LIFE ARCPROM

LIFE18 NAT/GR/000768

Improving human-bear coexistence
in 4 National Parks of South Europe

ASSESSMENT OF THE DISTRIBUTION AND NUMBERS OF BEARS IN THE PROJECT AREAS

Action A2

TECHNICAL REPORT WITH THE RESULTS OF THE BIO-INDICES, IR CAMERA TRAPS AND GENETIC ANALYSIS

31/12/2021

Authors

Drafted by (alphabetical order):

Antonio Antonucci – Maiella National Park
Pantelis Bagos – University of Thessaly
Charalambos Billinis – University of Thessaly
Giovanna Di Domenico – Maiella National Park
Stefanos Kyriakidis – Callisto
Yorgos Mertzanis – Callisto
Maria Satra – University of Thessaly
Evaggelia Stasi – University of Thessaly
Vasiliki Spyrou – University of Thessaly
Tzoulia Tsalazidou - Founta – University of Thessaly

Suggested citation

Assessment of the distribution and numbers of bears in the Maiella National Park, the Northern Pindos National Park, the Prespa National Park and the Rodopi Mountain Range National Park – Final Report Action A2 LIFE18 NAT/GR/768 ARCPROM.

Working Team:

Callisto: N. Karamoustos (biologist – internship, AUTH), M. Karapiperi (biologist – internship, AUTH), (St. Kyriakidis (MSc Forester), Y. Lazarou (field technician), Il. Marocco (MSc Biologist, volunteer - Italy), Y. Mertzanis (PhD Biology), An. Pyrovolos (Forester – Internship, AUTH), Ath. Tragos (biologist), Y. Tsaknakis (field technician).

Pindos NP: M.Tzaboura (warden), St. Psarras (warden), Ath. Karabina (warden) Ant. Stagkoyannis (warden), Z. Tsiotikas (warden), St. Zerva (warden), Al. Dafli (warden), I. Fitsiou (warden), Ath. Korakis (MSc Environmental Sc.), N. Petsis (GIS expert).

Prespa NP: L Anastasiadou (warden), Tr. Nitsopoulou (warden), V. Papadopoulos (warden).

Rodopi NP: P. Agorastos (warden technician), El. Gounari (warden), Elp. Grigoriadou (biologist), V.Konidari (warden), Ir. Kotsaki (warden), P. Psaltopoulos (GIS), P. Samara (warden), Al. Sotiriou (GIS).

UTH: P. Bagos (researcher), Ch. Billinis (Professor), Maria Satra (researcher), E. Stasi (researcher), V. Spyrou (researcher), Tz. Tsalazidou – Founta (researcher).

Maiella NP: A. Antonucci (biologist), G. Di Domenico (biologist), S. Angelucci (Veterinarian), G. Gavioli (biologist – student), I. Zuchegna (biologist – internship).

TABLE OF CONTENTS

EXECUTIVE SUMMARY IN GREEK –ΠΕΡΙΛΗΨΗ ΣΤΑ ΕΛΛΗΝΙΚΑ

Η παρούσα τεχνική αναφορά εντάσσεται στο έργο LIFE18 NAT/GR/00768 (LIFE ARCPROM) και αποτελεί παραδοτέο της προπαρασκευαστικής δράσης Α2 με τίτλο: «*Εκτίμηση της γεωγραφικής κατανομής και του πληθυσμιακού μεγέθους της καφέ αρκούδας (Ursus arctos*) στις περιοχές εφαρμογής του έργου*". Για την επίτευξη των στόχων της προαναφερόμενης δράσης χρησιμοποιήθηκαν (3) διαφορετικές μέθοδοι προσέγγισης ως εξής: α) γενετική ανάλυση βιολογικού υλικού αρκούδας, β) καταγραφή με την χρήση φωτοπαγίδων υπέρυθρου και θερμικού αισθητήρα και γ) καταγραφή βιοδηλωτικών ενδείξεων.

Στην **Ελλάδα** τα αποτελέσματα έδειξαν: πληθυσμιακό μέγεθος και συνδεσιμότητα: το μέσο μέγεθος πληθυσμού ήταν 191, 202 και 207 άτομα για το ΕΠ Πρεσπών, ΕΠ Πίνδου και ΕΠ Ροδόπης αντίστοιχα. Οι τρεις πληθυσμοί διακρίθηκαν επιτυχώς σε τρεις ομάδες με σαφή διαφοροποίηση μεταξύ του ανατολικού (Ροδόπη) και του δυτικού (Πίνδου) πληθυσμού. Ενδοαναπαραγωγή/γενετική ποικιλότητα: η γενετική ποικιλότητα σε όλους τους πληθυσμούς είναι σε ικανοποιητικά επίπεδα, ωστόσο υψηλά επίπεδα ενδοαναπαραγωγής παρατηρούνται στους πληθυσμούς αρκούδας στα ΕΠ Πρεσπών και Οροσειράς Ροδόπης. Επιπλέον, δεν εντοπίστηκε σημαντική γεωγραφική συμφόρηση σε κανέναν από τους (3) πληθυσμούς. Οι αυξημένες τιμές της ενδοαναπαραγωγής σε συνδυασμό με το χαμηλό Ne δείχνει ότι οι υποπληθυσμοί της Ροδόπης και των Πρεσπών είναι πιο ευάλωτοι σε σχέση με τον πληθυσμό της Πίνδου. Τα αποτελέσματα από τις φωτοπαγίδες έδειξαν ότι τα υψηλότερα επίπεδα σχετικής πυκνότητας καφέ αρκούδας (που κυμαίνονται από τιμές 2,28 – 3,98) ποικίλλουν στις (3) υποπεριοχές του έργου. Όσον αφορά τις περιοχές (σε km^2) με τις υψηλότερες σχετικές πυκνότητες επί της συνολικής επιφάνειας του κάθε ΕΠ αυτές κυμαίνονται από 26% στην περίπτωση του ΕΠ Οροσειράς Ροδόπης, 40% στην περίπτωση του ΕΠ Βόρειας Πίνδου και έως 59% της συνολικής επιφάνειας στην περίπτωση του ΕΠ Πρεσπών. Σε όλες τις περιπτώσεις οι τομείς με μεγαλύτερη σχετική αφθονία είναι σχετικά όμορες, ωστόσο αυτό δεν είναι πάντα ο κανόνας. Οι βιοδηλωτικές ενδείξεις αρκούδας στο ΕΠ Πρεσπών αποτυπώνουν (2) διακριτούς πυρήνες υψηλής έντασης παρουσίας και δραστηριότητας αρκούδας οι οποίοι εντοπίζονται και στις δύο πλευρές του οικοσυστήματος των ορεινών λιμνών δείχνοντας δύο πολύ σημαντικά στοιχεία: α) η λίμνη της Μικρής Πρέσπας δεν φαίνεται να λειτουργεί ως απόλυτος φραγμός μεταξύ των δύο πληθυσμιακών ομάδων που καταλαμβάνουν τους πέριξ ορεινούς όγκους β) Η παρουσία ενός πυρήνα υψηλής παρουσίας και δραστηριότητας της αρκούδας στο δυτικό τμήμα της λεκάνης της λίμνης της Μικρής Πρέσπας παρουσιάζει εξαιρετικό βιογεωγραφικό ενδιαφέρον δεδομένου του γεγονότος των ακατάλληλων συνθηκών περιβάλλοντος ενδιαιτήματος σε μεγαλύτερη γεωγραφική κλίμακα όπως: Β και Δ όπου οριοθετούνται από υδάτινες επιφάνειες (λίμνη Μεγάλης Πρέσπα) και ΝΔ όπου οριοθετούνται από ένανα κατά μεγάλο μέρος τομέα με ακατάλληλα/υποβαθμισμένα ενδιαιτήματα οικοτόπων που εισέρχονται στα αλβανικά εδάφη. Στο ΕΠ Οροσειράς Ροδόπης παρατηρείται ένας μοναδικός πυρήνας υψηλής έντασης παρουσίας και δραστηριότητας αρκούδας ο οποίος εντοπίζεται κοντά στη (δια)συνοριακή περιοχή με τη Βουλγαρία σε ένα συμπαγές και πολύ παραγωγικό οικοσύστημα ψυχρόβιων κωνοφόρων. Όλοι οι παραπάνω δείκτες και αποτελέσματα θα συμβάλουν καθοριστικά σε μια χωρικά βελτιστοποιημένη κα καλύτερα προσανατολισμένη εφαρμογή των δράσεων διατήρησης του είδους στο πλαίσιο του έργου.

Στην **Ιταλία**, στο Εθνικό Πάρκο της Maiella (MNP), η Δράση Α2 είχε ως στόχο την συγκέντρωση πληροφοριών σχετικά με το πού βρίσκονται οι αρκούδες, πόσα άτομα υπάρχουν και εάν υπάρχουν θηλυκά ή/και θηλυκά με μικρά. Οι (3) μέθοδοι που προβλέπονται στη Δράση Α2 εφαρμόστηκαν χρησιμοποιώντας έναν συνδυασμό συστηματικών και ευκαιριακών στρατηγικών, μια προσέγγιση που τα τελευταία 10 χρόνια αποδείχθηκε η καλύτερη ως προς το λόγο κόστους/οφέλους σε μια περιοχή, όπως το ΕΠ Maiella όπου μια διαδικασία «επαν-αποικισμού» από την αρκούδα βρίσκεται σε εξέλιξη. Τα δεδομένα που

συλλέχθηκαν πριν από την έναρξη του έργου LIFE ARCPROM έδειξαν μια μεταβλητότητα τόσο στον ετήσιο αριθμό των αρκούδων όσο και στην κατανομή τους, ένα αναμενόμενο αποτέλεσμα λαμβάνοντας υπόψη ότι ο εκ νέου αποικισμός από αρκούδες είναι μια μακρά διαδικασία που συνεπάγεται ότι τα άτομα κινούνται αμφίδρομα από την περιοχή- αφετηρία (μητρικός πληθυσμός) προς το ΕΠ Maiella προκειμένου να εξερευνήσουν διαφορετικές περιοχές πριν επιλέξουν πού θα εγκατασταθούντελικά. Για τους λόγους αυτούς, και προκειμένου να συλλεχθούν ακριβή και επικαιροποιημένα δεδομένα, η αξιολόγηση της κατανομής και του αριθμού ατόμων αρκούδας στο ΕΠ της Maiella πρέπει να γίνεται σε ετήσια βάση και αυτός είναι ο λόγος που στο πλαίσιο της δράσης Α2 έχουν συλλεχθεί δεδομένα τόσο το 2020 όσο και το 2021. Συνδυάζοντας τα αποτελέσματα που προέκυψαν χρησιμοποιώντας όλες τις μεθόδους, βρέθηκαν 318 αξιόπιστες βιοδηλωτικές ενδείξεις αρκούδας στο πλαίσιο της Δράσης Α2 (179 το 2020 και 139 το 2021). Το 2020 οι βιοδηλωτικές ενδείξεις αρκούδας εντοπίστηκαν κυρίως στο νότιο τμήμα της περιοχής παρακολούθησης ενώ το 2021 εντοπίστηκαν τόσο στο νότιο όσο και στο βόρειο τμήμα της περιοχής παρακολούθησης, συμπεριλαμβανομένου και του βορειοανατολικού τμήματος όπου λίγες ή καθόλου ενδείξεις είχαν εντοπιστεί τα τελευταία 10 χρόνια. Προκειμένου να κατανοηθεί καλύτερα το συνεχιζόμενο πρότυπο επαν-αποικισμού, τα δεδομένα που συλλέχθηκαν το 2020 και το 2021 έχουν επίσης αξιολογηθεί υπό το φως των δεδομένων που συλλέχθηκαν τα προηγούμενα έτη. Από το 2004 έως το 2021 ο αριθμός των βιοδηλωτικών ενδείξεων αρκούδας αυξήθηκε με ενδιάμεσες διακυμάνσεις και το 2011 φαίνεται να ήταν ένα σημείο καμπής μετά το οποίο ο αριθμός των ανιχνευθέντων βιοδηλωτικών ενδείξεων αυξήθηκε φτάνοντας τις μέγιστες τιμές το 2020 και το 2021. Συγκρίνοντας τις επιφάνειες των ελάχιστων κυρτών πολυγώνων (MCP) βιοδηλωτικών ενδείξεων που εντοπίστηκαν τις περιόδους 2004-2010 (όταν εντοπίστηκαν μερικές δεκάδες βιοδηλωτικές ενδείξεις/έτος), 2011-2019 (από το σημείο καμπής έως την αρχή του LIFE ARCPROM) και 2020-2021 (έτη εφαρμογής της Δράσης Α2), προκύπτει μια σαφής αλλαγή στην κατανομή των βιοδηλωτικών ενδείξεων και το πρότυπο που παρατηρήθηκε υποδηλώνει ότι η τρέχουσα παρουσία αρκούδας στο ΕΠ Maiella είναι το αποτέλεσμα μιας διαδικασίας επαν-αποικισμού που ξεκίνησε σχεδόν πριν από είκοσι χρόνια και δεν σταμάτησε ποτέ από τότε, κάνοντας τις αρκούδες να χρησιμοποιούν όλο και περισσότερους τομείς της περιοχής του ΕΠ κάθε χρόνο. Με βάση τα αποτελέσματα της γενετικής ανάλυσης βιολογικού υλικού αρκούδας (87 δείγματα που αναλύθηκαν από το αρμόδιο εργαστήριο, 39 το 2020 και 48 το 2021) και συνδυάζοντας αυτά τα αποτελέσματα με αυτά που προέρχονται από άλλες δραστηριότητες παρακολούθησης και δεδομένα τηλεμετρίας που συλλέγονται με κολάρα GPS, ο ελάχιστος αριθμός των αρκούδων ήταν 6 το 2020 (3F, 3M) και 13 το 2021 (5F, 8M). Παρατηρώντας την τάση του ελάχιστου αριθμού αρκούδων που εντοπίστηκαν από το 2012 (δηλαδή από τότε που είναι διαθέσιμες τυποποιημένες και συγκρίσιμες αναλύσεις γενετικών δειγμάτων) το πιο σημαντικό αποτέλεσμα είναι ότι ο αριθμός διπλασιάστηκε από το 2019 έως το 2020 και ξανά από το 2020 έως το 2021 και ότι επίσης ο ελάχιστος αριθμός των θηλυκών πέρασε από 2 σε 5 από το 2019 έως το 2021. Το 2020 και το 2021 δεν εντοπίστηκαν θηλυκά με μικρά στην περιοχή παρακολούθησης του ΕΠ Maiella, ωστόσο λαμβάνοντας υπόψη την αύξηση που παρατηρείται στον αριθμό των θηλυκών, θα δοθεί ιδιαίτερη προσοχή τα επόμενα χρόνια για την ανίχνευση κάθε πιθανού συμβάντος αναπαραγωγής. Προκειμένου να κατανοηθεί καλύτερα η δυναμική του επαν- αποικισμού, πραγματοποιήθηκε μια ανάλυση για το *εάν και πού* τα άτομα που είναι παρόντα στο ΕΠ Maiella έχουν ταυτοποιηθεί και σε άλλες περιοχές της ευρύτερης κατανομής της αρκούδας. Τα αποτελέσματα που προέκυψαν οδήγησαν στο συμπέρασμα ότι εισέρχονται νέα άτομα από τον πληθυσμό προέλευσης, ότι (με μεγάλη πιθανότητα) νέα άτομα γεννιούνται επίσης στην περιοχή παρακολούθησης του ΕΠ Maiella και ότι μια μετακίνηση πολλών ατόμων έχει σημειωθεί τα τελευταία 2-3 χρόνια στο διάδρομο σύνδεσης μεταξύ του μητρικού πληθυσμού και του ΕΠ Maiella προς το ΕΠ. Αυτό το συγκεκριμένο εύρημα, μαζί με την αυξητική τάση που παρατηρείται για τον αριθμό των

βιοδηλωτικών ενδείξεων , την τάση του ελάχιστου αριθμού αρκούδων που υπάρχουν στην περιοχή και τα δεδομένα σχετικά με τον αριθμό και τη θέση των θηλυκών, ενισχύουν την υπόθεση ότι η διαδικασία επαν-αποικισμού στο ΜΝΡ έχει εισέλθει σε μια πιο «ενεργή» φάση τα τελευταία 2-3 χρόνια.Τα δεδομένα που συλλέγονται μέσω της Δράσης Α2 θα συμβάλουν επίσης ενεργά στην υλοποίηση όλων των δράσεων διατήρησης που προβλέπονται στο πλαίσιο του έργου LIFE ARCPROM.

