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CONVENTION ON THE CONSERVATION OF EUROPEAN WILDLIFE AND NATURAL HABITATS

Standing Committee

32nd meeting Strasbourg, 27-30 November 2012

Other complaint

THREAT TO THE BROWN BEAR IN CROATIA

REPORT BY THE GOVERNMENT

Document prepared by the Ministry of Environment and Nature Protection of Croatia Class Mark: 612-07/11-44/0074 RegNo: 517-12-06 Zagreb, 2 April 2012

> COUNCIL OF EUROPE Directorate of Culture and of Cultural and Natural Heritage Biological Diversity Unit att. Ms Ivana d'Alessandro F-67075 STRASBOURG Cedex France

Subject: Report on the Brown bear (*Ursus arctos*) management in the Republic of Croatia

To Whom It May Concern,

In consideration of your letter from November 7th 2011 regarding a complaint from the NGO Udruga Animalia denouncing a possible breach of the Bern Convention with regards to the presumed unsustainable management of the brown bear population (*Ursus arctos*) in Croatia, the Ministry of Environment and Nature Protection, as the competent authority for the implementation of the Convention in Croatia, requested a report from the authorities responsible for issues mentioned in the complaint.

The Ministry of Agriculture, which is the competent authority for forestry, hunting and management of brown bear as a game species in Croatia, provided a response to the claims in the complaint in their jurisdiction, which is enclosed as **Annex I** of this Report. The Committee for the Elaboration of the Brown Bear Management Plan for the Republic of Croatia provided an explanation in regards to the brown bear management, and the state owned company Hrvatske šume d.o.o. (limited liability company) for forest and woodland management provided a report in regards to the forest management and forest infrastructure mentioned in chapters 4 and 5 of the complaint.

The Ministry of Environment and Nature Protection provided an answer in regards to the garbage dump problem mentioned in chapter 9 of the complaint and the statement from page 11 of the complaint that in Croatia there are no examples that the EIA study has expressed negative opinion regarding the planned investment. The complete answer is enclosed as Annex II of this report.

For information purposes we are also providing:

Annex III - list of scientific papers in peer reviewed journals and other publications and past and current projects dealing with brown bear and other large carnivores

Annex IV – scientific article "Genetic diversity of Dinaric brown bears (*Ursus arctos*) in Croatia with implications for bear conservation in Europe" (authors copy for internal non-commercial use, posting to third party websites is not allowed)

Annex V – detailed report on cases of poaching and poisoning

Annex VI – examples of brown bear mortality and age structure analyses

We hope this report provides all the relevant information requested from the Republic of Croatia in regards to the Brown Bear Management Plan and its results. For any additional inquiries, please feel free to contact us again.

Kind regards,

ASSISTANT MINISTER Nenad Strizrep

RESPONSE FROM THE COMMITTEE FOR THE ELABORATION OF THE BROWN BEAR MANAGEMENT PLAN FOR THE **REPUBLIC OF CROATIA**

INTRODUCTORY REMARKS:

Contrary to the initial statement that "the members of NGO Animalia are systematically engaged in monitoring, observing and exploring the bear population in Croatia and neighbouring Bosnia and Herzegovina for years" it has to be stated that their data sources are strictly opportunistic and as they have stated themselves originate from "...studying scientific articles, gathering information from people who get in touch with bears in everyday activities, the members of Animalia are also continuously in closed contact with those wild animals and their habitats".

We admit that, as nature and animal lovers, the Animalia members spend certain time in nature and they may occasionally whiteness something that could have remained undocumented. When received, each such information is welcomed, checked and used. Contrary to that, the Committee for the Elaboration of the Brown Bear Management Plan for the Republic of Croatia and the research team at the Biology Department of the Faculty of Veterinary Medicine in Zagreb through various international scientific projects consistently perform a full scale monitoring of the brown bear population in Croatia.

Here we present the broad platform which represents the framework for current bear management in Croatia. We want to convince the Standing Committee that our actions are not based on random guesswork, but are always thoroughly founded. Also, the outcome is very positive in sense of (1) bear population trend (annual growth around 7%), (2) acceptance of bears by local inhabitants (high with 80% in favour), and (3) the amount of damages (low at about 6000 EUR per year). We are aware that this equilibrium is very fragile and are ready to adapt in each moment when needed.

The list of established and fully functional brown bear management bodies in Croatia:

- Large Carnivores Monitoring Committee provides expertise to relevant Ministries (15 members) meets 3 to 4 time per year
- Committee for the Elaboration of the Brown Bear Management Plan for the Republic of Croatia– produces the Croatian Brown Bear Management Plan and prepares annual action plans which include quotas (8 members) – meets 6 – 10 times per year
- Brown Bear Intervention Group (10 members for local actions) meets for training every year

The list of agencies/institutions involved in Brown bear management:

- Ministry of Agriculture, Directorate for Forestry, Hunting and Wood Industry
- Ministry of Environment and Nature Protection, Nature Protection Directorate
- State Institute for Nature Protection
- Faculty of Veterinary Medicine of the University of Zagreb
- Croatian Hunters Association
- NGOs, public institutions governing national parks and nature parks, Faculty of Forestry, Faculty of Science
- Representatives from Slovenia and Bosnia and Herzegovina

Involvement of general public

- Representatives of all interest groups (including some current members of "Animalia") have been invited to workshops during the preparation of both editions of brown bear management plan (in 2004 and 2008)
- Before finalizing each edition of management plan a comprehensive public survey was conducted in 2002 and 2008 (see reference in Annex III: Aleksandra Majić, Agnese Marino Taussig de

Bodonia, Đuro Huber, Nils Bunnefeld (2011). Dynamics of public attitudes towards bears and the role of bear hunting in Croatia. Biological Conservation 144 (2011) 3018–3027.)

• Once every year a workshop with all institutions involved in bear management is organized as a preparation for the next year Action plan.

The list of relevant and fully implemented documents:

1. Brown bear management plans:

- Dečak, Đ., Frković, A., Grubešić, M., Huber, Đ., Iviček, B., Kulić, B., Sertić, D., Štahan Ž. (2005) Brown bear management plan for the Republic of Croatia. Ministry of Agriculture, Forestry and Water Management, Department for Hunting and Ministry of Culture, Department for Nature Protection. Zagreb. 90 pp.
- Đuro Huber, Zrinko Jakšić, Alojzije Frković, Željko Štahan, Josip Kusak, Dario Majnarić, Marijan Grubešić, Blaženka Kulić, Magda Sindičić, Aleksandra Majić Skrbinšek, Vladimir Lay, Maša Ljuština, Davor Zec, Robert Laginja, Ivica Francetić (2008) Brown bear management plan for the Republic of Croatia. Ministry of Regional Development, Forestry and Water Management, Directorate for Hunting and Ministry of Culture, Nature Protection Directorate. Zagreb. 86 pp.

2. The annual Brown bear action plans since 2004:

- Action plan for the brown bear management in the Republic of Croatia in 2004
- Action plan for the brown bear management in the Republic of Croatia in 2005
- Action plan for the brown bear management in the Republic of Croatia in 2006
- Action plan for the brown bear management in the Republic of Croatia in 2007
- Action plan for the brown bear management in the Republic of Croatia in 2008
- Action plan for the brown bear management in the Republic of Croatia in 2009
- Action plan for the brown bear management in the Republic of Croatia in 2010
- Antonija Bišćan, Ivica Budor, Ivica Francetić, Alojzije Frković, Stjepan Gospočić, Marijan Grubešić, Đuro Huber, Zrinko Jakšić, Magda Sindičić, Željko Štahan, Davor Zec (2011) Action plan for the management of brown bear in the Republic of Croatia in 2011. Ministry of Regional Development, Forestry and Water Management. 19 pp.

The list of scientific papers in peer reviewed journals and other publications and past and current projects dealing with brown bear and other large carnivores is provided in **Annex III**.

RESPONSE TO THE CLAIMS IN THE COMPLAINT BY CHAPTERS:

1. Current legal situation

This chapter of the complaint deals with the distribution of the brown bear in Croatia and the areas of the bear habitat where hunting is allowed.

The bear distribution areas in Croatia are categorized into areas with permanent bear presence and areas with occasional bear presence.

Habitats with permanent bear presence are areas in which bears satisfy all their food, water, space, tranquillity, cover, breeding and denning needs and in which bears are present all year round. In those areas all prescribed protective measures are implemented in order to ensure the stability of the population. Local inhabitants accept bears as part of their natural environment.

Habitats with occasional bear presence are areas with a sporadic presence of bears or areas in which the number of bears does not guarantee the continued existence of the species. Also, there are no permanent denning activities in these areas. In short, these are habitats where bears are returning and which are connected to permanent bear presence areas in Croatia, Slovenia or Bosnia and Herzegovina. Bears do occasionally cause damage in these areas. Within occasional bear presence habitats there are areas where bear presence is desirable and areas where bear presence is undesirable.

