruits and vegetables collected from the nearby forest) proved to be very successful, as until the time of their release both cubs gained significant weight and were, based on the results of their haematological, biochemical and parasitological exams, in a good health condition.

Haematological and biochemical exams are an important tool to assess health and understand the impact of disease on wildlife populations (Geffre et al., 2009); the baseline values of these exams may be affected by various host and ecological factors and therefore caution is advised when interpreting diagnostic data. As no baseline bear hematology and biochemistry studies have been conducted in Greece, our results were compared to other relevant data (Græsli et al., 2014) and were normal.

Bears are known to show exposure to a variety of disease pathogens. Active cases of disease are rare in the wild (Binninger et al., 1980), but there is always the possibility that released cubs may infect wild bear populations with disease pathogens or parasites they have been exposed to prior to or during the rehabilitation process. Orphan cubs brought into rehabilitation are often in a poor physical condition, due to dehydration or malnutrition and are therefore very susceptible to internal and external parasitic infestations. Internal parasites reported so far in orphan brown bears include protozoans, trematodes, cestodes, nematodes, while external parasites include arthropods (e.g., ticks, lice and mites) (Beecham, 2006). In our cases, the faecal samples of the animals at admission were free of parasites. In order to prevent spreading any parasites in the wild when released, a prophylactic treatment for endoparasites was administered in the beginning and continued again once every two months until the day of release and faecal samples were collected regularly for testing.

Bears, like other wild animals in captivity are prone to developing stereotypic behaviour (i.e., pacing or bawling) (Kolter and Zander, 1997; Langenhorst, 1998; Criswell and Galbreath, 2005). In an effort to minimize the chances for these types of behaviour to develop, and based on recommendations from previous bear rehabilitation efforts (Beecham, 2006), we placed objects found in natural bear habitat or constructed pools and climbing structures in our Rehabilitation Centre in order to stimulate the cubs. Furthermore, we also presented solid foods to our rehabilitated animals in a variety of ways (i.e., scattered, hidden), thus increasing behavioural enrichment in the enclosure and encouraging the animals to search for their food as they do in the wild (Ramanathan and Danilova, 2007). All this proved to be very successful as at the end of the rehabilitation period both bear cubs did not show any signs of stereotypic behaviour. Bears in captivity run also the risk of habituating to humans and getting into post-release conflicts (Huber, 2010). It is therefore important to take specific measures to reduce these risks (Beecham, 2006). In our cases, allowing the two bear cubs to

grow up together and socialize with a minimum of human contact proved to be successful, as neither cub showed any signs of habituation towards humans. This was true, not only for the time during the rehabilitation, but also after the release and during the one year of post-release monitoring (Beecham et al., 2015).

Anaesthetizing and handling bears has been an integral part of bear research for decades now and several studies have been carried out on the effects and dosages of drugs used during anaesthesia (Cattet et al., 2003a; b). In the present cases we used a combination of xylazine and ketamine. Although these drugs have been used extensively in anaesthetic protocols for wild and exotic animals, there is now a tendency to use other drug combinations, i.e. more potent α_{2} adrenergic agents instead of xylazine, combined with tiletamine (plus zolazepam) instead of ketamine in bear anaesthesia (Caulkett and Arnemo, 2007). Nonetheless we chose this particular drug combination in order to achieve more rapid recovery (Caulkett N, 2007): during the first anaesthetic episode we strived for rapid recovery because a second anaesthesia was going to follow and we would not have any access to the cubs after they had been placed in the den. Our decision to use this drug combination was supported furthermore by the short-term duration of the procedure, and the small size of the bears (Caulkett and Arnemo, 2007). About 45 min after Little John was anaesthetized for the first time, his buccal mucosa appeared cyanotic. Although respiratory rate and heart rate showed no change, we decided to accelerate recovery, especially since the procedure had been completed.

CONCLUDING REMARKS

In the present study we present information on the procedures followed during the first successful attempt of hand-rearing and release in the wild of two orphan brown bear cubs in Greece. The possibilities of finding orphan bear cubs in Greece are likely to increase in the future, since the bear population in the country and human-bear conflicts have been increasing (Karamanlidis et al., 2011; 2015). The procedures that were followed and the experience that was gained during this first successful rehabilitation and release attempt will prove valuable in the future management and conservation of this endangered species in Greece and other countries.

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CONFLICT OF INTEREST STATEMENT

The authors of this article declare that they do not have any conflicting interests.

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