EXECUTIVE SUMMARY IN ITALIAN – RIASSUNTO IN ITALIANO

La presente relazione tecnica fa parte del progetto LIFE18 NAT/GR/00768 (LIFE ARCPROM) e, in particolare, è il *deliverable* dell'azione preparatoria A2 intitolata: "*Assessment of the distribution and numbers of bears in the project areas*". Per raggiungere gli obiettivi della suddetta azione, sono stati utilizzati i seguenti (3) diversi approcci: a) analisi dei campioni genetici (peli ed escrementi), b) ricerca dei segni di presenza e c) video-trappolaggio.
In **Grecia** i risultati sono i seguenti. Dimensione della popolazione e connettività: la dimensione media della popolazione è di 191, 202 e 207 individui rispettivamente per Il Parco Nazionale di Prespa (PNP), Il Parco Nazionale del Pindos Settentrionale (PINDNP) e Il Parco Nazionale dei Monti Rodopi (RMNP). Consanguineità/diversità genetica: la diversità genetica in tutte le popolazioni è a livelli soddisfacenti, tuttavia si osservano alti livelli di consanguineità nelle popolazioni di orsi nelle montagne di Prespa e Rodopi. I valori più alti di consanguineità in combinazione con il basso Ne, mostrano che le sottopopolazioni di Rodopi e Prespa sono più vulnerabili rispetto alla popolazione di Pindos.
I risultati del fototrappolaggio mostrano che i livelli più alti di densità relativa dell'orso bruno (che vanno da 2,28 a 3,98) variano nelle (3) sotto-aree del progetto e, in termini di estensione, le aree a maggior densità di orso occupano il 26% dell'area totale di progetto nel caso del RMNP, il 40% nel caso del PINDNP e raggiungono il 59% dell'area totale nel caso del PNP. In tutti i casi le aree con i settori con il più alto valore di abbondanza relativa sono relativamente contigue, anche se questa non è sempre la regola.
I segni di presenza collezionati nel PNP hanno evidenziato (2) nuclei distinti di elevata presenza di orso che si trovano su entrambi i lati dell'ecosistema lacustre di montagna e mostrano due elementi molto importanti: a) il lago piccolo di Prespa non sembra agire come un barriera assoluta tra i due gruppi di popolazione che occupano le montagne circostanti b) la presenza di un nucleo di alta presenza di orso nella parte occidentale del bacino dei laghi di Prespa è di grande interesse biogeografico date le condizioni ambientali precarie o del tutto inadatte su scala più ampia. Infatti a nord e ovest è delimitato dall'acqua (lago grande di Prespa) e a sud-ovest è delimitato da un gran numero di habitat inadatti/degradati che entrano nel territorio albanese. Nel Parco Nazionale dei Monti Rodopi si osserva un nucleo unico di elevata presenza di orso che si trova vicino alla zona di confine con la Bulgaria in un ecosistema di conifere denso e molto produttivo. Tutti gli indicatori e i risultati di cui sopra contribuiranno in modo decisivo a una migliore implementazione, a livello spaziale, delle azioni di conservazione nell'ambito del progetto.
In **Italia**, nel Parco Nazionale della Maiella (PNM), l'azione A2 è finalizzata ad acquisire informazioni su a) la distribuzione dell'orso bruno marsicano e b) quanti individui sono presenti e se sono presenti femmine/femmine con cuccioli. I (3) metodi previsti nell'Azione A2 sono stati attuati utilizzando una combinazione di strategie sistematiche e opportunistiche, un approccio che negli ultimi 10 anni si è rivelato il migliore in termini di rapporto costi/benefici in un'area, come il PNM, dove è in corso un processo di "ricolonizzazione" da parte dell'orso. I dati raccolti prima dell'inizio del progetto LIFE ARCPROM hanno mostrato una variabilità sia sul numero annuo di orsi che sulla loro distribuzione, risultato atteso considerando che la ricolonizzazione da parte degli orsi è un processo lungo che implica che gli individui vadano avanti e indietro dall'area sorgente al PNM e che esplorino diverse aree prima di scegliere dove stabilirsi. Per questi motivi, al fine di raccogliere dati accurati e aggiornati, la valutazione della distribuzione e del numero degli orsi nel PNM deve essere effettuata su base annuale e questo è il motivo per il quale nell'ambito dell'azione A2 sono stati raccolti dati sia nel 2020 sia nel 2021. Combinando i risultati ottenuti utilizzando tutti i metodi, nell'ambito dell'Azione A2 sono stati trovati 318 segni di presenza di orso (179 nel 2020 e 139 nel 2021). Nel 2020 i segni di presenza sono

stati rinvenuti principalmente nella porzione meridionale dell'area di monitoraggio mentre, nel 2021, sono stati rilevati segni di presenza di orso sia nella porzione meridionale che in quella settentrionale dell'area di monitoraggio, compresa la porzione nord-orientale dove pochi o nessun segno era stato rilevato negli ultimi 10 anni. Al fine di comprendere meglio il *pattern* di ricolonizzazione in corso, i dati raccolti nel 2020 e nel 2021 sono stati osservati anche alla luce dei dati raccolti negli anni precedenti. Dal 2004 al 2021 il numero di segni di presenza di orso è aumentato (passando attraverso alti e bassi) e il 2011 sembra essere stato un punto di svolta dopo il quale il numero di segni rilevati è aumentato raggiungendo i valori massimi nel 2020 e nel 2021. Confrontando il Minimo Poligono convesso (MCP) dei segni di presenza rilevati nei periodi 2004-2010 (periodo in cui sono state rilevate poche decine di segni/anno), 2011-2019 (dal punto di svolta all'inizio del progetto LIFE ARCPROM) e 2020-2021 (anni di attuazione dell'Azione A2), è emerso un chiaro cambiamento nella distribuzione dei segni di presenza e il *pattern* osservato suggerisce che la presenza attuale dell'orso nel PNM è il risultato di un processo di ricolonizzazione iniziato quasi vent'anni fa e da allora mai interrotto, che ha portato gli orsi a utilizzare ogni anno sempre più porzioni dell'area di monitoraggio. Sulla base dei risultati dell'analisi dei campioni genetici (87 campioni analizzati dal laboratorio competente, 39 nel 2020 e 48 nel 2021) e combinando questi risultati con quelli provenienti da altre attività di monitoraggio e dai dati raccolti con i collari GPS, il numero minimo di orsi è di 6 nel 2020 (3F, 3M) e 13 nel 2021 (5F, 8M). Osservando l'andamento del numero minimo di orsi rilevati dal 2012 (ovvero da quando sono disponibili analisi standardizzate e comparabili di campioni genetici) il risultato più importante che emerge è che il numero è raddoppiato dal 2019 al 2020 e di nuovo dal 2020 al 2021 e che anche il numero minimo di femmine è passato da 2 a 5 dal 2019 al 2021. Nel 2020 e nel 2021 non sono state rilevate femmine con cuccioli nell'area di monitoraggio del PNM ma, considerato l'aumento osservato nel numero di femmine, nei prossimi anni si presterà particolare attenzione a rilevare ogni possibile evento di riproduzione.
Al fine di comprendere meglio la dinamica di ricolonizzazione, è stata effettuata, infine, un'analisi di *se* e *dove* gli individui presenti nel PNM sono stati campionati nelle altre porzioni dell'areale dell'orso. I risultati ottenuti hanno portato alla conclusione che nuovi individui stanno arrivando dalla popolazione sorgente, che con alta probabilità stanno nascendo nuovi individui anche nell'area di monitoraggio del PNM e che negli ultimi 2-3 anni si è verificato un aumento nel numero di individui in spostamento nel corridoio tra la popolazione sorgente e il PNM. Questo particolare dato, insieme all'andamento osservato per il numero di segni di presenza, l'andamento del numero minimo di orsi presenti nell'area e i dati relativi al numero e alla localizzazione delle femmine, avvalora l'ipotesi che il processo di ricolonizzazione nel PNM sia entrato in una fase più "attiva" negli ultimi 2-3 anni.
I dati raccolti attraverso l'Azione A2 contribuiranno fattivamente all'implementazione di tutte le Azioni concrete previste nell'ambito del progetto LIFE ARCPROM.

EXECUTIVE SUMMARY IN ENGLISH

This technical report is part of the project LIFE18 NAT/GR/00768 (LIFE ARCPROM) and, particularly, it is the deliverable to the preparatory action A2 entitled: "Assessment of the distribution and numbers of bears in the project areas". To achieve the objectives of the aforementioned action, (3) different methods of approach were used as follows: a) genetic analysis of bear biological material, b) recording of bear bio-signs and c) recording using infrared camera-traps and thermal sensor.
In **Greece** the results are the following. Population size and connectivity: the average population size was 191, 202 and 207 individuals for the Prespa NP, Pindos NP and Rodopi NP respectively. The three populations were successfully distinguished into three groups with a clear distinction between the eastern (Rodopi) and western (Pindos) populations. Inbreeding/genetic diversity: genetic diversity in all populations is at satisfactory levels, however high levels of inbreeding are observed in bear populations in the Prespa and Rodopi Mountains. In addition, no significant geographical congestion was detected in any of the (3) populations. The increased values of inbreeding in combination with the low Ne show that the subpopulations of Rodopi and Prespa are more vulnerable in relation to the population of Pindos.
The results from the photo traps showed that the highest levels of relative brown bear density (ranging from 2.28 - 3.98) vary in (3) sub-areas of the project. Regarding the areas (in km^2) on the total area of each NP range from 26% in the case of the Rodopi Mountain Range NP, 40% in the case of the Pindos NP and up to 59% of the total area in the case of the Prespa NP. In all cases the areas with the highest relative abundance value sectors are relatively contiguous, however this is not always the rule.
The biosigns in Prespa NP showed (2) discrete nuclei of high bear presence and activity intensity which are located on both sides of the mountain lake ecosystem and show two very important elements: a) the lake of Little Prespa does not seem to act as an absolute barrier between of the two population groups occupying the surrounding mountains b) The presence of a nucleus of high bear presence and activity in the western part of the basin of Lake Prespa is of great biogeographical interest given the fact of poor or completely unsuitable environmental conditions on a larger scale: N and W bounded by water (Lake Megali Prespa) and SW bordered by a large number of unsuitable / degraded habitats entering Albanian territory. In the Rodopi Mountains NP, a unique nucleus of high bear presence and activity intensity is observed, which is located near the border area with Bulgaria in a dense and very productive coniferous ecosystem. All the above indicators and results will contribute decisively to a spatially better oriented implementation of conservation actions within the project.
In **Italy**, in the Maiella NP (MNP), Action A2 was aimed at acquiring information on where bears are, how many individuals are present and if females/females with cubs are present. The (3) methods foreseen in Action A2 were implemented using a combination of systematic and opportunistic strategies, an approach that in the last 10 years proved to be as the best one in term of cost/benefit ratio in an area, like MNP, were a “re-colonization” process is ongoing. Data collected before the starting of the LIFE ARCPROM showed a variability on both the yearly number of bears and their distribution, an expected result considering that re-colonization by bears is a long process that implies individuals going back and forth from the source area to MNP and exploring different areas before choosing where to eventually establish. For these reasons, in order to collect accurate and up to date data, the assessment of bear distribution and numbers in MNP needs to be done on yearly basis and this is the reason why in the frame of action A2 data have been collected both in 2020 and 2021. Combining results obtained using all the methods, 318 reliable bear bio-signs were found in the frame of

Action A2 (179 in 2020 and 139 in 2021). Bear bio-signs in 2020 were found mainly in the southern portion of the monitoring area while in 2021 bear signs were found both in the southern and the northern portion of the monitoring area, including also the north-eastern portion where few or no signs at all had been detected in the last 10 years. In order to better understand the ongoing re-colonization pattern, data collected in 2020 and 2021 have been observed also in light of data collected in the previous years. From 2004 to 2021 the number of bear bio-signs augmented passing through peaks and hollows and 2011 seems to have been a turning point after which the number of detected bio-signs increased reaching the maximum values in 2020 and 2021. Comparing Minimum Convex Polygons of bio-signs detected in the periods 2004-2010 (when few dozens of bio-signs/year were found), 2011-2019 (from the turning point to beginning of the LIFE ARCPROM) and 2020-2021 (years of implementation of Action A2), a clear change in bio-signs distribution emerged and the pattern observed suggests that the current bear presence in MNP is the result of a recolonization process that started almost twenty years ago and never stopped since then, making bears use more and more portions of the area every year. Basing on the results of genetic analysis of bear biological material (87 samples analyzed by the competent laboratory, 39 in 2020 and 48 in 2021) and combining these results with the ones coming from other monitoring activities and data collected with GPS collars, the minimum number of bears was 6 in 2020 (3F, 3M) and 13 in 2021 (5F, 8M). Observing the trend of the minimum number of bears detected from 2012 (i.e. from when standardized and comparable analysis of genetic samples are available) the most important result is that the number doubled from 2019 to 2020 and again from 2020 to 2021 and that also the minimum number of females passed from 2 to 5 from 2019 to 2021. In 2020 and 2021 no females with cubs have been detected in the MNP monitoring area but, considering the increase observed in the number of females, particular attention will be paid in the next years to detect any possible reproduction event.

In order to better understand the re-colonization dynamic, an analysis of *if* and *where* individuals present in MNP have been sampled in other portions of the bear range was carried out. Results obtained led to the conclusion that new individuals are arriving from the source population, that (with high probability) new individuals are also being born in the MNP monitoring area and that a movement of several individuals has been happening in the last 2-3 years in the corridor between the source population and MNP. This particular datum, together with the trend observed for the number of bio-signs, the trend of the minimum number of bears present in the area and the data concerning number and location of females, bolsters the hypothesis that the recolonization process in MNP entered in a more “active” phase in the last 2-3 years.

The data collected through Action A2 will actively contribute to the implementation of all the concrete actions foreseen in the frame of the LIFE ARCPROM project.

PREFACE

Why Action A2

Wildlife management and conservation actions cannot effectively be implemented without knowing the status of the target taxon. This is a general rule which always apply but it becomes even more essential when actions are specifically addressed to the reduction of human-wildlife conflicts. The presence of the variable "human" in the already complicated framework of wildlife conservation, generates additional challenges which can only be handled having a detailed knowledge of the situation and the dynamics going on in a specific territory.
In the LIFE ARCPROM project Action A2, in synergy with Action A1 (*Identification-delineation of sectors with high risk of human-bear conflicts*), is thus essential to orientate the implementation of concrete conservation as well as communication/awareness raising actions. Even though in the project proposal it is stated that Action A2 will directly contribute to the implementation of Actions C5, C7, C8 and C9, it will actually directly contribute to almost all C actions (Table 1).

Table 1 . Contribution of Action A2 on additional concrete conservation actions with respect to those reported in the project proposal.

Action code and main topic	A2 Contribution
C1. Stakeholder consultation and involvement	Individuation of stakeholders to be actually involved in the platform basing not only on the actual bear distribution but also on the distribution "dynamic" observed. This last issue, essential to have a proactive approach, not only applies to project areas where bear range expansion is ongoing but also to those areas where the range is stable but still affected by some variables (e.g. habitat loss/degradation).
C3. Operation of anti-poison units	Individuation of the areas where poison baits could affect bear conservation to a greater extent (e.g. areas with female presence or areas where the genetic variability is lower than others).
C6. Mobilization of volunteers	Choice of the areas where to focus this activity in relation to where bears actually are and to where the human-caused mortality is possibly having an high impact on bear conservation (e.g. low detected genetic variability and genetic distance between individuals could be related to high levels of human-caused mortality).
C10. Bear friendly labelling	Individuation of the areas where to focus and, consequently, individuation of the key possible beneficiaries to optimize project objectives achievement.

Project areas background

Action A2 has been implemented in all the project areas, namely the Northern Pindos National Park (PINDNP), Maiella National Park (MNP), Prespa National Park (MBPNP) and Rodopi Mountain Range National Park (RMNP). Even though in the four Parks Action A2 has been implemented pursuing the same general scope (see below) and applying the same general method, differences in the specific objectives and methods applied are present between Greece and Italy. In order to better understand and interpret A2 methods and results reported

later on in this document, it is thus useful to briefly report here the background both for Greece and Italy.