According to the Brown bear management plan for the Republic of Croatia from 2008, the total bear distribution area in Croatia extended over $11.824,33 \text{ km}^2$ (1.237.217 ha). The permanent bear presence habitat extended over $9.253,47 \text{ km}^2$, while the occasional bear presence habitat extended over $2.570,86 \text{ km}^2$. Of the permanent bear presence area 94, 2% are hunting grounds, while 5,8% are parts of national parks, where bears are permanently protected and no hunting is allowed.

The statement in the complaint, that in the area of occasional bear presence there is a hunting quota, is not correct while in that area only intervention removals are allowed.

2. How the Management plan and Action plans were adopted?

General public was widely consulted in the process of preparation of each of the two bear management plans (2005 and 2008). Both times a wide, professionally conducted survey of local public opinion was performed (see reference in Annex III - Majić et al, 2011). The positions like the ones held by Animalia were in minority; most of the people see the bear population growing and do support hunting.

The workshop with all interest groups was held at the beginning and shortly before the end of the process of making each of the bear management plans.

3000 posters about bears were printed: about brown bear biology, implementation of the bear management and the advantages bears can bring to local communities.

The plan was presented to the public during five one-day "open house" information sessions held in Delnice (2 times), Mrkopalj, Gospić and Krasno.

The annual workshops with all stakeholders involved in bear management were held as a preparation for the next year Action plan. Here are the summaries of the last two:

 \geq 2010 - On June 30th 2010 a workshop on bear management was held in Risnjak National Park with representatives off all hunting grounds that manage bears. A total of 59 participants attended. The data was presented through seven presentations:

- Total mortality (Z. Jakšić)
- Sex and age distribution (M. Sindičić)
- Damages by bears (M. Sindičić)
- Bear counts at feeding sites and by genetic individual identification (D. Huber)
- Heavy metals analyses (D. Huber and M. Lazarus)
- Bear hunting at feeding sites in Croatia compared to stalking in Sweden (D. Huber)
- HUNT project activities (S. Reljić and V. Kereži)

In the subsequent discussion most participants agreed that there is no need for major changes in bear management regime. The participants were also called for continued and intensified collaboration on data and samples collection.



Figure 1. Bear management workshop in Risnjak NP on June 30th 2010

> 2011 - On June 15th 2011 a regular workshop on bear management was held with representatives off all hunting grounds that manage bears, only this time in Brinje, Lika region. About 40 people attended. The current data was presented through eight presentations:

- Introduction on the reason for this workshop (Davor Zec)
- Review on the bear mortality in 2011 (Zrinko Jakšić)
- Analyses of mortality by sex and age (Đuro Huber)
- Bear damages (Đuro Huber)
- Population trend (feeding sites counts) (Duro Huber)
- Genetic results (Đuro Huber)
- Results on heavy metals and pesticides (Maja Lazarus and Đuro Huber)
- HUNT project results and plans (Slaven Reljić and Vesna Kereži)

In the subsequent discussion, the issue of heavily male-biased bear mortality was recognised as a potential long-term problem. However, several participants objected the possible concept to put the pressure on killing more females, and also elaborated why so few were hunted. Anyhow, it was foreseen that the Action plan for 2012 will put the requirement for the hunting grounds that have more than one bear in quota per year, that 50% of bears shot must be bellow 100 kg.

3. How was the bear population size defined?

We are aware that bear population size estimates could be better (more precise) but feel safe that the estimate guarantees the minimum number of bears. All the calculations done with three different formulas (see the scientific article "Genetic diversity of Dinaric brown bears (*Ursus arctos*) in Croatia with implications for bear conservation in Europe" in **Annex IV**) actually gave numbers above 1000. Also it can be seen that only samples from the two paired years were used (not five as Animalia states). The full coverage of bear range and collection of about 3000 samples is planned for fall 2012 or 2013, depending on funding possibilities (cost of operation is estimated at about 150.000 EUR). With less than 10.000 EUR available in the last two years together we could do only a very limited study.

The estimates of bear numbers that were published in the past, as quoted by Animalia are correct and only indicate the continuous growth of population. The modelling shows an annual growth of about 7%, which means that the population is doubling in size every10 years. The current hunting did not cause an effect on the population size but it may had on the sex ratio. The population modelling done for the HUNT project is expected to evaluate the long-term effect of it.

4. Forest management and 5. Forest infrastructure

Modern approach to forestry in Republic of Croatia sees hunting as one in the line of activities in forestry auspice. Hrvatske šume d.o.o. (Croatian Forests limited liability company) as the main holder of hunting rights in Croatia pay special attention to the welfare of game animals, protected species as well as other animal species present in the forests.

On the ground of forest management plans Hrvatske šume d.o.o. manages over 2.000.000 hectares of forests and forest lands owned by the Republic of Croatia. Croatian forestry has a 250 year long tradition of making forest management plans and today we can say that we have forest management plans for all forests and forest lands managed by Hrvatske šume d.o.o. The basis for the planning of forest management is the Forest Management Basis, which includes data on former management and current state of all forests and forest lands on the territory of Croatia, as well as legislation for the upcoming ten years. Current Forest Management Basis for the territory of Croatia is valid for the time period from January 1st 2006 till December 31st 2015.

Forests and forest lands owned by Croatia are divided in over 600 management units for which forest management plans are being made. Each forest management plan includes Nature Protection Requirements issued by the ministry competent for nature protection with nature protection measures

elaborated by the State Institute for Nature Protection. Amongst other things, Nature Protection Requirements prescribe the quantity of dead wood that needs to stay in the forest.

Process of planning, construction and management of forest infrastructure is being done in accordance with technical and ecological conditions, as well as economic value of forest ground, plants, and game animals, bearing in mind their maximum protection. Forest roads are being constructed, managed and used in a way that it does not endanger ecologically important parts of forest ecosystems - parts of the ecological network, habitats of rare and endangered species, as well as without damaging areas important for feeding and denning of game animals.

Forest management plans are approved by the ministry competent for forestry. For management units that incorporate areas that are protected by nature protection regulations, a prior approval of forest management plans is given by the ministry competent for nature protection, before the approval by the ministry competent for forestry. Prior to activation of the procedure for approval of forest management plan, Hrvatske šume d.o.o. organize a public insight and public hearing where all interest parties can give their remarks and suggestions.

By checking the data in the Forest Management Basis for territory of Croatia and the data in plans and programs for forest management, both current and past, it can be seen in what state our forests are today and how it was decades ago, and then draw valid conclusions on our present forest management. From the above mentioned data, apart from the structural change in forests, it is visible that cutting in state owned forests is less than their accession, that being the basis of sustainable forest management.

A proof of proper forest management is also a prestige international FCS certificate which was given to Hrvatske šume d.o.o. in the year 2002 and which they manage to keep till today.

6. Poaching and poisoning

Radio-telemetry research has confirmed poaching of 5 bears since 1981 (last in 2005), among a total of 40 collared animals. The situations when only a signal was lost but a body or a collar never found (likely the electronic failure) are not taken into account. The incidence of poaching is not negligible but is much less than with wolves.

The Brown Bear Intervention Group has so far rescued 3 bears from poacher's snares. This confirms that poaching is present, though it was not targeted to bears but to other wildlife (wild boar and roe deer).

As an example, the report about one rescue operation from 2010 is provided in **annex V**.

Only one poisoned bear has been found recently. This case of carbofuran poisoning is mentioned in the complaint. The fact that the case was thoroughly studied and reported shows that there was no intention of hiding it; on the contrary it was fully covered as a negative example (thought a target species apparently was not the bear). The abstract about this case is provided in **annex V** and the complete article is in the process of being published.

7. Competence among the ministries

In 2005 the ministry competent for hunting (at that time the Ministry of Agriculture and Forestry) and the ministry competent for nature protection (at that time the Ministry of Environmental Protection and Physical Planning) have formed a Committee for the Elaboration of the Brown Bear Management Plan and the annual Action Plan. The Committee carries out revisions of the management plan and the action plans and is also responsible for reporting.

The Ministry of Agriculture and the Ministry of Environment and Nature Protection are both responsible for the implementation of the plan. However, the practical implementation is under the competence of the Ministry of Agriculture, since the brown bear is a game species regulated by the Hunting Act. Competences for the implementation of the Management Plan and the Action Plan are elaborated in detail in these documents.

In the complaint the case of an orphan bear cub that ended in bear sanctuary in Kuterevo is mentioned. In order to provide complete and correct information, we are providing a summary of actions taken in this case as reported by the Brown Bear Intervention Group:

A bear cub without mother has been seen in early April 2010 in the Kupa river valley. After seeing the bear alone for several days in a row, on April 12th 2010 a person from the local hunting club took it home. By doing that he violated the official procedure by not consulting the local member of the Brown Bear Intervention Group or the Department for Hunting of the Ministry of Regional Development, Forestry and Water Management. At that time the bear sanctuary in Kuterevo has not been officially registered to legally house brown bears so a request to place this bear in Kuterevo was declined. A decree to release the bear back to nature was issued and the bear was released on April 16th. Still, the animal was in constant contact with people and became totally habituated. In early September the Veterinary Department of the Ministry for Agriculture issued a temporary permit for Kuterevo to keep bears which enabled the placement of this bear in the sanctuary. On September 22nd 2010 the experts from the Faculty of Veterinary Medicine immobilized the bear, performed standard measurements, microchipping and blood sampling and the transfer was executed. Further inspection showed that the bear is doing fine in the sanctuary, although it is clearly much habituated to people and behaves very tame.