In **Greece** the Brown bear Ursus arctos (*) range consists of two (2) major population nuclei geographically separated but with recent signs of a first low level of communication through vagrants (Pylidis et al 2021). These two nulcei are located approximately 200 km apart in the north-western and north eastern part of the country and namely in Peristeri-Pindos mountain range and Rodopi mountain complex. Effective species distribution extends over 24,105 km^2 whereas the overall range is > 36,000 km^2 (Mertzanis et al. 2021). The Peristeri-Pindos range represents the southernmost distributional edge of the species range at a European scale, thus of outstanding bio-geographic importance.
The overall brown bear population in the country has shown positive trends at a local scale (mainly in Pindos range) reaching today 475-500 individuals minimum (Papamichael et al. 2015, Pylidis et al. 2015, Karamanlidis et al. 2018, Pylidis et al. 2021) with an expanding distribution over historical range (Mertzanis et al. 2009). At a biogeographical scale: the western population nucleus is directly connected to the Dinaric-Pindos biological brown bear population (covering 8 countries over the W. Balkans) and numbers 3.070 individuals (the 2nd largest brown bear population in Europe) whereas the RMRNP is connected to the East Balkan biological population which reaches 520 individuals minimum (Kaczensky et al. 2013). The brown bear population sizes in the targeted sub-areas of the proposed project are: Prespa Lakes NP (and wider area of Florina prefectural unit) estimated at an average (Nc) of 109 individuals (min. 52 – max 196, 95% CI) (Pilidis et al. 2015); N. Pindos NP: estimated at 140 individuals minimum (Karamanlidis et al. 2007, Karamanlidis 2011, Karamanlidis et al. 2017 and Mertzanis et al. 2008); RMNP 45-97 individuals (Pilidis et al. 2015). This sums an estimated size of circa 250 individuals which represents circa 50% of the total brown bear population in the country. Although total Ursus arctos* distribution covers large and continuous areas, both population nuclei are affected by either habitat disruption due to large infrastructure (mainly highways) or to inappropriate land use. In PINDNP sub-area, the eastern border of the area targeted by the project, Ursus arctos* habitat has suffered from 2005 to 2009 severe degradation and disruption due to the construction of the Egnatia highway (Mertzanis et al. 2009). In RMNP sub-area, the eastern part of the area will suffer from degradation due the current construction of another Egnatia highway stretch connecting Greece to Bulgaria. Additionally, small land ownership with farmland but also degraded oak forests due to over-exploitation, coupled to forest fires & over-logging are the most crucial factors of effective/potential Ursus arctos* habitat degradation in the sub-areas targeted by the project. Finally, a recent large scale oil extraction plans are threatening a large part of PINDNP area and related bear habitat with irreversible degradation and loss as well as do Wind Farms development plans in all (3) National Parks targeted by the project.

In **Italy** the project target is the Apennine brown bear (Ursus arctos marsicanus*), an endemic subspecies of the Central Apennines, classified as Critically Endangered in the IUCN red list (Kaczensky et al. 2013, Rondinini et al. 2013). Apennine brown bear (ABB) range in Central Italy reduced progressively (especially in the last 200 years) because of human persecution and bears now survive in a small, remnant population estimated in 50 (C.I. 45-69) individuals (Ciucci et al. 2015) living in a 5,000 Km^2 area (Ciucci et al. 2017). The main reproductive population survived during the 20th century in an area roughly corresponding to the Abruzzo National Park (PNA), one of the oldest National Parks in Italy (established in 1923) and the only National Park established in Abruzzo before 1991. Clearly, the protection of the territory through the PNA establishment played a role in avoiding ABB extinction and, in the same way, the establishment of the other protected areas (3 National Parks and 1 Regional Park in

Abruzzo) in 1991 played a role in favoring bear expansion to its historical range. In the Maiella National Park bears probably never disappeared but only in the last 10-20 years, data on bear presence became more and more abundant.
As stated at the beginning of this document, having accurate data on bear distribution and numbers is essential to plan and implement conservation actions but, when a re-colonization process is ongoing, collecting such data can be challenging and requires specific approaches to optimize the cost/benefit ratio. Particularly, systematic approaches are hardly applicable as they have high costs (in term of working hours, number of persons involved etc.) but benefits (i.e. data collected) can be really low if not absent at all. In fact, the last population estimation developed by Ciucci et al. (2015) using CMR models based on a systematic data collection design, refers only to the area with the highest bear density (MNP is thus not included in the estimation) and still the accomplishment of all the statistical assumptions was challenging. In the same way, the monitoring protocol specifically drafted for the expansion areas in the frame of the Apennine Brown Bear Monitoring Network (RMAM, established by MNP and PNA in 2017), individuated the opportunistic approach as the best one to be used in MNP in term of cost/benefit ratio.
Before the starting of the LIFE ARCPROM, data on bear distribution and numbers have thus been collected in MNP adopting an opportunistic protocol which, in 2017, was formalized through the institution of the RMAM. Data collected showed a variability on both the number of individuals and the distribution, an expected result considering that re-colonization by bears is a long process that implies individuals going back and forth from the source area to MNP and exploring different areas before choosing where to eventually establish. For these reasons, if accurate and up to date data are needed, the assessment of bear distribution and numbers in MNP needs to be done on yearly basis and this is the reason why in the frame of action A2 data have been collected both in 2020 and 2021.
Monitoring bear distribution and abundance implies to some extent the application of genetic samples analysis, a tool that beyond giving reliability to the results, also gives an amount of additional useful data. In the case of the ABB, due to the very low genetic variability and the high inbreeding rate among individuals (also assessed with the recent work of Benazzo et al. 2017) the only result currently possible is the attribution of each sample to a single genotype thus having the information of *which* bear is present and *which* is its sex. Information such as among-individual genetic variability and kinship are, however, impossible to obtain so far. In 2019-2020 MNP, together with other members of the RMAM, financed a specific study aimed at investigating the possibility to apply the SNPs technique to assess kinship among Apennine brown bear individuals. The study, carried out by ISPRA the Italian reference laboratory for ABB genetic analysis, had promising results opening the possibility to use SNPs to at least test some specific kinship hypothesis but the work is still ongoing thus impeding the inclusion of kinship analysis in the frame of Action A2.

Scope and objectives of Action A2

The general scope of Action A2 is to achieve information on bear population status and viability in the four National Parks in Greece and Italy. This general scope is pursued through the establishment of the following objectives:

1. Assess the number of bears
2. Assess population structure
3. Verify presence of females and family groups (Italy only)
4. Assess genetic variability and robustness (Greece only).
5. Assess bear distribution

Objectives 1, 2 and 5 are common for Greece and Italy, even though they will be achieved using different methods due to the differences in the current population status and distribution. Objective 3 and 4 are, however, specific for Italy and Greece respectively. In fact, presence of females and family groups is an indicator of how exactly is the re-colonization process going and what to expect to happen in the future so that it is a crucial information only for MNP (see §Report of activities in Italy). In the same way, the assessment of genetic variability and robustness is only possible in Greece as the low genetic variability of the ABB population doesn't allow the development of a such data analysis in Italy.
Objective 2 is common to Greece and Italy but in Italy it is constrained by results of the camera-trapping activity which, due to the current status of bear population in MNP, could give little if no results.

Tasks foreseen vs tasks actually implemented

The achievement of A2 objectives is based on the implementation of 3 non-invasive methods: 1) field collection of bear biological material (for DNA analysis), 2) field tracking surveys to detect bear bio-signs and 3) IR cameras operation. The first method is the one expected to produce the most robust outcome while the last two methods are to be considered complementary and auxiliary to method 1. These 3 methods imply the development of 4 main tasks: collection of genetic samples, genetic samples analysis, development of field surveys and IR camera positioning (Table 2). In PINDNP and RMNP some of the above mentioned tasks had already been implemented in the frame of the LIFE ARCPIN and National Monitoring projects respectively and were thus not to be implemented again in the frame of the LIFE ARCPROM (Table 2a). However, after the evaluation of the quality of the genetic samples collected in PINDNP and after and overall evaluation of the collected data, the collection of genetic samples and the IR camera network operation were actually implemented in PINDNP in the frame of Action A2 (Table 2b).

Table 2. Tasks foreseen in each National Park for the implementation of the 3 methods of Action A2 as reported in the project proposal (a) and as actually implemented (b).

*** Sampling network already installed during National Monitoring projects, only to be completed in the frame of the LIFE ARCPROM.**

a

Task	PINDNP	MBPNP	RMNP	MNP
Collection of genetic samples	NO	YES	YES*	YES
Genetic samples analysis	YES	YES	YES	YES
Field surveys	NO	YES	YES	YES
IR cameras	NO	YES	YES	YES

b

Task	PINDNP	MBPNP	RMNP	MNP
Collection of genetic samples	YES	YES	YES*	YES
Genetic samples analysis	YES	YES	YES	YES
Field surveys	NO	YES	YES	YES
IR cameras	YES	YES	YES	YES

INTRODUCTION

Non-invasive carnivore studies

Technological and methodological advances, and new techniques for data analysis, have contributed to a rapid increase in (NICS). These studies complement and extend inferences from traditional sampling regarding individuals, populations, and communities. Today, researchers can estimate size and survival rate for a population, estimate historic and current rates of movement across fragmented landscapes, and measure carnivore stress loads without ever catching, handling, or even seeing a single animal. Non-invasive sampling is the gathering of data without capturing, handling, or otherwise physically restraining individual animals. The techniques usually imply that a target animal is not observed during data collection and, presumably, is unaffected by data collection (Kelly et al. 2012).
Although direct animal observations for behavioural studies and for distance sampling may also be considered non-invasive, we do not include these direct observation methods. Non-invasive data-collection methods include sign surveys, diet analyses, camera trapping, DNA extraction, and endocrine or disease monitoring from scats and hair. Perhaps a better name is "less invasive" because we do not really know the impact, for example, of removing scat samples found in a jaguar (*Panthera onca*) habitat for 2 months. Such a study might disrupt marking behaviour and unknown impacts could arise in a study site from the presence of a trained scent-detecting dog locating scats. Nonetheless, the term has gained familiarity and become conventional (Long et al. 2008, *in Boitani & Powell 2013*)

Why use non-invasive sampling?

The advantages are numerous. Capture and handling are highly stressful and potentially dangerous to both humans and animals, especially with large carnivores. Invasive studies require more permitting, especially with endangered species, and often suffer issues with animal care and use committees. In addition, capture, handling, and subsequent monitoring are usually expensive, logistically difficult, time consuming, and result in small samples sizes, limiting population-level inferences, especially for elusive, low-density, or trap-shy animals like the brown bear in our case. By contrast, non-invasive techniques can produce larger sample sizes, reducing bias, increasing precision, and broadening the scope of potential hypotheses.
Non-invasive field sampling is often relatively simple to employ and to standardize, training inexperienced people can be easy and studies can cover large areas. Finally, non-invasive sampling is less likely to induce a trap response in animals, again reducing human-induced bias. While non-invasive techniques supply new information and hold great promise, we do not suggest that they should replace all traditional capture and handling studies, such as those to affix transmitters for studies of movements, home ranges, and habitat selection.

Brief review of non-invasive sampling methods

Sign surveys: Naturalists have sampled carnivores noninvasively for decades. Skilful, field-based identification of tracks, scats, kills, bones, and hair have illuminated much of what we know about distribution and habits of carnivores. In fact, identification of animal sign can be quite reliable in some instances. Misidentification of carnivore sign in the field occurs more often when the target species is rare (Prugh and Ritland 2005, *in Boitani & Powell 2013*). As with scat, identification of tracks in snow, dirt, and mud can be useful and at times reliable. However, identification problems can arise due to substrate quality and animal movements

(Heinemeyer et al. 2008, *in Boitani & Powell 2013*). If concerns about uncertainty can be ameliorated, track surveys can be effective and inexpensive for occurrence and distributional studies. Identifying signs is an excellent natural history skill, but sign surveys by themselves supply sometimes limited information, due to inability to distinguish individuals. Assuming, however, that species' identity from sign surveys is accurate, "occupancy modelling" allows researchers to combine detection/non-detection histories with spatial modelling to estimate and to predict species' occurrence across a landscape. By incorporating estimates of detectability from sign surveys directly, this approach corrects the inherent negative bias present in naïve occupancy estimates (MacKenzie et al. 2003; Tyre et al. 2003, *in Boitani & Powell 2013*).

Genetic sampling: Non-invasive collection of genetic samples is limited only by the creativity and natural history knowledge of the investigator. Carnivore hairs and scats are the two most commonly collected genetic samples. Hairs are often obtained via snags or rub devices. To sample bears, researchers have strung barbed wire around bait, and bears leave hair on the wire when approaching the bait (Woods et al. 1999; Mowat and Strobeck 2000; Kendall et al. 2009 *in Boitani & Powell 2013)*. Sampling bears' natural rub trees can detect bears not sampled by barbed wire corrals (Boulanger et al. 2008; Stetz et al. 2010 *in Boitani & Powell 2013*).
For some species, the amount of DNA left may be very small (e.g. single hairs or hair fragments). Hairs with follicles provide higher quality DNA extracts than do scats, which have more agents that inhibit and prevent amplification. A single hair, however, usually yields much less DNA than faeces. Multiple hairs can usually be pooled to increase DNA yield for species' detection studies, because diagnostic bands for multiple species can be simultaneously visualized. When individual identity is required, however, pooling multiple hairs is risky because it can create false, "new" genotypic individuals (Alpers et al. 2003; Roon et al. 2005 *in Boitani & Powell 2013*). Researchers must accept the low DNA yield from single hairs, or perhaps develop a hair snag that allows only one animal to rub it (Schwartz et al. 2006; Beier et al. 2005; Bremner-Harrison et al. 2006, *in Boitani & Powell 2013*).

Camera-trap sampling: Photographing wildlife via remotely triggered cameras (camera trapping) emerged in 1877 (Guggisberg 1977, *in Boitani & Powell 2013*) but was little used until the invention of infrared, automatically tripped cameras in the 1980s. Cameras became commercially available, lightweight, and easy to operate. In the mid-1990s, large-scale camera grids were linked with capture–mark–recapture analysis to estimate animal abundance (Karanth 1995; Karanth and Nichols 1998, *in Boitani & Powell 2013*). The 2000s brought digital camera technology. Widespread, remote camera use has resulted in an increase in carnivore inventories, due to the ability to photograph multiple species at the same site (Barea-Azcon et al. 2007; Datta et al. 2008; Tobler et al. 2008; Can and Togan 2009; Johnson et al. 2009; Pettorelli et al. 2009, *in Boitani & Powell 2013*). Two infrared trigger mechanisms exist in remote camera technology: active and passive infrared systems. An active infrared beam is triggered by an animal breaking an infrared beam that passes from a transmitting unit through the detection zone to a receiving unit. A passive infrared system is triggered by the heat difference between the animal and the environment as the animal moves past a heat and/or motion sensor (Kays and Slauson 2008, *in Boitani & Powell 2013*). Most modern studies use passive infrared systems. Digital camera options now include white flash and infrared flash, but image quality is still low with the latter. Digital camera durability and reliability are increasing and, most importantly, they do not have the 36-exposure limit of film cameras. Some passive digital can transmit images wirelessly to a base station or laptop computer. Additionally, many models can collect short video sequences.

REPORT OF ACTIVITIES IN GREECE

Methods

1. Field collection of bear biological material (genetic analyses)

This method focused on the genetic analysis of 10 DNA microsatellite loci received from hair samples of brown bears in the (3) project sub-areas in Greece: (MBPNP), (RMNP) & (PINDNP). The aim of the study was to obtain information regarding:
a) **Population structure** (number of bears present in the 3 sub-areas, sex ratio, e.tc) through the estimation of total (Nc) and effective (Ne) population size and testing for signatures of past bottlenecks
b) **Genetic variability** of and possible differentiation between the eastern and western subpopulations,
c) **Connectivity and migration** of bears between the above regions.
The results indicate the viability of each bear sub-the population and are expected to have a direct impact on CCA's implementation in the project sub-areas. They will also lead to a mid and long- term conservation plan, in order to establish a better coexistence between humans and brown bears in Prespa, Pindos and Rodopi National Parks.

1.1 Sampling

The samples were collected from the hair trap network which was established during the ARCPROM project from the areas: Prespa (MBPNP), Pindos (PINDNP) and Rodopi (RMNP) National Parks. Non-invasive genetic sampling was based on the ubiquitous marking and rubbing behaviour of bears on wooden poles of the telephone and electricity network (power poles) in Greece (Karamanlidis et al. 2007, 2015). The hair traps (coiled barbed wire) were placed on selected power poles according to the bear activity (signs of bites, scratches etc.) in the project areas. Power poles are made of wood (approximately 30–50cm in diameter and 10m high) and processed with a wood preservative (i.e., coal tar creosote, a substance derived from tars) to resist decay and insect damage. Depending on topography, poles are usually placed 50 to 100m apart, and vegetation is cleared 5m from each side of the power pole line (Karamanlidis et al. 2010). After collection, hair samples were placed in paper envelopes containing silica gel desiccant and stored at -20^{o}C. In total UTH received 522 samples that were used in this project: 96 samples from MBPNP, and 170 samples from PINDNP and 256 samples from RMNP.

1.2 DNA extraction

Each tuft of hair on a set of barbs was considered a sample. Hair samples were collected without contact to human skin, they were placed in uniquely numbered paper envelopes labelled with the exact location, coordinates, date and height of collection and then stored at -20^{o}C in zip-lock bags with silica gel until being analysed by UTH. For all the collected samples DNA extraction was performed using the DNA Mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions.

1.3 PCR amplification

DNA was amplified for 10 commonly used microsatellite loci G10H, Mu26, G1D, G10X, G1A, G10P, G10C, Mu59, G10L, Mu50 as well as ZFX and the SRY gene (Taberlet et al. 1996, Paetkau et al. 1998, Bellemain & Taberlet 2004). Gender identification is performed using specific primers (Table 3) which co-amplify a bear-specific Y marker (SRY gene) and a bear-specific X marker (ZFX gene), according to Pages et al. 2009 (Pagès et al. 2009). If the sample is a male bear 2 bands (144bp and 115bp) appear post electrophoresis (Figure 1) and 1 band (144bp) appears in the case of a female bear (Guichoux et al. 2011). The primers that were used for each locus are shown in table 3.

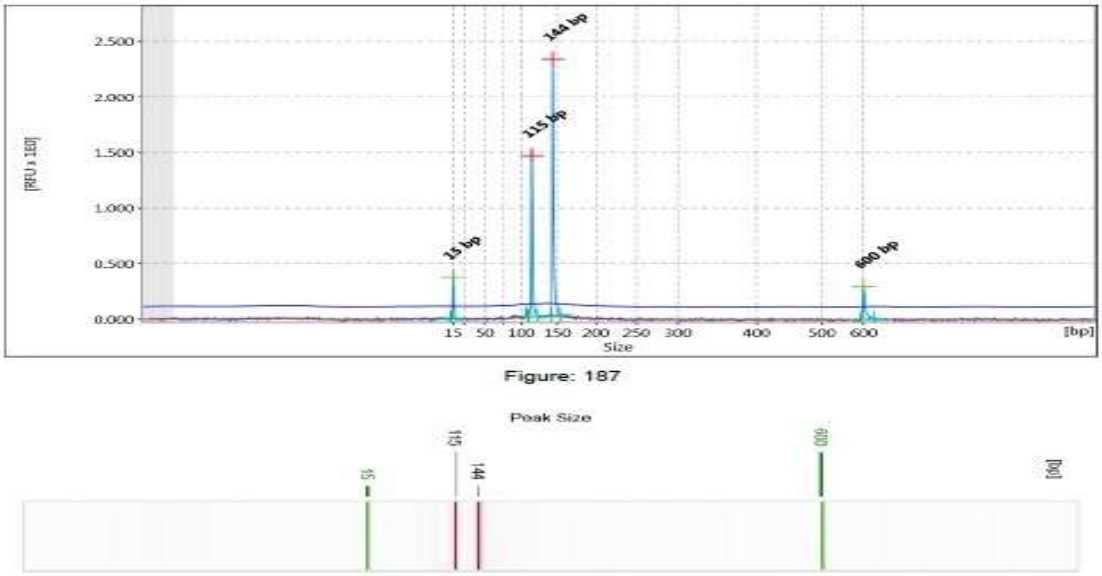


Figure 1. An XY Brown Bear: 2 bands appear post capillary electrophoresis via Qiaxcel (144bp and 115bp)

Table 3: Primer sequence and length of the 10 microsatellite loci

	Primer Sequence	length (bp)	Reference
G10H F	5'-CCCAACAAGAAGACCACTGTAA-3'	221–257	Paetkau et al. 1998
G10H R	5'-CCAGAGACCACCAAGTAGGATA-3'		
G10L F	5'-TGTACTGATTTAATTCACATTTCCC-3'	153–163	Paetkau et al. 1998
G10L R	5'-GAAGATACAGAAACCTACCCATGC-3'		
Mu50 F	5'-GTCTCTGTCATTTCCCCATC-3'	110-130	Bellemain&Taberlet 2004
Mu50 R	5'-AACCTGGAACAAAAATTAACAC-3'		
Mu26 F	5'-GCCTCAAATGACAAGATTTC-3'	182-200	Taberlet et al. 1997
Mu26 R	5'-TCAATTAAAATAGGAAGCAGC-3'		
G10P F	5'-TACATAGGAGGAAGAAAGATGG-3'	145-159	Paetkau et al. 1998
G10P R	5'-AAAAGGCCTAAGCTACATCG-3'		
Mu59 F	5'-TGCTGCTTTGGGACATTGTAA-3'	219-251	Taberlet et al. 1997
Mu59 R	5'-CAATCAGGCATGGGGAAGAA-3'		
G10C F	5'-AAAGCAGAAGGCCTTGATTTCCTG-3'	97-116	Paetkau et al. 1998
G10C R	5'-GGGACATAAACACCGAGACAGC-3'		

G1D F	5'-ATCTGTGGGTTTATAGGTTACA-3'	172-184	Paetkau et al. 1998
G1D R	5'-CTACTCTTCCTACTCTTTAAGAG-3'		
G10X F	5'-CCCTGGTAACCACAAATCTCT-3'	132-154	(16, Paetkau et al. 1998)
G10X R	5'-TCAGTTATCTGTGAAATCAAAA-3'		
G1A F	5'-GACCCTGCATACTCTCCTCTGATG-3'	180-190	Paetkau et al. 1998
G1A R	5'-GCACTGTCCTGCGTAGAAGTGAC-3'		
SRY-F	5' -TGGTCTCGTGATCAAAGGCGC-3'	115	Pagès et al. 2009
SRY-R	5'-GCCATTTTTCGGCTTCCGTAAG-3'		
ZF-F	5'-GACAGCTGAACAAGGGTTG-3'	144	Pagès et al. 2009
ZF-R	5'-GCTTCTCGCCGGTATGGATG-3'		

The amplification conditions used were: denaturation at 94°C for 5min, 40 cycles at 94°C for 30sec, primer hybridization at 58°C for 45sec and elongation at 72°C for 1min. The final elongation was performed at 72°C for 8min. The reactions were performed in total volume 15μl using HotStarTaq Master Mix (contains 0,1 units/μl HotStarTaq DNA Polymerase, PCR Buffer with 3 mM MgCl2, and 400 μM of each dNTP), 50ngr DNA, 1 p/μl for each primer and RNase-Free Water. Thermal cycling was performed using an MJ Research (Peltier Thermal Cycler) PTC-200 thermocycler with 96-well "gold" blocks. Because of the low quantity of DNA, the samples were not electrophorized after DNA extraction process.
To evaluate the amplification of the specific locus all PCR products were electrophorized using 2% agarose gel. In order to identify the exact length of each microsatellite locus that was successfully amplified we performed capillary electrophoresis through the QIAxcel Advanced system.

1.4 Capillary electrophoresis

High-resolution capillary electrophoresis was performed using a QIAxcel® DNA high resolution kit (Qiagen) on a QIAxcel Advanced System (Qiagen), according to the manufacturer's instructions. A QX DNA Size Marker (Qiagen) with 10 fragment sizes ranging in size from 25 to 500 bp was used to size PCR products. A QX Alignment Marker (Qiagen), which consisted of 15-600 bp fragments, was injected onto the cartridge with each sample. The 0M800 method in the ScreenGel® software (Qiagen) was used for all analyses; this corresponds to a 10 sec sample injection time at 5 kV and 800 sec separation time at 3 kV. The QIAxcel system injected 0.1 μl of 20 μl PCR products onto a cartridge for analysis. The retention time of the PCR fragments relative to the 15-bp and 600-bp QX Alignment Marker fragments was calculated using the ScreenGel software (Qiagen). The PCR product sizes were then determined by comparing the retention time with the QX DNA Size Marker. The ScreenGel software produces a digital gel image and an electropherogram for fragment analysis.
The high detection sensitivity provided by the QIAxcel Advanced System enables robust results even with low concentrations of nucleic acid. QIAxcel Advanced system has a high resolution for fragments smaller than 0.5 kb and ensures great accuracy and confidence in data interpretation. Sample consumption is less than 0.1 μl per analysis, saving precious samples for further downstream analysis.

1.5 DNA Fragment Analysis

The 10 microsatellite loci were also analyzed in the ABI 3500 genetic analyzer (Applied Biosystems®), which allows the process of 1-5 different loci in the same time. This method was used in 10 samples to verify the size of the bands in each loci and to prove the accuracy of Qiaxcel capillary electrophoresis. The main stages in this method are: 1. PCR amplification with fluorescent dyes, 2. Sample preparation with formamide and a marker, 3. Capillary electrophoresis in the Analyzer, 4. Length analysis (Peak Scanner Software v.10.).

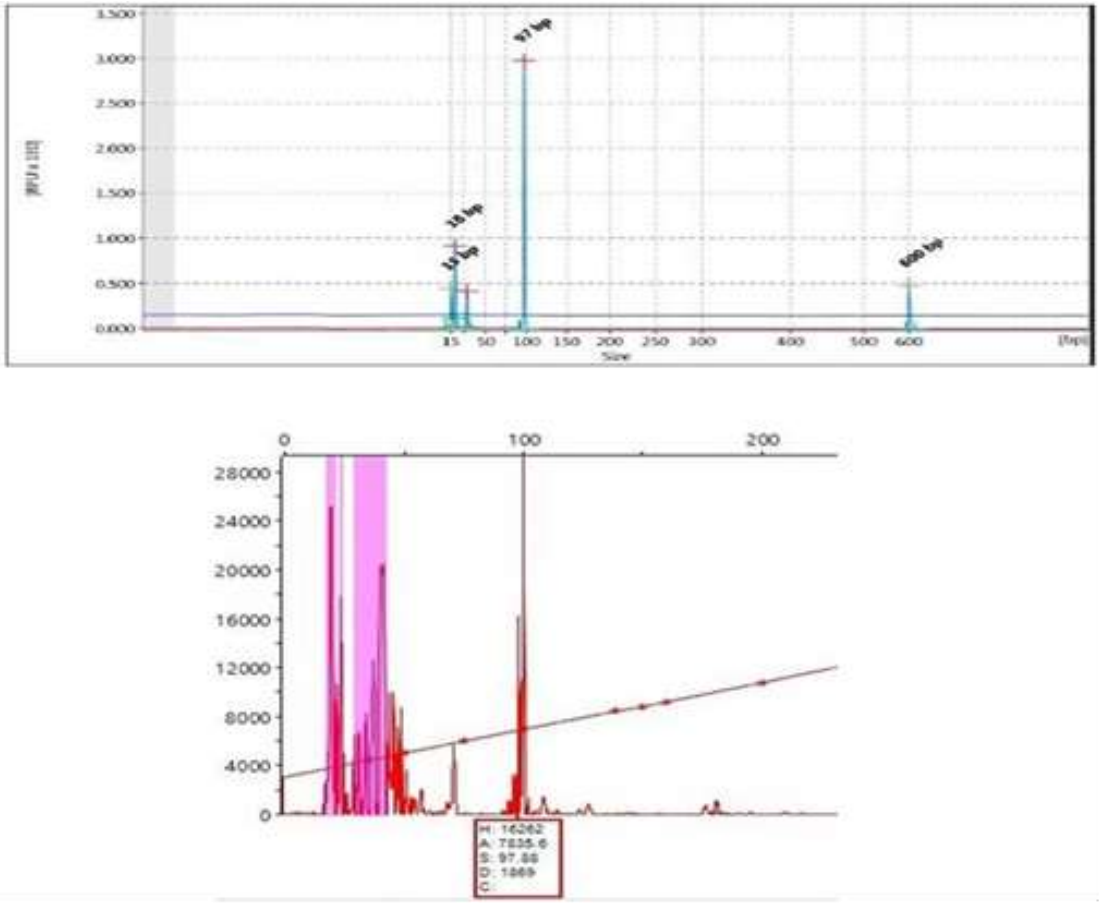


Figure 2: Homozygous sample for allele 97bp of the G10C locus defined both by Qiaxcel Advanced System and ABI3500 genetic analyzer.

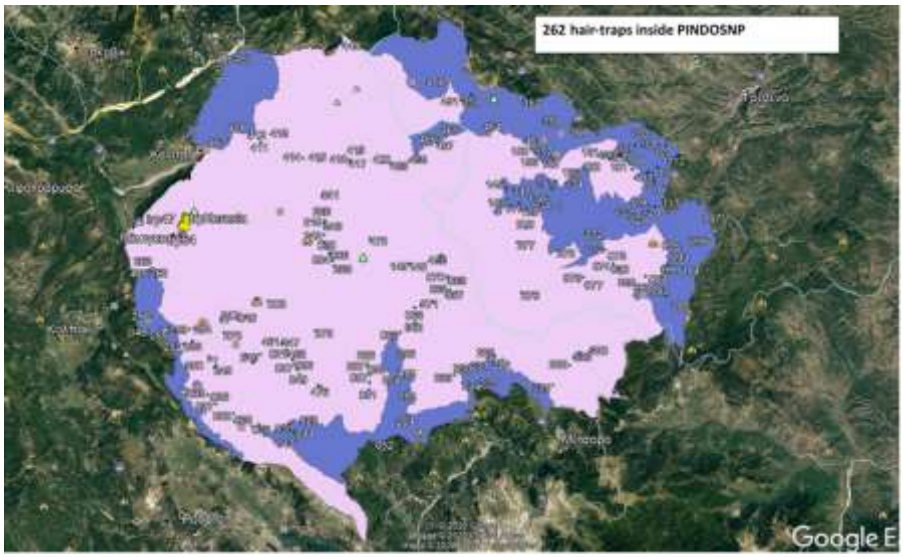
1.6 Statistical tools for estimation of population size and genetic analysis

In order to find the possibilities of identification between siblings, we use Gimlet software (Valière 2002). In the next step of our analysis, we used Cervus 3.0, to calculate various summary statistics for each locus, such as the number of individuals typed, the number of alleles, and the polymorphic information content (PIC) (Tessier et al. 1999 , McKelvey &Schwartz 2005, Kalinowski et al. 2007). For the calculation of the unique genotypes in our sample, we used Dropout. The deviation from the Hardy-Weinberg equilibrium was calculated with the Genepop 4.6 software (Rousset 2008), with the exact Fisher's tests and the p-values are coming from the Markov Chain method. Again, we used Genepop for the calculation of the observed and expected heterozygosity of each locus.

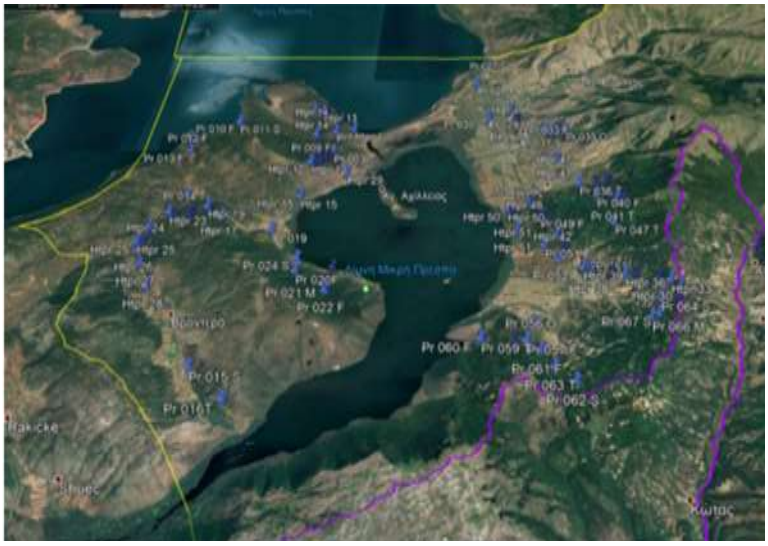
The program BOTTLENECK computes for each population sample and for each locus the distribution of the heterozygosity expected from the observed number of alleles (k), given the sample size (n) under the assumption of mutation-drift equilibrium (Piry et al. 1999). We also used NeEstimator 1.3, a tool for estimating contemporary effective population size (Ne) using multi-locus diploid genotypes from population samples and was calculated by (Peel et al. 2003). The Ne value was calculated with the option "linkage disequilibrium method". Moreover, mark-recapture studies showed that an individual can be captured once per sampling session, but in non-invasive genetic sampling, like ours, individuals are counted multiple times as the sampling takes place in a single session (Puechmaille & Petit 2007). In order to incorporate data from a single session in non-invasive sampling or multiple sessions 'Capwire' was established (Pennell et al. 2013) and is capable of estimating population sizes (in small populations <100 individuals) from different species (Piggott & Taylor 2003, Arrendal et al. 2007). ECM assumption (equal capture model) stands that individuals are allowed to come from different rate classes (Botstein et al. 1980, Weir & Cockerham 1984, Boulanger &McLellan 2001, Miller et al. 2005). There is a second model, which is called Two-Innate Rates Model (TIRM), were the population is assumed to contain individuals that are easy to be captured (Na) and those individuals that are not (Nb) (Pennell et al. 2013). We can also run bootstrap, to estimate confidence intervals for the MLE. Individuals in the present research analysis were identified with microsatellite loci, so we assume that the population contains a mixture of individuals with two distinct capture probabilities (TIRM model) (Pennell et al. 2013). Finally, we used "Documentation for STRUCTURE software: Version 2" in order to make an estimation of population structure (Evanno et al. 2005).