Figure 2. Releasing the cub in an enclosure in Kuterevo

8. Garbage dump problem

As a part of LIFE project "Improving coexistence of large carnivores and agriculture in Southern Europe" (LIFE04NAT/IT/000144) an activity entitled "Prevention of bear access to garbage" was implemented. As a part of this activity recommendations for "bear-safe" garbage management were given to local authorities and communal companies and two types of "bear proof" bins have been designed – smaller $0,70 \text{ m}^3$ garbage baskets and bigger 5 m³ garbage containers. The bins are made of metal and have lids which bears cannot open and that can be opened only by humans. Seven small baskets and nine big bear proof containers were donated and also national parks, nature parks, local units and communal companies in the bear habitat have been invited to use the same bear proof containers. Also, a campaign was launched (presented by two press conferences) with the goal of educating the public and raising awareness about this problem. The logo "Garbage kills bears" (which is still used to promote the issue) and educational leaflets have been designed. Management of problematic bears that are habituated to feeding on garbage is of a special concern in our bear management and each case is evaluated separately and necessary actions are taken in coordination with Brown Bear Intervention Group. During the years electrical fencing for one of the most problematic dumpsters Sović Laz in Gorski kotar was performed, on several occasions translocations of problematic cubs, adverse conditioning of problematic animals and when necessary removal of individuals was organized.

CLOSING REMARKS

Contrary to claims of Animalia, the bear population in Croatia is growing and the main management goals are:

- To keep the population within the "social carrying capacity". That means to prevent the further growth which is currently at the doubling rate at 10 years interval.
- To maintain the positive attitude of people. The profit from hunting is one of the ways that gives the value to bears.
- To keep the damages as low as they are now.

• To minimize the appearing of problem bears and to act when necessary (including intervention shooting).

Mortality monitoring

One of the key tools to ensure the proper management is to carefully monitor each bear mortality (the example from the year 2010 is attached as **Annex VI**), which includes taking measurements and samples (tooth for age, muscle for DNA, liver, kidney and muscle for heavy metals, fat for pesticides). The 2010 Action Plan assigned 100 bears for hunting and up to 40 for other losses. Hunting took 85 (61 males, 24 females) and other losses 33 (19 males, 12 females, 2 unknown). Among other losses 27 were due to traffic, 3 to interventions, 1 was poached (poisoned), 1 orphan cub was placed to the Kuterevo sanctuary and 1 cause of death was not determined.

The evident bias towards higher mortality of male bears is under careful investigation through advanced modelling within the HUNT project (FP7). The age structure of bear population and the influence of hunting on age pyramid are also under study. Additional concern is the bear mortality in Slovenia which is above the sustainable level. Data from both countries are gathered, including the age from tooth sections, and the first useable results are expected at the end of 2011.

Bear age structure

The age of 167 bears from 2009 and 2010 has been determined and that was the base for the age structure analyses (table with the results provided in **Annex VI**). The average age of bears killed in quota in Croatia was $5,47\pm0,27$ years (n=167). For comparison, in Slovenia average age of shot bears was $2,82\pm0,12$ years (n=418). Current obstacle in the implementation of bear management is that hunters regularly fail to use the given quota. As seen in the table below, since the beginning of Bear Management Plan implementation only 75% of expected bear mortality occurred. On one side this indicates relatively low hunting pressure and no need for poaching. Hunters try to sell each bear (what increases the pressure on bigger animals and males) and the market (demand) mostly regulates hunting. The Action Plan for 2012 requires that close to 50% of the bears shot must be below certain body mass category (100 kg) and that the quota is fulfilled.

Year	Hu	nting	Other	Other losses			
	Plan	Realized	Expected	Occurred			
2005.	80	31 (39%)	20	21 (105%)	52 (52%)		
2006.	70	49 (70%)	30	36 (120%)	85 (85%)		
2007.	70	50 (71%)	30	8 (27%)	58 (58%)		
2008.	70	64 (91%)	30	47 (156%)	111 (111%)		
2009.	100	86 (86%)	40	24 (60%)	110 (79%)		
2010.	100	86 (86%)	40	33 (82%)	119 (85%)		
2011	100	68 (68%)	40	14 (35%)	82 (59%)		
Total	590	434 (74%)	230	183 (80%)	617 (75%)		

In conclusion, we hope that we have replied to the concerns listed in your letter. However, we will be glad to provide any additional information if requested.



REPUBLIC OF CROATIA MINISTRY OF ENVIRONMENT AND NATURE PROTECTION 10000 Zagreb, Ulica Republike Austrije 14 Tel: 01/3782-111, faks: 01/3782-157

Class: 351-01/11-02/780 Reg.Num.: 517-12-2 Zagreb, 10 January 2012

> Ministry of Environment and Nature Protection Directorate for Nature Protection Runjaninova 2, Zagreb

MATTER: Unsustainable management of Brown bear (*Ursus arctos*) in Croatia Answer, is given

Ministry of Environmental Protection, Physical Planning and Construction has received a memorandum from the Ministry of Culture (today they both fall under the same Ministry of Environment and Nature Protection) regarding the complaint from the NGO Udruga Animalia denouncing a possible breach of the Bern Convention with regards to the presumed unsustainable management of the Brown bear population.

In Chapter 9 there is mention of the garbage dump sites problem which attract bears who then get used on human food, lose the fear of humans and get in closer contact with them. We hereby recognize the problem and wish to inform you that, according to Article 18 of the Waste Act (Official Gazette No. <u>178/04</u>, <u>111/06</u>, <u>60/08</u>, <u>87/09</u>) the City of Zagreb, town or municipality shall ensure the removal and disposal and/or recovery of waste discarded by an unknown person into the environment in their respective areas. If the person responsible for providing municipal sanitation services in a town or municipality does not dispose of the waste that an unknown person has discarded into the environment, the waste in question shall be disposed of by the county at the expense of the town or municipality budget. If the person responsible for providing municipal sanitation services in the City of Zagreb does not dispose of the waste that an unknown person has discarded into the environment, this waste shall be disposed of by the City of Zagreb at the expense of its budget. The county, City of Zagreb, town and municipality shall have the right to a return of expenses from the person who has illegally discarded waste into the environment. If the garbage dump sites are highly burdened with hazardous waste by an unknown person, a person who has ceased to exist or if it has no legal successors, the State shall ensure the remediation of such environment according to Article 18a of the Waste Act.

The statement from page 11 of the complaint that "in Croatia we do not have an example that the EIA study has expressed negative opinion regarding planned investment" is not true as we have had EIA studies with negative opinions. The environmental impact assessment procedures are conducted in compliance with the Regulation of environmental impact assessment (Official Gazette No. 64/08, 67/09).

If the NGO Animalia knows the locations of the mentioned garbage sites, it should report them to the Directorate for Inspection Affairs of the Ministry of Environment and Nature Protection as well as other violations of the Environmental Protection Act (Official Gazette No. 110/07), the Waste Act and/or regulations adopted on their basis.

DEPUTY MINISTER

Hrvoje Dokoza

LIST OF SCIENTIFIC PAPERS IN PEER REVIEWED JOURNALS AND OTHER PUBLICATIONS, AS WELL AS PAST AND CURRENT PROJECTS DEALING WITH BROWN BEAR AND OTHER LARGE CARNIVORES

SCIENTIFIC PAPERS:

- Huber Đ., Kusak J., Majić-Skrbinšek A., Majnarić D., and Sindičić M., 2008. A multidimensional approach to managing the European brown bear in Croatia. Ursus 19, pp.22-32.
- Kocijan, I. and Huber, D., 2008. Conservation genetics of brown bears in Croatia. Final report. Project Gaining and maintaining public acceptance of Brown bear in Croatia (BBI-Matra/2006/020 through ALERTIS).
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- Modrić, Z. and Huber, D. 1993. Serologic survey for leptospirae in European brown bears (Ursus arctos) in Croatia. Journal of Wildlife Diseases. 29: 608-611.
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- Frković, A., Huber, D. Kusak, J. 2001. Brown bear litter sizes in Croatia. Ursus 12:103-106.
- Clark, J. D., Huber, D. Servheen, C. 2002. Reintroducing bears: lessons and challenges. Invited paper. Ursus. 13:153-163.

- Kusak, J., Barić Rafaj, R., Žvorc, Z., Huber, D., Foršek, J., Bedrica, L., Mrljak, V. 2005. Effects of sex, age, body mass and capturing method on hematologic values of brown bears in Croatia. Journal of Wildlife Diseases. 41(4): 843-847.
- Kaczensky, P., Huber, D., Knaer, F., Roth, H., Wagner, A., Kusak, J. 2005. Activity patterns of brown bears (*Ursus arctos*) in Slovenia and Croatia. Journal of Zoology. 269: 474-485.
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PROJECTS DEALING WITH BROWN BEAR AND OTHER LARGE CARNIVORES:

- Improving coexistence of large carnivores and agriculture in S. Europe –COEX (LIFE project). Institute for Applied Ecology, Rome.
- Building capacity to meet the challenges of multi-level democracy: the case of conserving species with transboundary populations. Norwegian Institute for Nature Research (NINA), Trondheim, Norway.
- Gaining and maintaining public acceptance of brown bear in Croatia BBI MATRA project. ALERTIS fund for bear and nature conservation, Rhenen, The Netherlands.
- Transboundary cooperation in management, conservation and research of the Dinaric lynx population (DinaRis). INTERREG IIIA project. Neighbourhood Programme Slovenia-Hungary-Croatia 2004-2006.
- Conservation of large carnivores in Croatia. (Co-funding for DinaRis project) EURONATUR, Germany.
- Contract on genetic analyses of Croatian brown bear population. Ministry of Agriculture, Forestry and Water Management.
- Monitoring animal crossings bandwidth on the Zagreb Split freeway, Hrvatske autoceste d.o.o.
- Contract about co-financing the continuation of the project activities on enforcement of the Plan for Wolf Management in Croatia. State Institute for Nature protection.
- Contract about co-financing the continuation of the project activities on enforcement of the Plan for Lynx Management in Croatia. State Institute for Nature Protection.
- "Recreation of the BALKAN NET, a network of conservation bodies in countries sharing continuous large carnivore populations" SERA ERA project Reference number 06 1000031 10491. Leader for the Faculty of Veterinary Medicine prof. dr. sc. Đuro Huber
- "HUNTING FOR SUSTAINABILITY (HUNT)" FP7 project, European Commission.
- Study on animal crossings bandwidth on the Rijeka Zagreb freeway, Autocesta Rijeka Zagreb d.o.o.
- Business Cooperation Agreement of large carnivores monitoring (wolf, bear and lynx) on the preliminary Ecology Network NATURA 2000 areas. State Institut for Nature Protection. Associates from the Faculty of Veterinary Medicine: Prof. dr. sc. Đuro Huber and doc. dr. Josip Kusak
- "The role of natural resources in sustainable rural livelihoods in the western Balkans, the distribution and flow of costs and benefits", The Norwegian Institute for Nature Research (NINA), PO Box 5685 Sluppen, NO-7485 Trondheim, Norway.

All these projects were obtained through rigorous international competitions or within Croatia through public bids (opened to everyone including NGO Animalia). It should be noted that none of the projects were financed, directly or indirectly, through hunting organizations.

SCIENTIFIC ARTICLE "GENETIC DIVERSITY OF DINARIC BROWN BEARS (URSUS ARCTOS) IN CROATIA WITH IMPLICATIONS FOR BEAR CONSERVATION IN EUROPE"

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Pdf format available at the Secretariat

CASES OF POACHING AND POISONING

Rescue and collaring the bear in the poacher's trap

Following the phone call by local Bear Emergency Team member on 15 October 2010 an expert from the Faculty of Veterinary Medicine in Zagreb handled the brown bear captured in the poacher's snare near Pazarište in Lika. Upon arrival on site the bear was seen in the hedge along the local road. It was immobilized with a dart gun.



Figure 1. Bear captured in the poacher's snare near Pazarište in Lika on 15 October 2010

Animal was agitated after darting and jumped around as much as the 7m long cable allowed (5 mm diameter) what luckily did not result in injuries.

The bear was a female weighing 82 kg and about 3 years old. All needed measures and samples were taken. The bear was marked with a microchip and a GPS/UHF radio collar was put on.



Figure 2. An immobilized bear.



Figure 3. Poacher's snare.

Upon leaving the bear at the forest edge all members of the rescue team left the site. The map of the movements in the first week shows that the bear recovered and resumed using the current range.

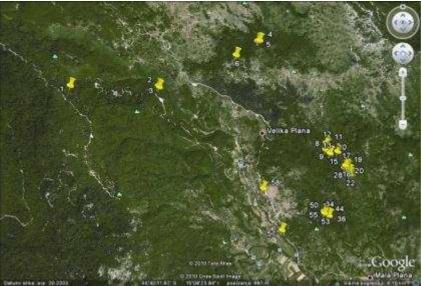


Figure 4. The first week of movements of the bear in subject

A case of brown bear poisoning with carbofuran in Croatia

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On April 19th 2010 in the hunting ground adjacent to the Velebit Nature Park near natural water hole the jackal (*Canis aureus*) corpse was found, and the day later a dead European brown bear at a distance of 200 m was found as well (coordinates: X 5545067, Y 4906858). There were no visible injuries on bear carcass; only on the front legs the vomited watery content was found. On three spots near the water found were baits consisting of meat, bones, and dark blue compact granules, which

indicated that they were placed after the last rain, or a maximum of 6 days ago. On April 21st the veterinarian from the Faculty of Veterinary Medicine Zagreb accompanied by police officers visited the site. Veterinarian performed an autopsy and found a bloating corpse, moderate autolysis and congestion of organs. Liver and kidney tissue samples and entire ligated stomach were taken. All baits found along the water hole were collected too. Frozen tissue samples and baits were sent to the police forensic laboratory. In the stomach a small amount of liquid bluish content was found. By the gas chromatography-mass spectrometry (GC-MS) the presence of carbofuran (insecticide from the carbamate group) was determined in the stomach content and in the baits. In kidney and liver tissue the presence of the carbofuran was proven by high performance liquid chromatography-mass spectrometry (LC-MS) with mean measured concentrations of 2.695 and 12.650 ppm, respectively. Based on the findings of high concentrations of carbofuran in liver and kidney tissue, a short distance between baits along the water hole and the place where the corpse was found, with certainty we claim the animal was per acutely poisoned by this compound. This is the first proven record of a poisoned bear in Croatia, although the bait was probably not intended for bears.

BROWN BEAR MORTALITY AND AGE STRUCTURE ANALYSES

No	AnimalID	Sex	Search type	Date	Time	SiteName	Age* (est.)
1	RH0075-10	М	Legal hunting	06.03.2010	20:03:00	Karlovci, VELIKI VUJNOVAC IX/20	3
2	RH0040-10	F	Legal hunting	08.03.2010	16:30:00	Žage, KUPJAČKI VRH VIII/10	2
3	RH0019-10	М	Legal hunting	13.03.2010	18:15:00	Staja, KUPIČKI VRH VIII/111	4
4	RH0028-10	М	Legal hunting	23.03.2010	19:32:00	Potočine, BJELOLASICA VIII/2	6
5	RH0090-10	М	Legal hunting	24.03.2010	10:20:00	Atlinovac, OTOČAC IX/106	
6	RH0055-10	М	Legal hunting	24.03.2010	18:50:00	Zeleno Bilo, RIČIČKO BILO VIII/29	4
7	RH0048-10	М	Legal hunting	24.03.2010	21:20:00	Karnica, SMREKOVA DRAGA VIII/21	7
8	RH0029-10	F	Legal hunting	25.03.2010	19:05:00	Gluhe drage, BJELOLASICA VIII/2	3
9	RH0049-10	М	Legal hunting	26.03.2010	19:15:00	Prapotna draga, SMREKOVA DRAGA VIII/21	4
10	RH0030-10	F	Legal hunting	26.03.2010	22:30:00	Slavica, BJELOLASICA VIII/2	12
11	RH0056-10	Μ	Legal hunting	26.03.2010	22:30:00	Kozjan, GOLO TRLO IX/2	7
12	RH0031-10	М	Legal hunting	27.03.2010	23:04:00	Malo Duboko, BJELOLASICA VIII/2	7
13	RH0032-10	М	Legal hunting	28.03.2010	20:32:00	Potočine, BJELOLASICA VIII/2	6
14	RH0063-10	F	Legal hunting	28.03.2010	20:40:00	Crni vrh, KREKOVAČA IX/7	4,5
15	RH0069-10	М	Legal hunting	28.03.2010	23:15:00	Lipovača, SJEVERNI VELEBIT IX/14	6
16	RH0022-10	М	Legal hunting	30.03.2010	2:00:00	Mrzle drage, MRKOPALJ VIII/114	3
17	RH0017-10	М	Legal hunting	30.03.2010	19:50:00	Gmajna-Doline, CRNA GORA VIII/110	12
18	RH0024-10	Μ	Legal hunting	07.04.2010	19:25:00	Dimovac, KUPA VIII/116	3
19	RH0080-10	М	Legal hunting	07.04.2010	22:01:00	Bubinica, SREDNJI VELEBIT IX/30	13,5
20	RH0064-10	М	Legal hunting	08.04.2010	20:15:00	Trošelj Seline, LUKOVO ŠUGARJE IX/9	7
21	RH0046-10	F	Legal hunting	09.04.2010	19:55:00	Suha Rečina, RISNJAK VIII/19	6
22	RH0070-10	М	Legal hunting	10.04.2010	18:50:00	Okrugi, SJEVERNI VELEBIT IX/14	4
23	RH0045-10	F	Legal hunting	11.04.2010	20:35:00	Čopov laz, RISNJAK VIII/19	3,5
24	RH0023-10	М	Legal hunting	13.04.2010	19:30:00	Dedinski Vrh, PETEHOVAC VIII/115	15
25	RH0047-10	М	Legal hunting	14.04.2010	19:10:00	Čopov laz, RISNJAK VIII/19	7
26	RH0052-10	М	Legal hunting	16.04.2010	19:40:00	Vršice, "SNJEŽNIK" VIII/22	6
27	RH0003-10	M	Legal hunting	19.04.2010	20:40:00	BUKOVAČA - IV/3	11
28	RH0065-10	M	Legal hunting	19.04.2010	21:30:00	RAMINO KORITO - IX/10	5
29	RH0002-10	М	Legal hunting	20.04.2010	20:45:00	Trojvrške livade, BRŠLJANOVICA IV/2	11
30	RH0018-10	М	Legal hunting	21.04.2010	23:30:00	Gmajna-Doline, CRNA GORA VIII/110	10
31	RH0057-10	М	Legal hunting	22.04.2010	1:00:00	Baljkuša, GOLO TRLO IX/2	6
32	RH0096-10	М	Legal hunting	23.04.2010	2:20:00	Lisina-Crni vrh, MASLOVARA XIII/16	7,5
33	RH0004-10	F	Legal hunting	23.04.2010	19:55:00	Kozji Kamen, KLEK IV/5	7,5
34	RH0033-10	М	Legal hunting	24.04.2010	20:25:00	Malo Duboko, BJELOLASICA VIII/2	7
35	RH0050-10	М	Legal hunting	24.04.2010	22:03:00	Gržin laz-čeka, SMREKOVA DRAGA VIII/21	5