1.7 Sampling - Field work

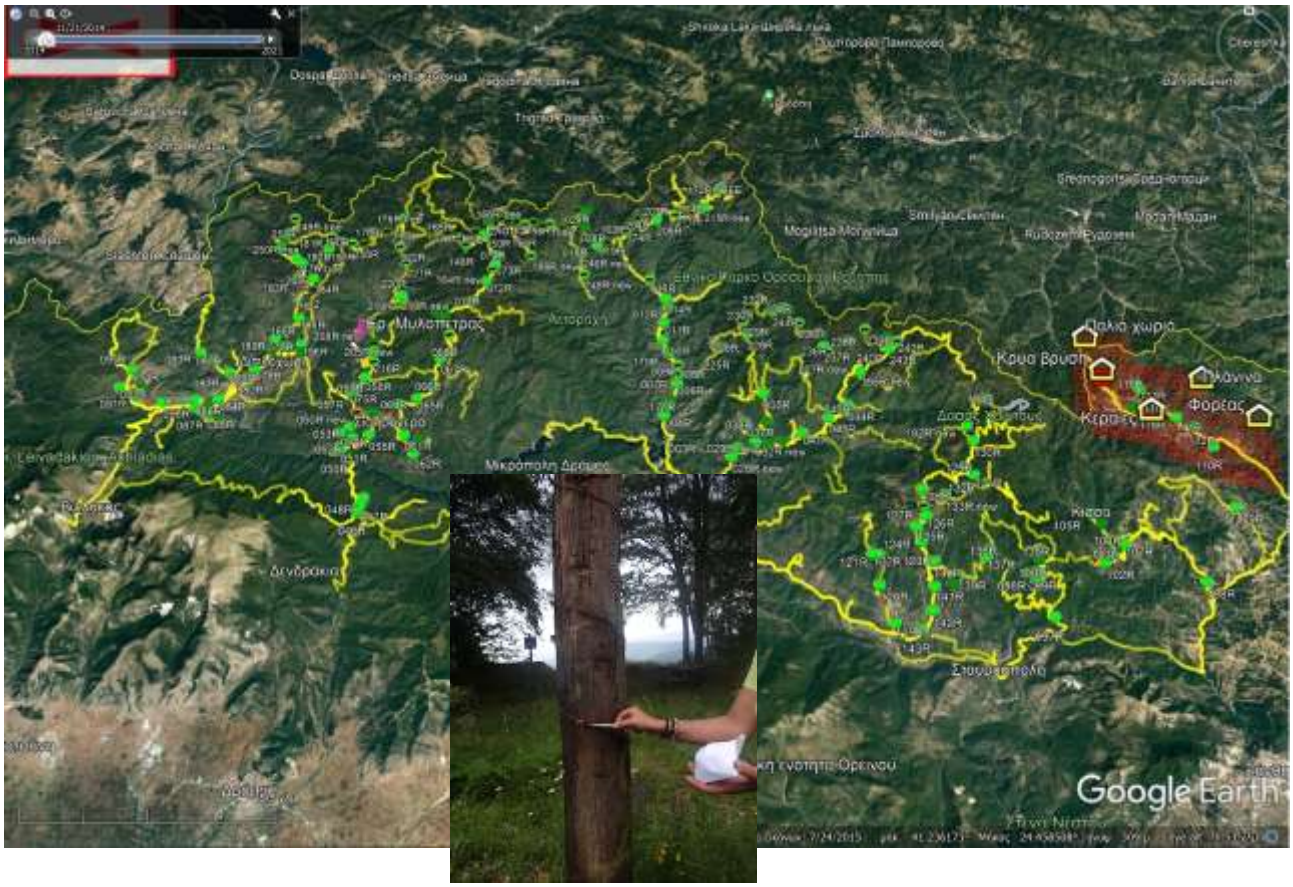
The samples were collected from the hair trap network which was established during the ARCPROM project in the three project sub-areas: Prespa (MBPNP), Pindos (PINDNP) and Rodopi (RMNP) National Parks. In PINDNP this action took place in the frame of LIFE ARCPIN but as mentioned before the sample quality was very poor. Therefore, after budget modification this action was repeated in the frame of LIFE ARCPROM using the pre-existing hair-trap network which was also updated. Non-invasive genetic sampling was based on the ubiquitous marking and rubbing behaviour of bears on wooden poles of the telephone and electricity network (power poles) in Greece (Belant 2003, Karamanlidis et al. 2007, Karamanlidis et al. 2015).
The hair traps (coiled barbed wire) were placed on selected power poles according to the bear activity (signs of bites, scratches etc.) in the project areas. Power poles are made of wood (approximately 30–50cm in diameter and 10m high) and processed with a wood preservative (i.e., coal tar creosote, a substance derived from tars) to resist decay and insect damage. Depending on topography, poles are usually placed 50 to 100m apart, and vegetation is cleared 5m from each side of the power pole line (Karamanlidis et al. 2010).
After collection, hair samples were placed in paper envelopes containing silica gel desiccant and stored at -20^{o}C. In total UTH received 522 samples that were used in this project: 96 samples from MBPNP, and 170 samples from PINDNP and 256 samples from RMNP. Personnel from NP's and Callisto was involved in samples collection following 3 rounds: a) collection during hair-traps installation late spring, b) in early summer and c) in mid-summer. (see maps and photos 1,2,3 &4).



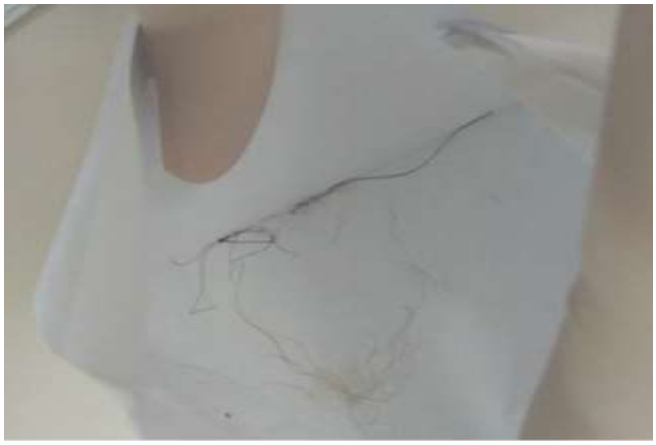
Map (1): Hair-traps network (262) in PINDNP and photos (1 & 2) : installation of hair-trap with barbed wire.



Map (2) Hair-traps network (n=50) in MBPNP and photo (3) : installation of hair-trap with barbed wire.



Map (3) Hair-traps network (256) in RMNP (green dots) and photo (4):sample collection

Photos (5, 6, 7 & 8): bear hairs initial processing at the laboratory (UTH)

2. Camera trapping – brown bear relative abundance

2.1. Cameras network installation

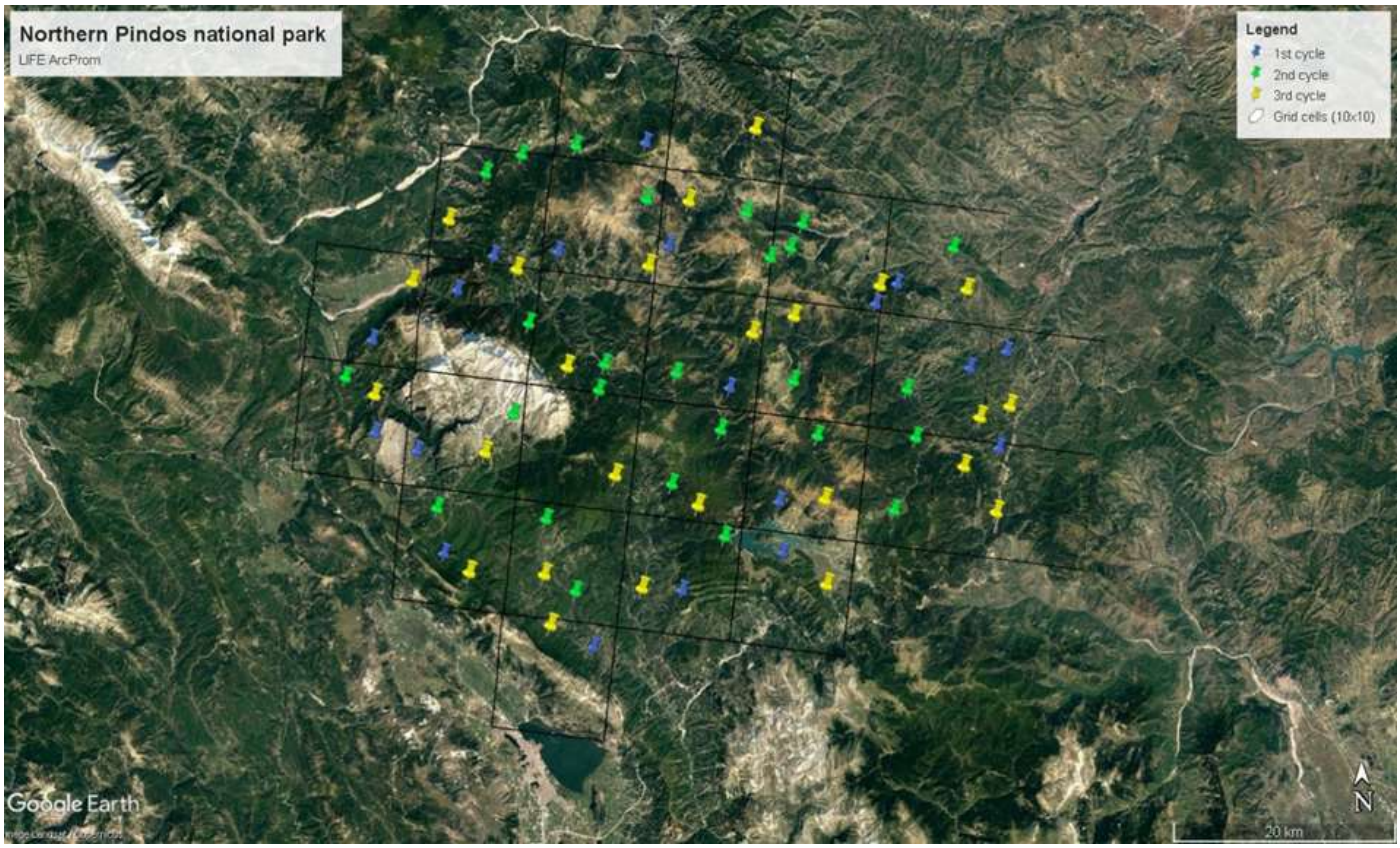
Camera trapping was used to assess the relative abundance of brown bears in the (3) National Parks (project sub-areas (of Northern Pindos (PINDNP), Prespa (MBMBPNP) and Rodopi Mountain-Range (RMNP).
Regarding the sampling protocol and the needs in IR cameras: according to a) previous specifications and standards set under LIFE ARCPIN project (but not implemented in the field due to project's termination), b) the 6year report on mammals species of Community interest in RMNP (2016), c) the surface of the (3) project sub-areas and d) the available time margins in the frame of LIFE ArcProm project (time schedule of actions (A), it was decided to use:
a) twenty-six cameras (26) were in PINDNP, b) eighteen (18) in MBPNP and c) twenty-seven (27) in RMNP.
The camera models used in PINDNP were: a) Browning Dark Ops HD Pro X, b) Reconyx RC60 RapidFire Covert IR, and c) Bushnell Trophy Brown HD . The camera model used in MBPNP was "Bushnell Trophy Cam Aggresor HD no Glow 24MP Camo, and the camera models used in RMNP were: a) Bushnell Trophy Cam Aggresor HD no Glow 24MP Camo, b) Bushnell Core DS No Glow Trail 32LED, c) Denver WCM-8010 Cam 12MP, d) Reconyx HyperFire 2 HF2X, e) Victure HC100 16MP, f) Reolink Go 4G LTE Camera, g) Apeman H40 and h) Browning BTC-8E Spec Ops Edge. Due to the variety of camera brands and models, a standardised procedure was used to minimise potential technical errors as much as possible.

For PINDNP: PINDNP covers an area of ~2.000 km^2 and was divided in twenty-six (26) grid cells covering 100 km^2 (10x10 km^2) based on male brown bears' average home range surface and in order to cover the entire area of the national park with the maximum representativity of habitat types but also in order maximize the bear detection probabilities. The sampling period covered ~six (6) months, and the protocol included a rotation of every camera inside the same grid cell in three (3) or four (4) different locations.
For the grid cells in the NP periphery which included areas outside the administrative borders of PINDNP, the cameras rotation was performed only twice (2) (placement in two different locations). The (2) extra cameras were used in grid cells nearby the central part of PINDNP where the sampling effort had to be enhanced due to terrain ruggedness and difficult

accessibility to the pre-selected locations for cameras rotation. The timeline of the sampling period was as follows:

- 14/04/2021 – 05/07/2021: First placement of the cameras (first cycle)
- 05/07/2021 – 17/08/2021: First rotation (second cycle)
- 17/08/2021 – 03/10/2021: Second rotation and end of sampling period

Personnel from Callisto CB was leading the whole activity of cameras protocol installation and checking operation. Personnel from PINDNP followed the operation in the field in order to gain necessary experience and know-how in order to replicate this activity in the future for monitoring purposes on wildlife species in the NP. The area of PINDNP and the grid cells network used for implementing the camera traps sampling protocol are shown on map (4), along with the different (successive) camera trap locations for each rotation cycle.



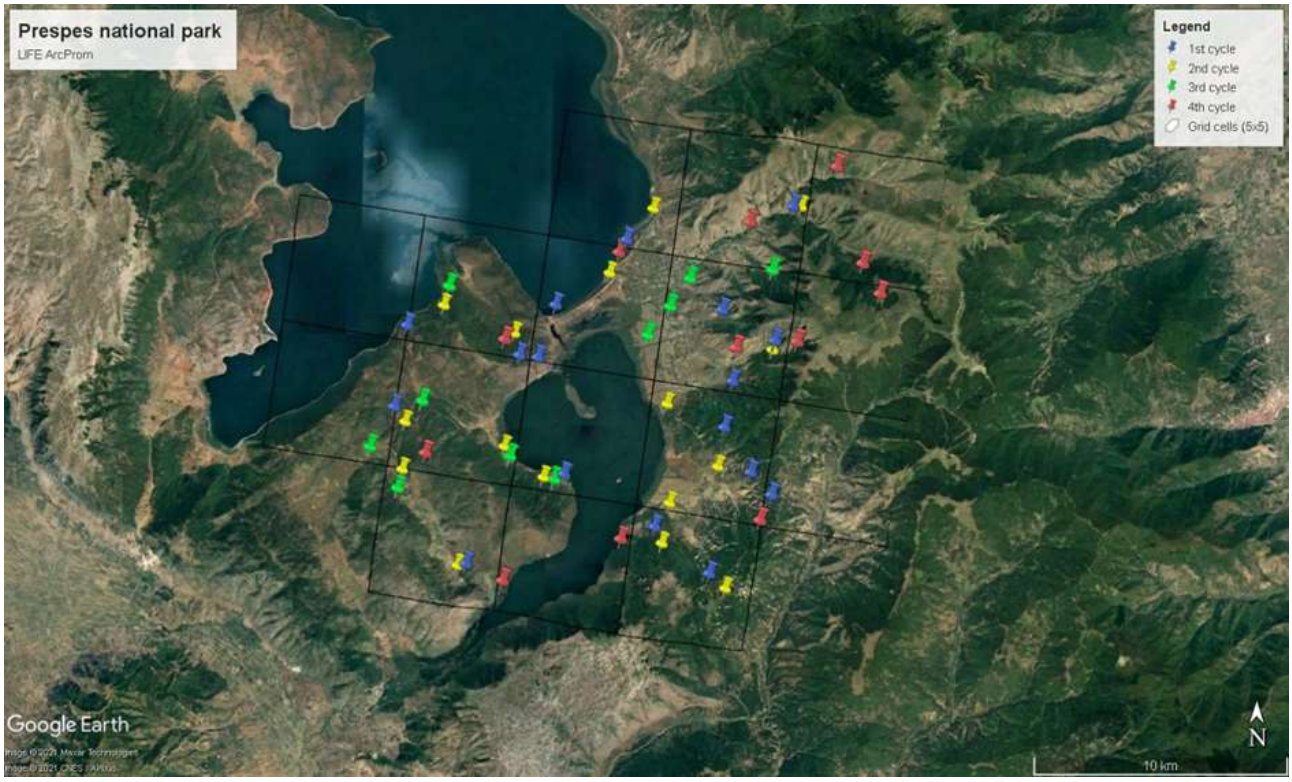
Map (4) : IR cameras sampling grid (10X10 km) in PINDNP and location of cameras in the (3) rotation cycles.

For MBPNP: MBPNP covers a surface of ~278 km^2 and due to the park's smaller size, the grid cell scale and coverage was adapted accordingly. The sampling area was divided in eighteen (18) grid cells covering 25 km^2 (5x5 km^2), based on the home ranges of female brown bears with cubs of the year ("FCOY") litter of the same year (evidenced with telemetry data in central Pindos area of Grevena). Here again a sampling period of ~six (6) months was implemented, with a rotation of each camera inside the same cell in four (4) different locations. It is also worth mentioning that the extreme south part of the MBPNP (two 5X5 grid cells – see results part) was not covered evenly due to its inaccessibility (no forest road network allowing a minimum access to these two sectors). The average duration of each rotation phase was 1.5-2 months maximum. Again in this case, grid cells with part of their

surface outside the NP's administrative borders were covered by less rotations. The timeline of the sampling period was as follows:

- 01/04/2021 – 30/05/2021: First placement of the cameras (first cycle)
- 24/05/2021 – 09/07/2021: First rotation (second cycle)
- 09/07/2021 – 03/09/2021: Second rotation (third cycle)
- 03/09/2021 – 19/10/2021: Third rotation and end of sampling period

The area of MBPNP and the grid cells used are shown on map (5), along with the camera trap locations of each cycle.