Table 1. Bear mortality due to hunting and other reasons in 2010

36 37	RH0020-10	F	т 11 /			VRANJAK VIII/112	
37		Г	Legal hunting	26.04.2010	19:30:00	(LIČ-FUŽINE)	3
	RH0027-10	М	Legal hunting	27.04.2010	20:30:00	Medvetka, LIPOV VRH VIII/119	2
38	RH0088-10	М	Legal hunting	28.04.2010	21:35:00	Jukina krčevina, BILO IX/39	3,5
39	RH0077-10	М	Legal hunting	29.04.2010	22:00:00	Kik, PLJEŠIVICA IX/26	6
40	RH0098-10	М	Legal hunting	30.04.2010	3:15:00	Ošljak, SV. BRDO XIII/29	3
41	RH0012-10	F	Legal hunting	16.09.2010	19:50:00	Debeli lug, DEBELI VRH IV/18	5,5
42	RH0067-10	М	Legal hunting	17.09.2010	1:06:00	SENJ - IX/13	11
43	RH0042-10	М	Legal hunting	06.10.2010	20:30:00	LITORIĆ - VIII/12	3
44	RH0060-10	М	Legal hunting	07.10.2010	19:05:00	Radić Uvala, GODAČA IX/5	10
45	RH0108-10	М	Legal hunting	12.10.2010	19:10:00	RAMINO KORITO - IX/10	4
46	RH0081-10	М	Legal hunting	21.10.2010	21:15:00	Bubinica, SREDNJI VELEBIT IX/30	15
47	RH0084-10	Μ	Legal hunting	21.10.2010	21:25:00	Kojnovac, LJUBOVO IX/35	8,5
48	RH0100-10	М	Legal hunting	21.10.2010	21:45:00	Zeleno Bilo, RIČIČKO BILO VIII/29	8
49	RH0034-10	F	Legal hunting	22.10.2010	0:15:00	Bunarine, BJELOLASICA VIII/2	4
50	RH0109-10	М	Legal hunting	22.10.2010	20:10:00	Gmajna-Doline, CRNA GORA VIII/110	6
51	RH0071-10	F	Legal hunting	22.10.2010	21:50:00	Golo brdo	5
52	RH0059-10	F	Legal hunting	23.10.2010	18:30:00	Dulibe, CRNO JEZERO IX/4	4
53	RH0061-10	F	Legal hunting	24.10.2010	0:35:00	Grabarje, JABLANAC IX/6	4,5
54	RH0073-10	М	Legal hunting	25.10.2010	0:30:00	SVETI JURAJ - IX/17	
55	RH0068-10	М	Legal hunting	25.10.2010	4:30:00	Lukovica, SENJ IX/13	
56	RH0013-10	М	Legal hunting	27.10.2010	21:43:00	Dubine Tom, DEBELI VRH IV/18	5,5
57	RH0107-10	F	Legal hunting	28.10.2010	9:20:00	GOLO TRLO - IX/2	4
58	RH0078-10	М	Legal hunting	31.10.2010	19:30:00	Tičevo, PLJEŠEVICA IX/26	7
59	RH0087-10	F	Legal hunting	11.11.2010	19:15:00	Tisov vrh, MARKOVIĆ-RUDINE IX/38	5
60	RH0014-10	F	Legal hunting	12.11.2010	17:03:00	Sekulinka, DEBELI VRH IV/18	4,5
61	RH0053-10	М	Legal hunting	14.11.2010	20:15:00	Šanjsko Pleće, ZAVRŠJE VIII/26	6
62	RH0026-10	М	Legal hunting	15.11.2010	18:00:00	Miletka, JELENSKI JARAK VIII/118	5
63	RH0037-10	М	Legal hunting	16.11.2010	19:30:00	CETIN - GLOŽAC - VIII/3	3
64	RH0106-10	М	Legal hunting	19.11.2010	1:45:00	Klokoč 1, BITORAJ IX/24	4,5
65	RH0041-10	F	Legal hunting	19.11.2010	18:05:00	Rogi-čeka, KUPJAČKI VRH VIII/10	8
66	RH0051-10	F	Legal hunting	19.11.2010	20:00:00	Karnica čeka, SMREKOVA DRAGA-GUMANCE VIII/21	5
67	RH0102-10	F	Legal hunting	19.11.2010	21:30:00	Mrzle drage, MRKOPALJ VIII/114	3
68	RH0105-10	М	Legal hunting	20.11.2010	19:24:00	Begovača, SJEVERNI VELEBIT IX/14	6
69	RH0025-10	F	Legal hunting	20.11.2010	19:40:00	Račkova žaga 2	5
70	RH0035-10	F	Legal hunting	20.11.2010	22:10:00	Malo Duboko, BJELOLASICA VIII/2	5
71	RH0155-10	F	Legal hunting	23.11.2010	18:30:00	Gmajna-Doline, CRNA GORA VIII/110	4
72	RH0086-10	М	Legal hunting	27.11.2010	17:10:00	Korita, VRH JELOVI IX/37	3,5
73	RH0007-10	F	Legal hunting	27.11.2010	17:15:00	Tisovac, VELIKA KAPELA IV/11	4
74	RH0066-10	М	Legal hunting	11.12.2010	21:15:00	Pećinski vrh (Zelenike), RISOVAC IX/12	4
75	RH0076-10	М	Legal hunting	11.12.2010	22:30:00	Pločanska poljica, VREBAC IX/23	5
76	RH0093-10	M	Legal hunting	12.12.2010	10:30:00	Tavani, JELOVI TAVANI XIII/6	5
77	RH 0062-10	M	Legal hunting	13.12.10.		Drž. lov. br: IX/6 "JABLANAC"	3-4
78	RH 00153-10	М	Legal hunting	13.12.10.		Drž. lov. br: VIII/19 – "RISNJAK"	6

CONSERVATION GENETICS OF BROWN BEARS IN CROATIA

FINAL REPORT

by Ivna Kocijan, University of Zagreb, Faculty of Science, Division of Biology, Croatia and by Đuro Huber, Veterinary Faculty, University of Zagreb, Croatia Zagreb, 25 September 2008

INTRODUCTION

Brown bear (*Ursus arctos*) population in Croatia is estimated roughly at 600-1000 individuals. This estimation has no firm scientific background, but is used for management decisions, including hunting quotas (Dečak, Đ., A. Frković, M. Grubešić, Đ. Huber, B. Iviček, B. Kulić, D. Sertić, Ž. Štahan. 2005. Brown bear management plan for the Republic of Croatia. Ministry of agriculture, forestry and water management. Zagreb. 90 pp., Đuro Huber, Zrinko Jakšić, Alojzije Frković, Željko Štahan, Josip Kusak, Dario Majnarić, Marijan Grubešić, Blaženka Kulić, Magda Sindičić, Aleksandra Majić Skrbinšek, Vladimir Lay, Maša Ljuština, Davor Zec, Robert Laginja, Ivica Francetić. 2008. Plan gospodarenja smeđim medvjedom u Republici Hrvatskoj. Ministarstvo regionalnog razvoja, šumarstva i vodnoga gospodarstva, Uprava za lovstvo Ministarstvo kulture, Uprava za zaštitu prirode, Zagreb, 86 pp).