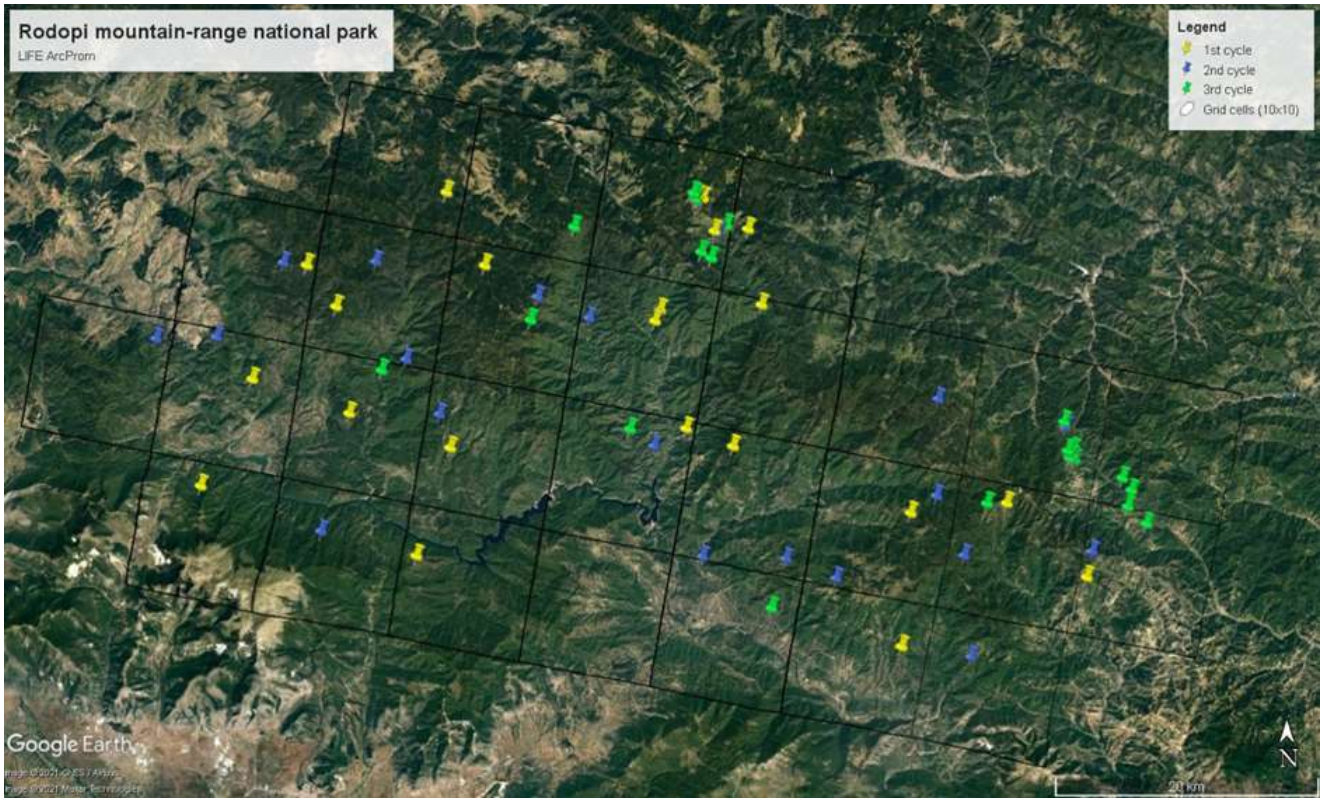
Map (5): IR cameras sampling grid (5X5 km) in MBPNP and location of cameras in the (4) rotation cycles

For RMNP: RMNP covers an area of ~1.750 km^2 and was divided in twenty-five (25) grid cells covering 100 km^2 each (10x10 km^2) based in male brown bears' average home range surface (as estimated with telemetry data in previous studies and projects). This grid cells dimension was also selected for the following additional criteria: a) the NP 's large surface (like in PINDNP), b) to cover a maximum of the national park surface – maximize sampling effort, c) to maximize bear detection probabilities and d) to maximize sampling representativity with coverage of all habitat types. The sampling period lasted for a total of nine (9) months, with the first six (6) months taking place in 2020 (as RMNP had already available the IR cameras equipment) with 1 rotation and the last three (3) months taking place in 2021 with the last rotation. The timeline of this sampling period was:

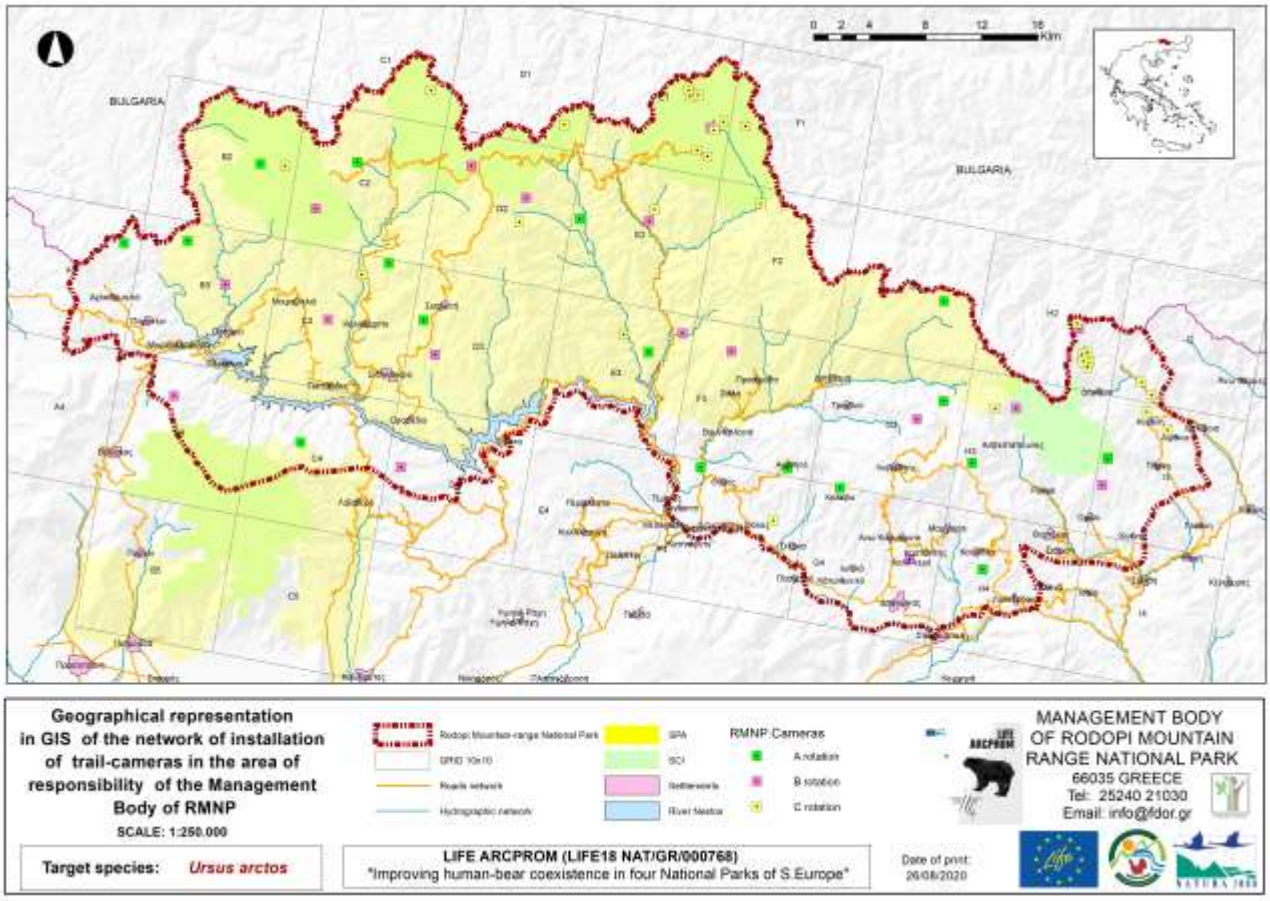
- 01.07.20 – 29.09.20: First placement of the cameras (first cycle)
- 30.09.20 - 03.12.20: First rotation (second cycle)
- 02.03.21 – 31.05.21/*27.10.21*: Second rotation and end of sampling period (third cycle).

As we may note, in the case of RMNP the protocol regarding the timeline length of each rotation/cycle was different for the ones implemented in the other (2) NP's (project sub-areas). In fact, here each cycle lasted circa 3 months and covered the entire length of each of the most important biological seasons for the bears (summer and fall 2020 and spring 2021). For 2021, and in order for the statistical analyses to yield comparable results with the other two investigated project sub-areas, we retained data only from the spring period (March to May 2021). The reason for this elongation of the rotation cycle duration is because according to previous data (results from genetic analyses in the 6years report from RMNP -2016) the RMNP area had showed very low bear population densities. Therefore, by prolonging the camera operation at the same location also maximized the overall sampling effort within one biological season.

The area of RMNP and the grid cells used for sampling are shown on maps (6 & 7), along with the camera trap locations for each cycle.



Map (6-7) : IR cameras sampling grid (10X10 km) in RMNP and location of cameras in the (3) rotation cycles (2020-21) .



Map (6-7): IR cameras sampling grid (10X10 km) in RMNP and location of cameras in the (3) rotation cycles (2020-21.

The installed camera traps were set to operate 24 hours per day and most of the camera traps were set on photo mode and set up to capture two (2) or three (3) photos after each activation. Several camera traps had the option for a hybrid mode and in order to gather further information on the morphological characteristics of each animal, these camera traps were set on hybrid mode, capturing both photos and videos after each activation. Trigger speed for each camera brand/model was different, ranging from 0.2 seconds to 0.6 seconds and no attractants or lures were used at the camera trap sites. Some unexpected problems occurred, such as some cameras' sensitivity levels were higher than expected, which resulted in numerous false alarms and overload of the memory cards, leading to the decrease of the total trapping days.

The placement of the cameras was performed by field researchers and field technicians/personnel from Callisto (CB) as well as by personnel from the management bodies of each national park. Moreover, students doing their internships (and hosted by Callisto CB) also contributed in the field work activities and the camera traps survey protocol gaining useful experience for their academic future and relevant research topics in similar disciplines and survey protocols.

Photos (9-12) IR cameras installation in MBPNP, RMNP & PINDNP respectively.

Unfortunately, the loss of equipment was inevitable in all (3) project sub-areas: in PINDNP, one camera was stolen during the third cycle, resulting in loss of data and less total trapping days. In MBPNP, seven (7) cameras were stolen during the third cycle, which also resulted in loss of data and less total trapping days. In RMNP, (4) IR cameras were stolen with the same detrimental effects on sampling effort results. In all cases, the thefts were reported to the local police authorities but without any recovery of the lost equipment. Filed work for camera traps placement is shown in the following photos (9,10,11 & 12).

2.2 Camera sampling protocol standardization and locations selection criteria

The size of the study areas, the amount of available camera traps and the size of the grid cells used in each national park defined created the conditions for the maximum possible "capture" of individuals of the native bear subpopulations. Each grid cell was divided into three (3) or four (4) sampling cycles and each camera was rotated after each cycle in order to maximize the sampling effort in terms of area coverage and bear detectability maximization. The main criteria used for the location choice of each camera inside the grid were defined as follows:

- Distance from settlements and human infrastructure.
- Distance from agricultural lands.
- Occurrence/presence of bear bio-signs in the surrounding area.
- Distance from secondary roads (forestry roads of categories A and B) and the county road network
- Testimonies of residents located in the surrounding villages on brown bear occurrences in the area.
- Correct placement of camera traps in order to ensure the optimum vision field. This included the correct placement based on aspect, slope and sunlight conditions (avoidance of direct exposure), and surrounding vegetation.
- Minimise the likelihood of detection by people and theft. The cameras were installed mostly by strapping them on trees, 50-350 cm above the ground and camouflaged with leaves and branches taken from the surrounding vegetation.
- Cleaning of the vegetation inside the camera vision field was meticulously done for each camera trap placement, maximising the output of each camera. Moving vegetation by the wind and the sudden changes in ground temperature might also trigger unavoidable false alarms.

2.3 Setup and statistical analysis of camera trapping data

Camera trapping photos were carefully screened, and animals were identified up to species level. Age and sex of brown bears was not identified. One independent event was defined as consecutive photographs of individuals of the same species within a time-period of 15 minutes (Blankenheim 2018, Mertzanis et al. 2018, Kyriakidis 2021). Photographs with more than one individual of the same species in the frame were counted as a single detection for that species (O'Brien et al., 2003). Moreover, individuals of different species within the 15-minute interval were counted as a separate event (Muhly et al., 2011).
For each event, the time, date, camera coordinates and species of the first photo were recorded in MS Excel format data base (Table 3). All data entry and species identification was done manually by researchers from Callisto CB, the personnel from each corresponding national park, as well as by internship students hosted by Callisto CB, using Microsoft Excel software. All events were standardised by using the total amount of events of a given species per 100 trapping days, also referred to as relative abundance index (RAI), a widely used index for camera trapping data standardization (O'Brien, 2011, O'Connell et al. 2010, Sollmann et al. 2013). Additional processing in .xls format produced (2) types of data: a) cycles with bear events (Table 4) and b) standardized cycles per camera (Table 5).

Table 3. Snapshot from first stage data entry for species (brown bear) events

Cycle	Camera	Latitude	Longitude	Species	Nb_Ind	Scientific nam	Hunter	Vehicle	Nb_Veh	Temperature	Filename	Date	Time
2	K2	40.141551	21.090673	Wildcat	1	Felis sylvestris				15	IMAG0129.JPG	18/07/2021	21:38:38
2	K2	40.141551	21.090673	Brown bear	1	Ursus arctos				25	IMAG0219.JPG	25/07/2021	13:56:46
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	28	IMAG0222.JPG	25/07/2021	19:15:00
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	30	IMAG0234.JPG	26/07/2021	18:46:08
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	19	IMAG0237.JPG	27/07/2021	05:36:52
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	19	IMAG0240.JPG	27/07/2021	05:50:50
2	K2	40.141551	21.090673	Human		Homo sapiens	Yes	Yes	1	20	IMAG0246.JPG	27/07/2021	06:20:22
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	19	IMAG0249.JPG	27/07/2021	06:53:56
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	19	IMAG0252.JPG	27/07/2021	07:26:02
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	32	IMAG0297.JPG	27/07/2021	18:24:22
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	29	IMAG0324.JPG	28/07/2021	18:53:52
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	20	IMAG0546.JPG	01/08/2021	07:15:58
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	28	IMAG0549.JPG	01/08/2021	09:14:34
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	37	IMAG0657.JPG	01/08/2021	18:28:12
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	33	IMAG0822.JPG	03/08/2021	14:10:50
2	K2	40.141551	21.090673	Human		Homo sapiens	Yes	Yes	1	27	IMAG0837.JPG	04/08/2021	09:01:02
2	K2	40.141551	21.090673	Human	1	Homo sapiens				35	IMAG0909.JPG	04/08/2021	18:16:30
2	K2	40.141551	21.090673	Human		Homo sapiens	Yes	Yes	1	28	IMAG1236.JPG	11/08/2021	09:01:14
2	K2	40.141551	21.090673	European badger	1	Meles meles				17	IMAG1296.JPG	13/08/2021	05:39:58
2	K2	40.141551	21.090673	Human		Homo sapiens		Yes	1	17	IMAG1299.JPG	13/08/2021	07:35:34
2	K3	40.076334	20.773532	Human	1	Homo sapiens		Yes	1	31	IMG_0020.JPG	01/08/2021	13:02:06
2	K3	40.076334	20.773532	Human	1	Homo sapiens		Yes	1	34	IMG_0021.JPG	01/08/2021	14:30:16
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	37	IMG_0032.JPG	01/08/2021	15:56:04
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	33	IMG_0051.JPG	01/08/2021	20:25:34
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	35	IMG_0073.JPG	02/08/2021	14:37:48
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	37	IMG_0118.JPG	02/08/2021	16:52:26
2	K3	40.076334	20.773532	Human	1	Homo sapiens		Yes	1	25	IMG_0135.JPG	03/08/2021	10:29:22
2	K3	40.076334	20.773532	Human	1	Homo sapiens				30	IMG_0223.JPG	04/08/2021	20:31:48
2	K3	40.076334	20.773532	Human	1	Homo sapiens				35	IMG_0319.JPG	05/08/2021	15:57:04
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	31	IMG_0458.JPG	06/08/2021	16:14:42
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	32	IMG_0465.JPG	06/08/2021	16:48:38
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	31	IMG_0469.JPG	06/08/2021	17:02:26
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	32	IMG_0551.JPG	07/08/2021	16:06:02
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	32	IMG_0558.JPG	07/08/2021	16:48:38
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	34	IMG_0571.JPG	08/08/2021	15:53:36
2	K3	40.076334	20.773532	Human		Homo sapiens		Yes	1	18	IMG_0587.JPG	09/08/2021	07:23:48

Table 4. Snapshot from data base with bear and other species standardized events per camera and per cycle.