Although conventional methods such as direct counting, telemetry, trapping etc. are used and accepted in the scientific community, they may be difficult or expensive to use in case of large and elusive animals, such as bears. Moreover, such surveys are longitudinal in time and provide a cumulative estimate of population size that includes animals which have been born, have migrated or have died. Recently, molecular genetic methods are available to ecologists and allow them to detect and count animals in the wild through non-invasive sampling of faeces, hair, feathers etc. In contrast to conventional methods, this approach can be designed in such a way that it gives a relatively instantaneous point count of population as the samples are collected in great number in a short period of time over previously determined areas. Not only is this approach much less time consuming than conventional methods, but it is also more accurate and feasible.

The goal of the project was to estimate brown bear population size by means of molecular genetic analysis of faecal (scat) DNA. By analysing scats collected over three study areas, we would be able to identify each individual bear and make an estimate of brown bear population size.

SAMPLES AND METHODS

We have received and analysed the total of 709 samples belonging to brown bears: 547 scat samples collected in three study areas and 159 tissue samples of culled or accidentally killed bears. The samples were kept in 96 % ethanol at -20°C until DNA extraction. Every sample was designated an ID number and recorded in our database which includes details on date and time of collection, location and GPS coordinates.

DNA was extracted from all samples using commercially available kits and following manufacturers instructions: "Wizard Genomic DNA Purification Kit" (Promega) for tissue samples and "QIAamp DNA Stool Mini Kit" (Qiagen) for scat samples. Afterwards, scat DNA extracts were screened for DNA content and with a pair of bear specific microsatellite primers. The samples which had not yielded a product were excluded from further analysis because of poor DNA content (Fig. 1). The remaining scat samples and all of the tissue samples were then genotyped at 6 and 13 microsatellite loci, respectively, by means of polymerase chain reaction – PCR and ABI PRISMTM 3100-Avant Genetic Analyzer (Table 1).

Although it was not one of the aims of this project, tissue samples were also analysed for mtDNA control region sequence which, like microsatellite markers, can indicate the level of genetic diversity

within the population. We decided to do this analysis in order to get a better insight in the current state of the population.

Results

The study areas were mapped for all samples collected and later only for samples successfully genotyped. The surfaces were calculated as Minimum Convex Polygon (MCP) and as 95% Kernel (Figs. 2, 3, 4, 5). The areas are shown in Table 2. Considering the fact that the bears have large home ranges, a buffer zone was drawn around each study area, and the total surface calculated. The mean axes of bears tracked by radio-telemetry (N=13) was calculated (15.2 km) and then half of it (7.6 km) was added to MCP. The goal was to include all the bears that had a realistic chance to enter the original study area and leave the sample there. We expect that the chance that additional bear (from even farther area) had the similar chance to leave the mark as was the chance that some bears using partly our study area as a part of their home range did not leave it (Fig. 6). Those enlargements resulted in surfaces of 937.9 km² for Gorski kotar North, 1000.3 km² Gorski kotar South, and 1158.7 for Velebit. However, as part of Velebit area enlargement reached the Adriatic Sea, the terrestrial part of buffer gave 997.8 km². The sum of these three areas is 2936.0 km².

All tissue samples were successfully genotyped, whereas 328 scat samples gave quality genotypes and 219 had been excluded from further analysis due to low DNA content and/or quality (example: Figure 1). Scat genotyping success rate was therefore 67 %, which is in accordance with other data published in scientific literature.

Table 3 shows how many alleles were found at each microsatellite locus (allelic diversity) and the difference between observed and expected heterozygosity. The mean allelic diversity per microsatellite locus is 7.46 and ranging from 5 to 10 alleles. These parameters are similar to those found in Scandinavian brown bear population and they indicate relatively high genetic diversity of Croatian brown bear population.

Mitochondrial DNA (mtDNA) control region sequences were analysed in the total length of 267 base pairs for 71 bears. The analysis of mtDNA control region sequences revealed only two haplotypes that differ by two nucleotides. One haplotype was found in 13 individuals and the other in 58, which means that the frequencies of haplotypes are 0.18 and 0,82. This extremely low mtDNA and haplotype diversity could be explained by very low dispersal habits of females (mtDNA is maternally inherited) and/or by stochastic events (extinction of other haplotypes in small populations), or it may indicate a population bottleneck in the recent past by severe hunting and should therefore be explored further.

Among 328 genotypes obtained from scat DNA, we identified 210 unique genotypes that represent 210 individual bears. This also means that a certain number of bears had been *recaptured*, i.e. that their genotype had been found more than once among analysed scat samples. The distribution of bears over three study areas and sampling periods together with recapture data is shown in Table 3. Between-years recapture data is shown in Table 4. No genotypes (bears) were recaptured between three study areas.

The data show that the recapture rate is very low in all 3 study areas, within the same year and also between years. The recapture rate, however, is necessary for population size calculations and data modelling. Hence the calculations done and presented here are only for orientation value, and may be mostly used for future data collections and elaborations. Here we present the three approaches for calculation.

1. Minimum number of bears

As stated above 210 unique genotypes that represent 210 individual bears were found among scat samples. In addition, the 145 tissue samples of dead bears were not previously found through scat samples. However, for this calculation we used only the individuals identified through scat samples. The samples collected in the period 2003-2004 in GK South were excluded (N=44) as for the same area we had data for 2006-2007. Hence, a minimum of 166 different bears lived in the total area of 2936 km2 (all three study areas with buffer zone). This cumulative area represents 30.66% of the total range of permanent bear presence in Croatia. We consider the density of bears in the Gorski kotar South to be the highest in Croatia, in Gorski kotar North as the average one and in Velebit as a little

below average. All three areas together may represent the average bear density for their permanent presence in Croatia. In that case the calculation shows probable 541 bears in the entire permanent range of Croatia. Due to the fact that in the periods of two years there was certain mortality and new reproduction, we can roughly subtract 20% of that number. The result gives a minimum of 433 bears in Croatia. The factor of multiplication to get the actual population size is unknown, but may be above 2.5 up to close to 3. Hence, it can be speculated that a total population of 1000 bears is quite likely.

2. Mark – recapture calculation

This calculation suffers of the low number of recaptures, especially as only the ones after the demarcation date could be used.

We have performed the *Lincoln-Petersen* mark-recapture model for study area GK-North in the year 2004, which has the highest recapture rate, and obtained a calculation in the range of 36 - 123 (SD=22) bears in the area. This range is, however, too wide for drawing definite and reliable conclusions about population size in the GK-North area.

Further we attempted calculations for the pairs of years for the three study areas. Only the recaptures in the second year were used. For the accurate calculation the share of recaptures should be 2.5 to 3 times higher than the total expected population size. In our case the marked portion of population was below one third. Therefore the numbers given below contain the great margin of error and can be used only for orientation. With the included rough factor of population flow over the two years the orientation numbers are for Gorski kotar South 150, Gorski kotar North 133 and for Velebit 136, or the total 419 bears. Extrapolating this number to the entire permanent bear range in Croatia gives 1366 bears. Considering the big error it can be speculated that a total population of 1000 bear is quite likely.

3. "Rarefaction" curve

Rarefaction curve is another procedure that asks for a large number of recaptures. The attempt to draw the rarefaction curve provided lines without approaching sigmoid part (Figs. 7, 8, 9), thus are not real "rarefaction" curves. The software used ("Gimlet" and "R") provided certain mean estimated numbers and the standard deviations (SD) which are quite large as expected.. For the reason of safety the conservative approach was used. Therefore the mean numbers minus SD are shown: GK South (2006-2007) 182, GK North (2003-2004) 102, Velebit (2003-2004) 99, or the total of 383 bears. Extrapolating this number to the entire permanent bear range in Croatia gives 1249 bears. As already stated in the minimum number and mark-recapture calculations, it can be speculated that a total population of over 1000 bear is quite likely even when considering the big error of the rarefaction method.

Here we would like to warn that using these numbers as definite may be misleading in population management. Overestimation of the population size could lead to increase in hunting quotas and subsequent damage to bear population, therefore, in terms of bear conservation, a more sensitive approach would be to choose the smaller number as a guidance in population management and hunting quotas recommendations.

The reasons behind the very low recapture rates could be all or any of the following: inadequate sampling intensity, too large sampling areas, more bears than previously expected or too extended sampling period.

When comparing scat genotypes to genotypes obtained by tissue DNA analysis of culled or accidentally killed bears, a match was found for 14 samples. This means that 14 bears that had been previously identified by scat DNA analysis, have eventually been culled or killed. None of the remaining 145 tissue samples were matched to scat samples. From this a conclusion can be drawn that in the period 2003 - 2008 we have identified a total of 355 individual bears.