Cycle1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Camera name	Browning1	Browning2	Browning3	Browning4	Browning5	Reconyx6	Reconyx7	Browning7b	Browning8	Browning9	LTLAcorn10	Reconyx11	Reconyx12	Browning13	Browning14
Grid cell	K1	K2	K3	K4	K5	K6	K7	K7B	K8	K9	K10	K11	K12	K13	K14
Starting date	25/05/21	25/05/21	18/05/21	18/05/21	18/05/21	14/04/21	18/05/21	14/04/21	18/05/21	17/05/21	19/05/21	14/04/21	20/04/21	28/05/21	17/05/21
Ending date	10/07/21	10/07/21	09/07/21	09/07/21	10/07/21	12/07/21	13/07/21	09/07/21	07/07/21	09/07/21	13/07/21	12/07/21	11/07/21	11/07/21	09/07/21
Duration (Trapping days)	46	46	52	52	53	89	56	86	50	53	55	89	82	44	53
Julian Date	-44364	-44364	-44360	-44360	-44360,5	-44344,5	-44362	-44343	-44359	-44359,5	-44362,5	-44344,5	-44347	-44366	-44359,5
Latitude Y	40.13272	40.13526	40.04737	40.04958	40.05197	40.01388	40.01853	39,98378	40.02174	39,959	39.94043	40,00317	39,95166	39,96386	39,91089
Longitude X	20.94816	21.04258	20.81988	20.88574	20.99942	21.19874	21.23198	20.69372	20.78094	20.88864	21.05849	21,20946	21,30524	21,34367	20,69558
Number of photos	5759	0	12603	2592	13034	0	747	10938	451	0	4653	453	948	3767	13967
Brown bear Events			2		1		3	2			1	9	5	1	
Wolf Events								38				1	1	1	1
Human Events	5		36	38			19	19	2		34	7	31	43	402
Dog Events	3			2							5	1	5		59
Cattle Events	6										19	1			9
Smaller livestock Events	28			1			9						4		15
Red fox Events	4			4	1		9	23	1		2	3	6	5	19
Marten Events														1	
European badger Events							9	2							
Wild goat Events							1								
Roe deer Events			2	4			2		1		3	12	5	2	
Wild boar Events				2			2	24			1	13	4		
Hare Events	4			4	10		14	173			2		1	6	4
Wildcat Events								4				2	18	2	
Otter Events															
Hedgehog Events															
Horse Events								42							
Bird Events	25		8	4	1		14	10	15		9	3		23	9
Reptile/Amphibian Events								1					1	6	
Rodent Events					10		4		1		1	11		33	
Total Events	**75**	**0**	**48**	**59**	**23**	**0**	**86**	**338**	**20**	**0**	**77**	**63**	**81**	**123**	**518**

Table 5. Snapshot from data base with 5 days period events with brown bears detection per camera & per cycle.

5-Day Period Events																																						
Camera	p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	p11	p12	p13	p14	p15	p16	p17	p18	p19	p20	p21	p22	p23	p24	p25	p26	p27	p28	p29	p30	p31	p32	p33	p34	p35			
K1_1	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K3_1	NA	NA	NA	NA	NA	NA	0	2	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K4_1	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K5_1	NA	NA	NA	NA	NA	NA	0	0	1	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K7_1	NA	NA	NA	NA	NA	NA	0	2	0	0	0	0	0	0	1	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K7B_1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K8_1	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K10_1	NA	NA	NA	NA	NA	NA	NA	0	0	1	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K11_1	1	1	1	2	1	0	1	1	1	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K12_1	NA	1	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K13_1	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	1	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K14_1	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K15_1	NA	NA	NA	NA	NA	NA	0	0	0	1	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K18_1	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K20_1	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K21_1	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K23_1	NA	NA	2	1	0	0	0	0	0	0	1	0	0	0	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K24_1	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K25_1	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K2_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	0	0	1	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA			
K3_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA			
K4_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K5_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	1	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K6_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	1	0	0	0	0	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA			
K7_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA			
K7B_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K8_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K9_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	1	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K10_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	1	0	2	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K11_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA			
K12_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	1	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K13_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K14_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K15_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K16_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K17_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K18_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K19_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K20_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA			
K21_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K22_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	1	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K23_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K24_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K25_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA			
K1_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0				
K3.1_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	3	0			
K3.2_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	2	0	0	0	0	0	0			
K4_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0			
K5.1_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	1	0	0	0	0	1	0			
K5.2_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0			
K6.1_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	2	1	3	1	5	0	1	0			
K6.2_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4	5	4	7	3	3	8	1	1			
K7_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0			
K8_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	1	0	0	0	0	0	0	0			
K9_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	NA			
K10_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	1	0	0	0	0	0	0	0			
K11_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0			
K12_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3	1	0	0	0	0	0	0			
K14_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	NA			
K15_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	NA			
K16_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	NA			

Data analysis for the relative abundance of brown bears in each surveyed area was performed with R version 4.0.4 (R Core Team, 2021), by using "unmarked" package (Fiske and Chandler, 2011) and the Royle-Nichols (RN) **n-mixture model** (Royle and Nichols 2003; Moeller et al., 2018). This model is based on detection/non-detection data and considers the imperfect detection and the detection probability of a species at a given site (Sollmann et al. 2013). Furthermore, the RN model takes into consideration that the detection probability of a species is higher in sites with more individuals present. Thus, it assumes that the detection probability (r) at a given site is dependent on the local abundance (N) (Denes et al. 2015). The anthropogenic and environmental variables used for estimating relative abundance in the national parks and the confounding variables used for estimating detection probability are shown in the next table (6).

For the assessment of the variables for measuring relative abundance, the ArcMap 10.7.1 (Esri Inc., 2020) was used. Pair-wise correlation among all the variables was tested to avoid collinearity by using the correlation matrix provided in "corrplot" package (Wei and Simko 2017) for R version 4.0.4. No significant correlation was found among the variables. Firstly, a "Log standardization" was applied to the values of continuous variables measured in meters, such as "distance from settlements". Moreover, a "z-score standardization" was applied to the values of the remaining continuous variables, a necessary step for occupancy models. All variables were fit into univariate models and were tested against the null model, which included no variables for detection probability and abundance. Only the models of the variables that had a lower AIC value than the null model were retained for further analyses. Following that, a Likelihood Ratio Test (LRT) was run, comparing the univariate models with a lower AIC to the null model, in order to assess any significant difference between them. The best, significant models retained after that step are shown in table (7). Further, data exploration and model selection was done by using and "dredge" function in "MumIn" package (Barton, 2020).

Table 6. Set of environmental and anthropogenic variables used for estimating bear detection probability in the sampled areas.

Anthropogenic variables	Environmental variables	Variables for detection probability
Distance from settlements (m)	Distance from rivers	Operation time (in days)
Distance from main roads	Distance from water bodies	Camera model
Distance from secondary roads	Distance from shrubland	Julian date
Distance from agriculture	Distance from coniferous forests	
Distance from Natura 2000 areas	Distance from broad-leaved forests	
Human RAI	Distance from mixed	
Road density	Elevation	
Land cover	Slope	
	Aspect	
	Average temperature	

Table 7. Variables combination from the best significant (fittest) models by survey area (3 NP's).

Northern Pindos National Park	Prespes National Park	Rodopi Mountain-Range National Park
Camera model (r)	Julian date (r)	Average temperature (r)
Julian date (r)	Distance from agriculture (N)	Distance from shrublands (N)
Distance from settlements (N)	Slope (N)	Distance from agriculture (N)
Distance from rivers (N)		Distance from Natura 2000 areas (N)
		Road density (N)

3. Bear biosigns surveys

The methodology used is based on the systematic recording of biodetective indications (of all categories) of the presence and activity of the bear by systematic scanning of sections of the forest road network on permanent and fixed routes (type of "stratified sampling") (Camarra 1982, Harris 1986). With this method data are obtained, both for the spread and for the frequency of presence but also for the type of activity of the species under study as well as for the use of the habitat. The sampling routes for recording biomarkers were distributed to all representative habitat types found in the project area as well as to all geographical areas both in the central project area and in the wider area (map 8).
The three main categories of biomarkers of bear presence and activity were recorded again, such as:

- movements biosigns
- food search and foraging biosigns
- resting and reproduction biosigns

For each bear biosign, a point recording was made with a satellite navigator (GPS) and the information was entered in a cartographic digital file and then their final recording in the GIS cartographic background. Field personnel from the NP's and Callisto CB conducted the surveys (see map 8 and photos 13 & 14).

For each biosign recorded the observer recorded also (in a radius of 150-200m around the location) the habitat characteristics based on the main biotic and abiotic parameters (relief, vegetation, etc.). To better visualize the degree of spatial concentration of biosigns and therefore to determine the habitat areas with the highest frequency of bear presence, we used the statistical tool "Kernel density estimator - KDE". This statistical tool determines a gradient of the concentration density in space of a specific variable (in this case bear biosigns) with random distribution and allows us to geographically identify those parts of the habitat where the presence and activity of the bear is highest. The primary data of biosigns indications were stored in a configured database in .xls format (Table 8).

Map (8): Spatial distribution of transects for bear bio-signs survey in RMNP.

Photos 13 & 14: bear biosigns collection in RMNP and MBPNP (scats and footprints).

Table 8. Snapshot from xls data base with records on bear biosigns in RMNP.

code	DATE	YEAR	MONTH	ENTOS_EKTOS	REGIONAL_UNIT	MUNICIPALITY	LOCATION	X	Y	HEIGHT	GRID10x10	GRID5x5	TYPE_OF_SOURCE	METHOD	BIOSIGN	INDIVIDUALS	DESCRIPTION
S01	23/1/2020	2020	ΙΑΝΟΥΑΡΙΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Μυλοκορφή	541547,00000	4578065,00000	880	F3	E5530N2140	ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΚΟΠΡΑΝΑ	1	Περιττώματα αρκούδας
T01	12/2/2020	2020	ΦΕΒΡΟΥΑΡΙΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Ζήτα	536418,00000	4593825,00000	910			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΙΧΝΗ	1	
T02	12/2/2020	2020	ΦΕΒΡΟΥΑΡΙΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Καρυδόρεμα	538046,00000	4594990,00000	1115			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΙΧΝΗ	1	
B01	12/2/2020	2020	ΦΕΒΡΟΥΑΡΙΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Καρυδόρεμα	537411,00000	4594924,00000	1079			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΒΙΟΔΗΛΩΤΙΚΑ	1	Νυχιές στην κολόνα 220R
S02	7/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Αυχένας	541318,00000	4594913,00000	1432			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΚΟΠΡΑΝΑ	1	κόπρανα φρέσκα
T03	13/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Δράμα	Καλύβια Βρίζα	522652,00000	4587246,00000				ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΙΧΝΗ	1	Πάτημα αρκούδας
B02	19/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Δράμα	Βαθύρεμα	518778,00000	4587885,00000				ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΒΙΟΔΗΛΩΤΙΚΑ	1	Νυχιές στην κολόνα 218R
B03	19/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Δράμα	Βαθύρεμα	518778,00000	4587885,00000				ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΒΙΟΔΗΛΩΤΙΚΑ	1	Νυχιές στην κολόνα 219R
B04	19/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Δράμα	Βαθύρεμα	518778,00000	4587885,00000				ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΒΙΟΔΗΛΩΤΙΚΑ	1	Νυχιές στην κολόνα 258R
S03	25/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Δράμα	Στραβόρεμα	529437,00000	4591818,00000	1181			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΚΟΠΡΑΝΑ	1	Κόπρανα αρκούδας παλιοκαιρισμένα (κουκούτσια-κράνα)
S04	25/5/2020	2020	ΜΑΪΟΣ	ΕΚΤΟΣ ΕΠΟΡ	Δράμα	Παρανέστι	Στραβόρεμα	529437,00000	4591818,00000	1181	D2	D22	ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΚΟΠΡΑΝΑ	1	
H01	27/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Δράμα	Σκαλωτή	527337,00000	4595485,00000	1400			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΗΧΗΤΙΚΗ ΚΑΤΑΓΡΑΦΗ	1	Ακούστηκαν 2 μουγκρίσματα αρκούδας
B05	28/5/2020	2020	ΜΑΪΟΣ	ΕΠΟΡ	Δράμα	Δράμα	Μ.Παναγιά	517085,00000	4593238,00000				ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΒΙΟΔΗΛΩΤΙΚΑ	1	Νυχιές στην κολόνα 179R
S05	23/6/2020	2020	ΙΟΥΝΙΟΣ	ΕΚΤΟΣ ΕΠΟΡ	Ξάνθη	Ξάνθη	Ερύμανθος	559291,00000	4577225,00000		E554N214	E5545N2145	ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΚΟΠΡΑΝΑ	1	Εντοπίστηκαν κόπρανα στην άκρη δασικού δρόμου,1-2 ημερών
U01	28/6/2020	2020	ΙΟΥΝΙΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Θερμιά	536359,00000	4591692,00000	754	E2	E5520N2155	ΦΔΟΡ	ΣΗΜΕΙΑΚΗ ΚΑΤΑΓΡΑΦΗ	ΟΠΤΙΚΗ ΠΑΡΑΤΗΡΗΣΗ	1	Εντοπίστηκε αρκούδα 1 έτους νεαρή από την Χαραλαμπίδου (διδακτορικό)
S06	3/7/2020	2020	ΙΟΥΛΙΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Αμισηνό	547549,00000	4572326,00000	615			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΚΟΠΡΑΝΑ	1	κόπρανα ωρών σε δασικό δρόμο
T04	3/7/2020	2020	ΙΟΥΛΙΟΣ	ΕΠΟΡ	Δράμα	Παρανέστι	Αμισηνό	548130,00000	4572475,00000	644			ΦΔΟΡ	ΕΜΜΕΣΗ ΠΑΡΑΤΗΡΗΣΗ	ΙΧΝΗ	1	ίχνος+πέρασμα

Results

1. Genetic analyses

1.1 Prespa NP

1.1.1 Samples that were analyzed

In the present study, UTH received 96 hair samples of brown bears from Prespa NP. An attempt was made to isolate DNA from all samples, followed by the application of PCR protocols for the 10 microsatellite loci as well as ZFX and SRY genes. Regarding hair samples, the quality and quantity of the hair roots determine the outcome of the microsatellite loci amplification. Finally, 6 or more genetic loci were successfully amplified in 59 of the 96 samples (61,5%).

1.1.2 Unique genotypes and sex ratio

A total of 53 unique individuals were identified based on their complex genotype for the 10 microsatellite sites, meaning that we found 5 samples with the exact same complex genotype. Moreover, regarding the sex ratio, males were 2,5 times more than the females (38 males /15 females).

1.1.3 Genetic diversity

Microsatellite data of 10 microsatellite loci revealed abundant genetic diversity in the population "Prespes". Table 9 shows the number of homozygotes and heterozygotes that are present at each locus of all samples. The number of alleles ranges from 4 (for Mu26) to 13 (for G10C) (Table 9). The allele with the highest frequency (Figure 2) in locus G10H is the 260, in Mu26 is the allele 192, in G1D is the allele 184, in G10X is the allele 146, in G1A is the allele 193, in the G10P is the allele 160, in G10C are the alleles 112 and 122, in Mu59 is the allele 231, in G10L is the allele 168 and in Mu50 is the allele 116.

Table 9. Number of homozygotes, heterozygotes and alleles that are present at each locus.