For 67 bears it was possible to determine sex by means of genetic analyses of scat samples (Figure 10). We assume that there was no bias towards any sex in collecting the scat samples and that they truly represent the population sex ratio. Out of 67 samples 34 were females and 33 males, indicating the 50:50 males versus females in the population. Considering the fact that hunting mortality is heavily leaning towards male side one could expect fewer males in the population. The

presented result requires bigger sample size and additional checks, but at least gives no warning about current hunting effects.

CONCLUSION

We have analysed all scat and tissue samples that we had received. All the samples containing DNA were successfully genotyped which enabled us to recognize each bear individually. By recognizing each bear, we managed to determine the minimum number of individual bears per study area and sampling period. DNA analysis revealed the current level of genetic diversity within the population, which could be described as relatively high as compared to other European brown bear populations.

The revealed population sex ratio seems to be close to 50:50, thus showing no evident negative effect of hunting predominantly male bears. This, however, needs to be taken with precaution because a confirmation on a larger sample size is needed.

The goal of obtaining a reliable population size estimate was only partly reached due to very low recapture of bears. Nevertheless, the three approaches used for calculating population size have pointed towards the upper limit of our starting estimate of 600-1000 bears indicating a total population of around or over 1000 bear is quite likely. By obtaining the minimum number of bears and revealing the level of genetic diversity, we have given a firm scientific foundation for management guidelines and further research of brown bears in Croatia. The current hunting quotas do not seem to be a limiting or threatening factor to the present population.

Figure 1. An image of samples that yielded a PCR product and those that did not. A band shows a PCR product. (DNA-ladder is in the first column; it enables product size determination).

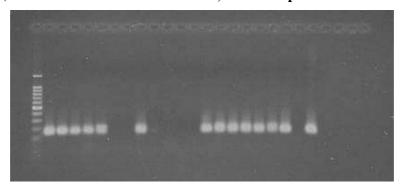


Table 1. Microsatellite loci used in our research:

Locus	Microsatellite sequence	Used for genotyping
Mu10	(f) attcagatttcatcagtttgaca	tissue and scat
	(r) tcagcatagttacacaaatctcc	
Mu23	(f) gcctgtgtgctattttatcc	tissue and scat
	(r) tagaccaccaaggcatcag	
Mu50	(f) gtctctgtcatttccccatc	tissue and scat
	(r) aacctggaacaaaaattaacac	
Mu51	(f) agccagaatcctaagagacct	tissue and scat
	(r) aaagagaagggacaggaggta	
Mu59	(f) gctcctttgggacattgtaa	tissue and scat
	(r) tgactgtcaccagcaggag	
G10L	(f) actgattttattcacatttccc	tissue and scat
	(r) gatacagaaacctacccatgcg	
G10B	(f) gccttttaatgttctgttgaatttg	tissue
	(r) ggatggaggtttctgtgatttgtc	
G10C	(f)aaagcagaaggccttgatttcctg	tissue
	(r) gctgtctcggtgtttatgtccc	
G1D	(f) gatctgtgggtttataggttaca	tissue
	(r) ctcttaaagagtaggaagagtag	
G10J	(f) gatcagatattttcagcttt	tissue
	(r) gaagtggagtgtgaggggtt	

G10M	(f) ttcccctcatcgtaggttgta	tissue
	(r) attatttggaaacacatgatc	
G10P	(f) agttttacataggaggaagaa	tissue
	(r) ttcagagtatttccccacatga	
G10X	(f) ccctggtaaccacaaatctct	tissue
	(r) ttttgatttcacagataactga	
SRY	(f) gaacgcattcttggtgtggtc	tissue and scat
	(r) tgatctctgagttttgcatttg	

Table 2. The sizes of the 3 study areas in the permanent bear range i	n Croatia
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	GORSK	KI KOTAR N	NORTH	GORSK	I KOTAR S	SOURH	VELEBIT			
	95%	100%	100%	95%	100%	100%	95%	100%	100%	
PERIOD	KERNEL	MCP	MCP	KERNEL	MCP	MCP	KERNEL	MCP	MCP	
	(KM^2)	(KM^2)	$(KM^{2}) +$	(KM^2)	(KM^2)	(KM^2)	(KM^2)	(KM^2)	$(KM^{2}) +$	
			7.6 km			+7.6km			7.6 km	
			buffer			buffer			buffer	
2003 + 2004	160.6	240.1	937.9	455.5	283.7	-	380.8	390.5	997.8*	
2006 + 2007	-	-	-	378.7	278.1	1000.3	-	-	-	

* Only terrestrial part

Table 3.. Observed (H_o) and expected (H_e) heterozygosity and allelic diversity (A) in Croatian brown bear population (sample size=156)

Locu	G10B	G10C	G1D	G10J	G10L	G10M	G10P	G10X	Mu10	Mu23	Mu50	Mu51	Mu59	mean
S														
He	0,70	0,72	0,80	0,67	0,62	0,77	0,84	0,86	0,62	0,80	0,80	0,64	0,87	0,75
Но	0,71	0,69	0,70	0,72	0,65	0,32	0,75	0,89	0,58	0,80	0,79	0,60	0,87	0,70
А	8	10	7	6	6	6	9	10	5	7	8	5	10	7,46

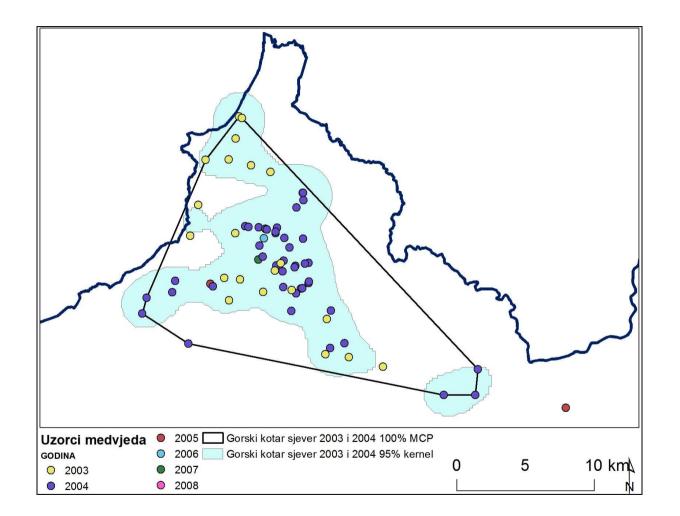
Table 4.. The distribution of bears and recapture data for three study areas and sampling periods.

Study area	Year	Sample total	Unique genotypes (Individual bears)	Number of recaptured bears	Maximum number of recapture occasions	% recapture	Sampling duration (days)
GK-South(Bjelolasica)	2003	38	31	4	4	12,90%	100
GK-South (Bjelolasica)	2004	17	16	1	2	6,25%	60
GK-South (Bjelolasica)	2006	56	42	14	3	33,33%	217
GK-South (Bjelolasica)	2007	41	34	8	3	23,53%	53
GK-North	2003	22	18	4	2	22,22%	124
GK-North	2004	61	37	13	5	35,14%	133
Velebit	2003	46	33	11	3	33%	223
Velebit	2004	47	33	6	5	18,18%	125

Study area	Year	Unique genotypes (as in Table 1)	new genotypes	recaptured genotypes	Maximum number of recapture occasions
GK-South (Bjelolasica)	2003	31	31	-	-
GK-South (Bjelolasica)	2004	16	13	3	1
		2-year total:	44		
GK-South (Bjelolasica)	2006	42	31	11*	1
GK-South (Bjelolasica)	2007	34	27	7**	1
		2-year total:	58		
GK-North	2003	18	18	-	-
GK-North	2004	37	32	5	1
		2-year total:	50		
Velebit	2003	33	33	-	-
Velebit	2004	33	25	8	1
		2-year total:	58		
		GRAND TOTAL:	210		

* 9 genotypes recaptured from year 2003; 2 from year 2004.
** 1 genotype recaptured from year 2003; 2 from year 2006; 1 from years 2004 and 2006; 3 from years 2003 and 2006.

Figure 2. Gorski kotar North sampling area showing only the samples that were successfully genotyped in 2003 and 2004. Shown is the Minimum convex polygon (MCP) and the 95% Kernel area.



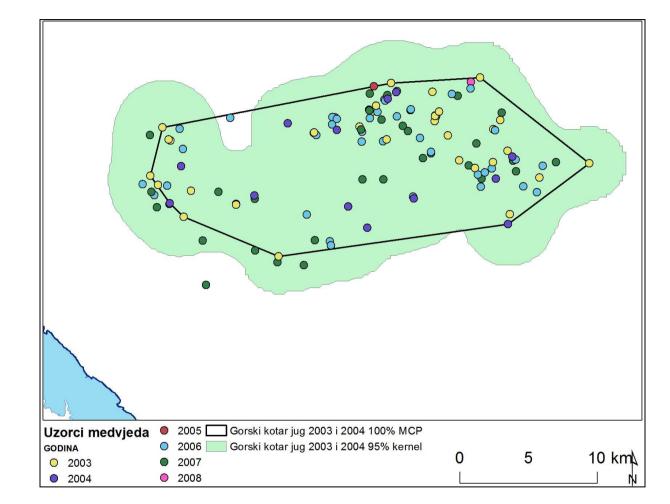


Figure 3. Gorski kotar South sampling area showing only the samples that were successfully genotyped in 2003 and 2004. Shown is the Minimum convex polygon (MCP) and the 95% Kernel area.

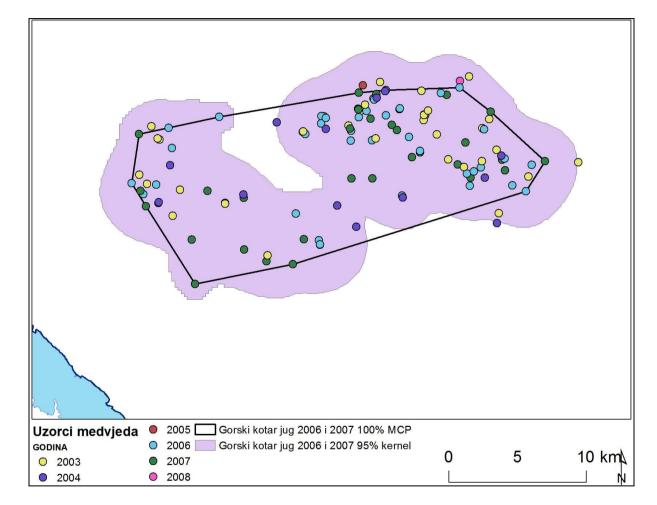
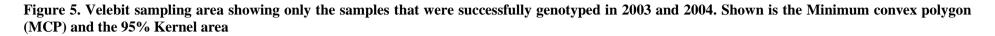
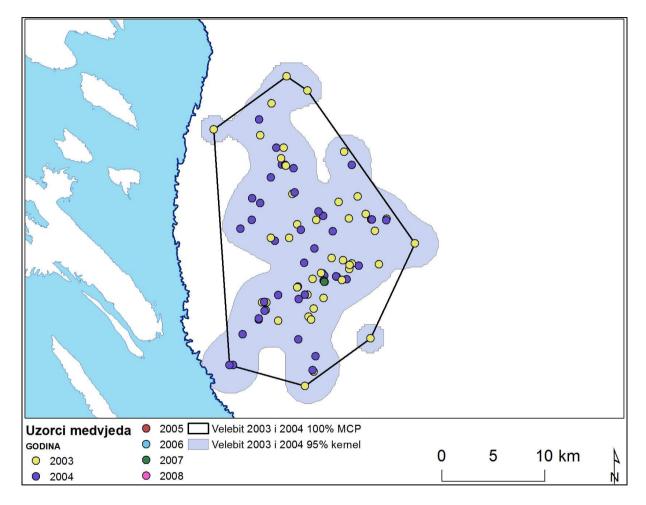
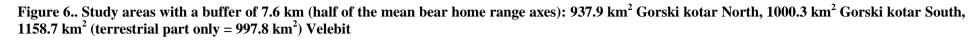


Figure 4. Gorski kotar South sampling area showing only the samples that were successfully genotyped in 2006 and 2007. Shown is the Minimum convex polygon (MCP) and the 95% Kernel area







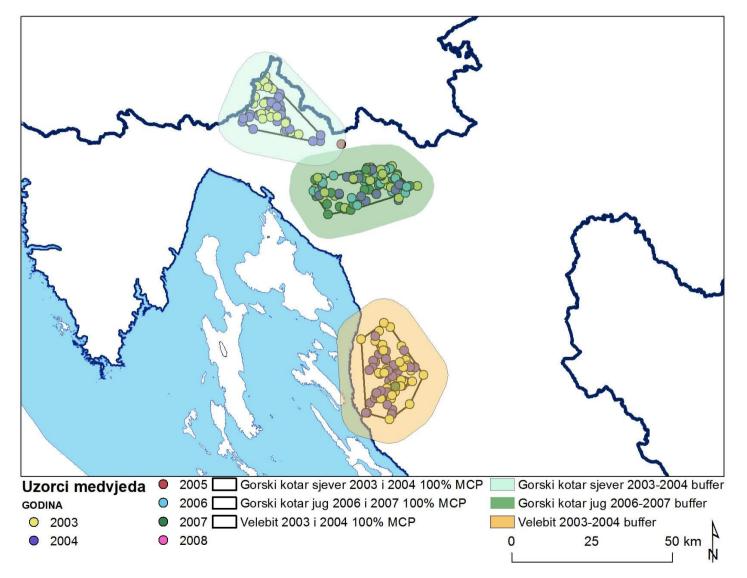
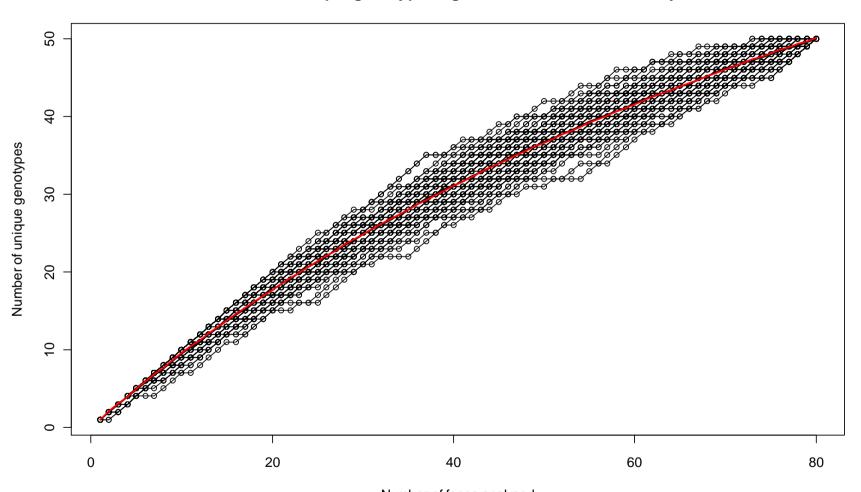


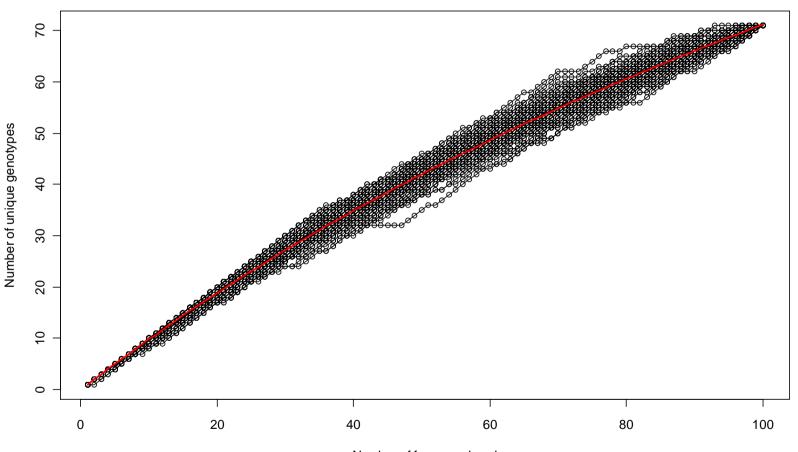
Figure 7. Gorski kotar North



Number of unique genotypes against number of feces analysed

Number of feces analysed observed= circles; mean of observed= black line; Kohn's eq= red line

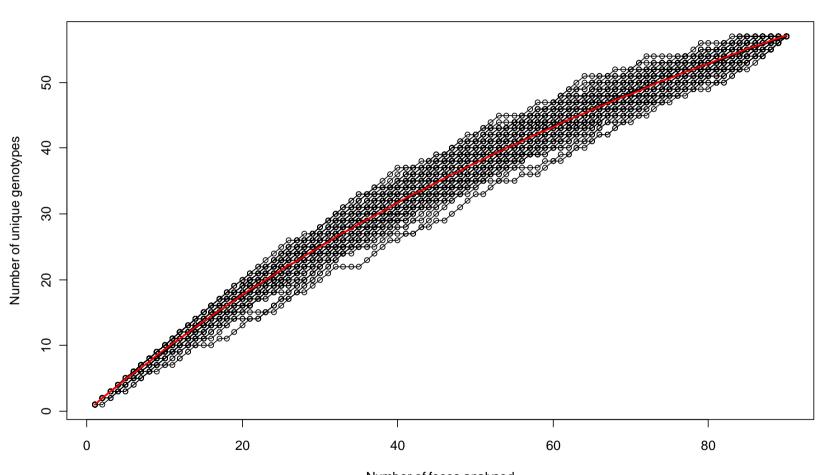
Figure 8. Gorski kotar South



Number of unique genotypes against number of feces analysed

Number of feces analysed observed= circles; mean of observed= black line; Kohn's eq= red line

Figure 9. Velebit



Number of unique genotypes against number of feces analysed

Number of feces analysed observed= circles; mean of observed= black line; Kohn's eq= red line