Locus	Individuals	Heterozygotes	Homozygotes	Number of alleles
G10H	42	13	29	8
Mu26	39	25	14	4
G1D	30	11	19	7
G10X	30	17	13	5
G1A	45	23	22	7
G10P	36	21	15	9
G10C	50	34	16	13
Mu59	34	5	29	5
G10L	47	32	15	5
Mu50	52	44	8	7

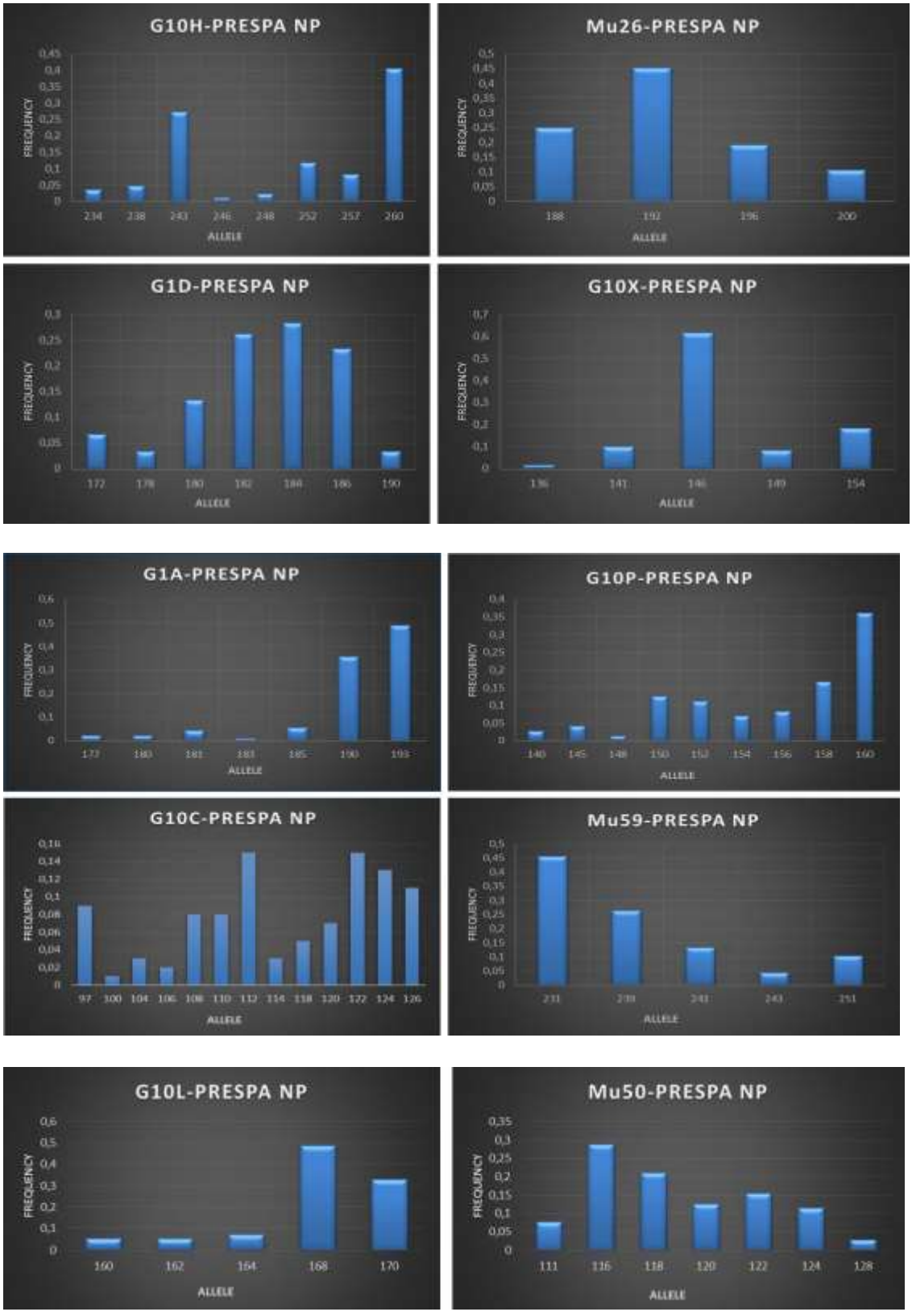


Figure 2. Allele freqeuncy of each miscrosatellite locus of the Brown Bear population in Prespa NP.

The average observed heterozygosity (*Ho)* was 0.5287 (range 0.310-0.846), and the average estimated heterozygosity (*He)* was 0.6865 (range 0.579-0.904). The *PIC* (21) at each microsatellite locus was always larger than 0.5 (range 0.529 to 0.886), a threshold value considered to be highly informative for the evaluation of genetic variance.
Regarding the Hardy-Weinberg equilibrium, we can see that the locus G10H, G1D, G10C, MU59, G10L, have significant deviations from the HWE equilibrium ($p<0.001$). We estimate the observed (Ho) equal to 0.4244 and expected Heterozygosity (He) equal to 0.726, for the 53 unique individuals respectively (Table 10). In our analysis all loci seem to have a Fis value larger than 0.15 (excluding the loci G10X, G10L and Mu50). All other Fis results for the microsatellite loci declare that there are high inbreeding levels in the population.

Table 10. Genetic information of the Brown Bear population from Prespa NP. Number of alleles (A), Allele size per bp (R), Expected Heterozygosity (He), Observed Heterozygosity (Ho), P-value for Hardy-Weinberg Equilibrium (pHW), Inbreeding Marker (Fis), Prorabilities of Indetity ($P_{ID\text{-}sib}$), (Frequency of null Alleles (Fnull), Polymorphic Information Content (PIC)

locus	*A*	*R (bp)*	*He*	*Ho*	*pHW*	*Fis*	$P_{ID\text{-}sib}$	*Fnull*	*PIC*
G10H	8	234-260	0.74	0.25	0.0000	0.5873	4.088e-01	0.4320	0.699
MU26	4	188-200	0.69	0.47	0.0550	0.1430	1.820e-01	0.0788	0.633
G1D	7	172-190	0.79	0.21	0.0000	0.5501	6.758e-02	0.3696	0.763
G10X	5	136-154	0.57	0.32	0.0180	0.0209	3.528e-02	-0.0118	0.529
G1A	7	177-193	0.63	0.43	0.0020	0.1975	**1.717e-02**	0.1130	0.563
G10P	9	140-160	0.80	0.40	0.0038	0.2833	**6.280e-03**	0.1559	0.777
G10C	13	97-126	0.90	0.64	0.0000	0.2501	**1.930e-03**	0.1381	0.886
MU59	5	231-251	0.69	0.09	0.0000	0.7931	**8.479e-04**	0.6598	0.646
G10L	5	160-170	0.64	0.60	0.0000	-0.0522	**4.045e-04**	-0.0281	0.581
MU50	7	111-128	0.81	0.83	0.6495	-0.0315	**1.451e-04**	-0.0210	0.788
Mean	7		0.73	0.42		0.28			0.69

1.1.4 Bottleneck status

We used the stepwise mutation model (SMM), which is more suitable for microsatellite data (Pennell et al. 2013) and our analysis shows that the population has not been recently decreased, because the p-value in Wilcoxon test is equal to 0.72168. Therefore, in Prespes no signature of significant bottleneck was detected. Also, L-shaped distributions of allele's frequencies are shown in Figure 3.

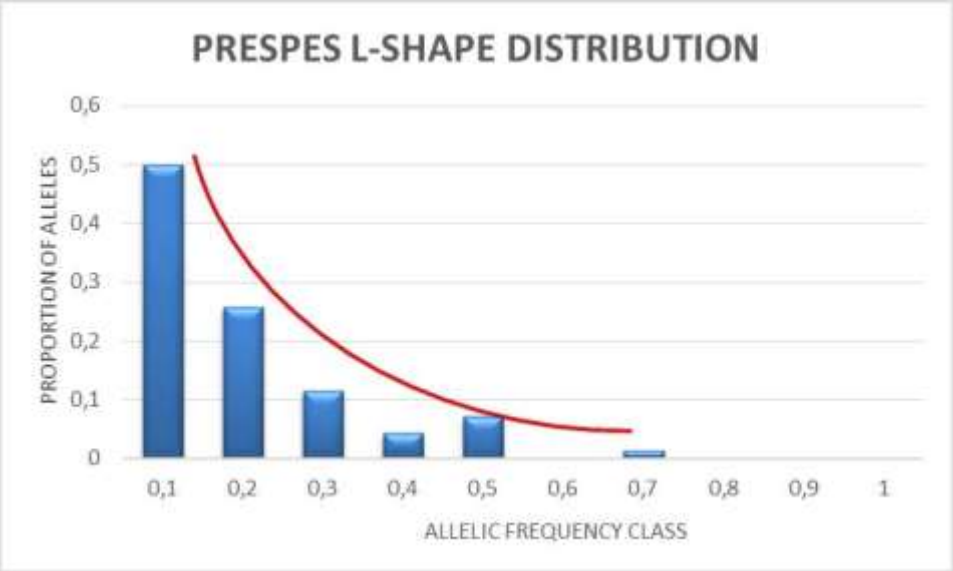


Figure 3. L-shaped distributions of alleles' frequencies

1.1.5 Total population size (Nc) and effective population size (Ne)

For the estimation of the effective population size (Ne) we used linkage disequilibrium method. Therefore, regarding Prespes and we found that Ne=35 (95% confidence interval is from 25 to 52 individuals). Moreover, using the Capwire program in order to estimate the average of population size (N_C), we found that Nc=191 individuals (95% confidence interval is from 150 to 222 individuals). Moreover, the mean arrest / sample ratio was 1.11 since 48 of the 53 samples were captured once, 4 samples were captured twice and 1 sample was captured three times (Table 11).

Table 11. Sample Recaptures for Prespa NP.

Sample Code	**1st capture (date)**	**2nd capture (date)**	**3rd capture (date)**	**Gender**	**Max Distance between captures (km)**
247	7/21	7/21		Male	0
253	7/21	7/21		Male	0
264	7/21	7/21	7/21	Female	0
268	7/21	7/21		Male	0
271	7/21	7/21		Male	0

1.2. Pindos National Park

1.2.1 Samples that were analyzed

In the present study, UTH received 170 hair samples of brown bears from Pindos NP. An attempt was made to isolate DNA from all samples, followed by the application of PCR protocols for the 10 microsatellite loci as well as ZFX and SRY genes. Regarding hair samples, the quality and quantity of the hair roots determine the outcome of the microsatellite loci amplification. Finally, 6 or more genetic loci were successfully amplified in 77 of the 170 samples (45,3%).

1.2.2 Unique genotypes and sex ratio

A total of 65 unique individuals were identified based on their complex genotype for the 10 microsatellite sites, meaning that we found 9 samples with the exact same complex genotype. Moreover, regarding the sex ratio, males were 2 times more than the females (44 males / 21 females).

1.2.3 Genetic diversity

Microsatellite data of 10 microsatellite loci revealed abundant genetic diversity in the population "Pindos". Table 12 shows the number of homozygotes and heterozygotes that are present at each locus of all samples. The number of alleles ranges from 5 (for Mu26, G1D, G1A, G10P) to 10 (for G10H) (Table 12). The allele with the highest frequency (Figure 4) in locus G10H is the 260, in Mu26 is the allele 192, in G1D is the allele 184, in G10X is the allele 146, in G1A is the allele 193, in the G10P is the allele 160, in G10C is the allele 97, in Mu59 is the allele 239, in G10L is the allele 170 and in Mu50 is the allele 111.

Table 12. Number of homozygotes, heterozygotes and alleles that are present at each locus.

Locus	Individuals	Heterozygotes	Homozygotes	Number of alleles
G10H	53	34	19	10
Mu26	53	40	13	5
G1D	23	9	14	5
G10X	56	25	31	4
G1A	56	34	22	5
G10P	52	16	36	5
G10C	63	57	6	6
Mu59	41	19	22	7
G10L	56	32	24	6
Mu50	64	58	6	9

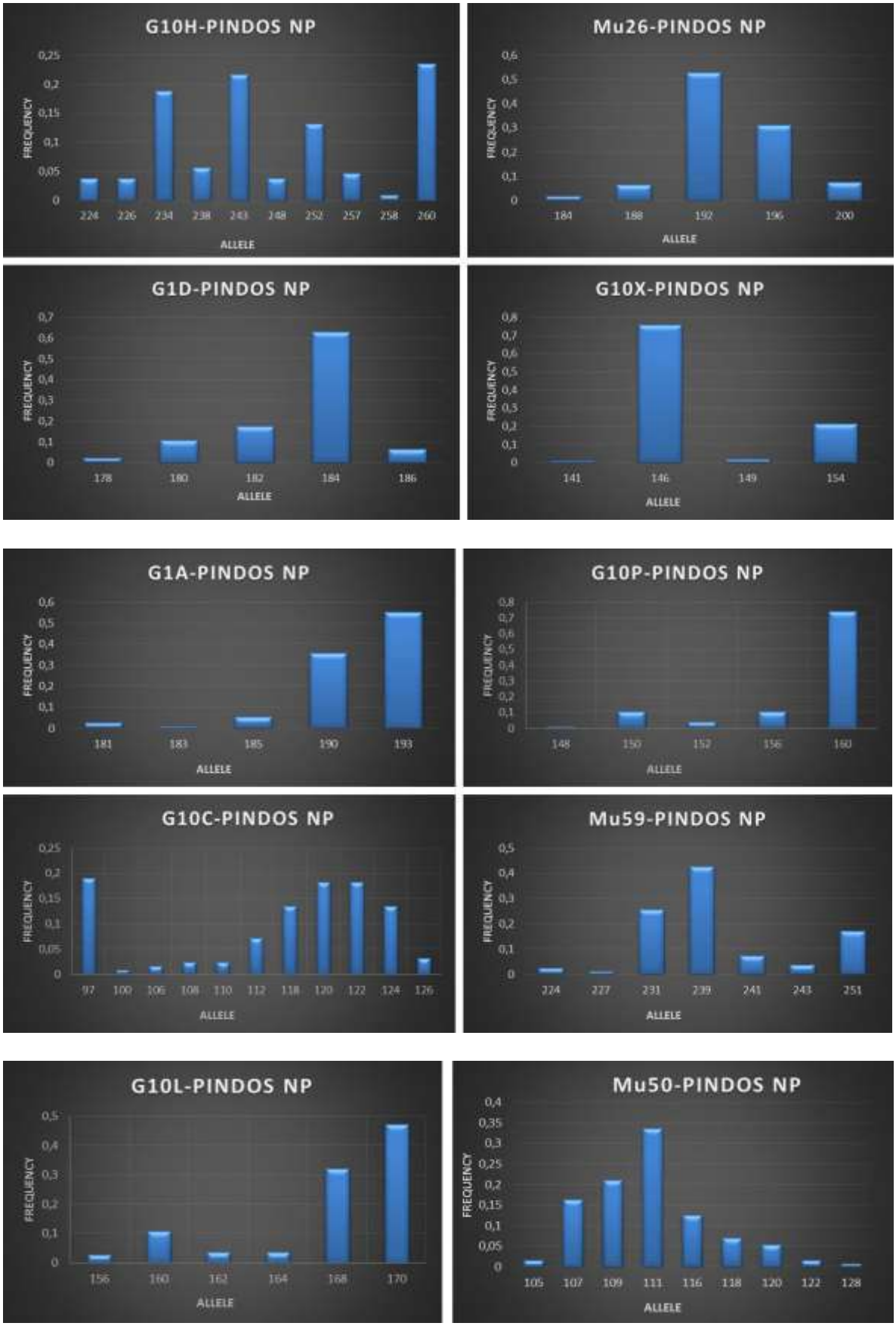


Figure 4. Allele freqeuncy of each miscrosatellite locus of the Brown Bear population in Pindos NP.

The average *Ho* was 0.5088 (range 0.391-0.906), and the average *He* was 0.6457 (range 0.381-0.860). The *PIC* [21] at each microsatellite locus was always larger (except from locus G10P) than 0.5 (range 0.324 to 0.836), a threshold value considered to be highly informative for the evaluation of genetic variance (Table 13).
Regarding the Hardy-Weinberg equilibrium, we can see that the locus G10H, Mu26, G1A, G10P, G10C, Mu59, G10L, have significant deviations from the HWE equilibrium ($p<0.001$) (Table 13). Regarding the Fis marker for the inbreeding existence (Weir & Cockerham 1984), 5 loci seem to have a Fis value smaller than 0.15: Mu26, G10X, G1A, G10C, and Mu50.

Table 13. Information about the population of Brown Bear from Pindos NP. Number of alleles (A), Allele size per bp (R), Observed Heterozygosity (Ho), Expected Heterozygosity (He), P-value for Hardy-Weinberg Equilibrium (pHW), Inbreeding Marker (Fis), Prorabilities of Indetity (PID-sib), Frequency of null Alleles (Fnull), Polymorphic Information Content (PIC).

locus	*A*	*R (bp)*	*He*	*Ho*	*pHW*	*Fis*	$P_{ID\text{-}sib}$	*Fnull*	*PIC*
G10H	10	234-260	0.842	0.642	0.0000	0.2402	3.447e-01	0.1280	0.814
MU26	5	188-200	0.619	0.755	0.0000	-0.2210	1.710e-01	-0.1286	0.552
G1D	5	172-190	0.568	0.391	0.0040	0.3161	9.081e-02	0.2107	0.517
G10X	4	136-154	0.381	0.446	0.5072	-0.1732	6.096e-02	-0.0939	0.324
G1A	5	177-193	0.567	0.607	0.0000	-0.0707	3.274e-02	-0.0349	0.481
G10P	5	140-160	0.432	0.308	0.0000	0.2898	2.045e-02	0.2260	0.401
G10C	11	97-126	0.860	0.905	0.0000	-0.0526	**6.813e-03**	-0.0337	0.836
MU59	7	231-251	0.724	0.463	0.0001	0.3632	**2.882e-03**	0.2086	0.673
G10L	6	160-170	0.664	0.571	0.0000	0.1404	**1.338e-03**	0.0657	0.602
MU50	9	111-128	0.798	0.906	0.0439	-0.1371	**4.976e-04**	-0.0758	0.764
Mean	6.7		0.65	0.6		0.13			0.6

1.2.4 Bottleneck status

We used the stepwise mutation model (SMM), which is more suitable for microsatellite data and our analysis shows that the population has not been recently decreased, because the p-value in Wilcoxon test is equal to 0.99854. Therefore, in the Pindos population no signature of significant bottleneck was detected. Also, L-shaped distributions of allele's frequencies are shown in Figure 5.

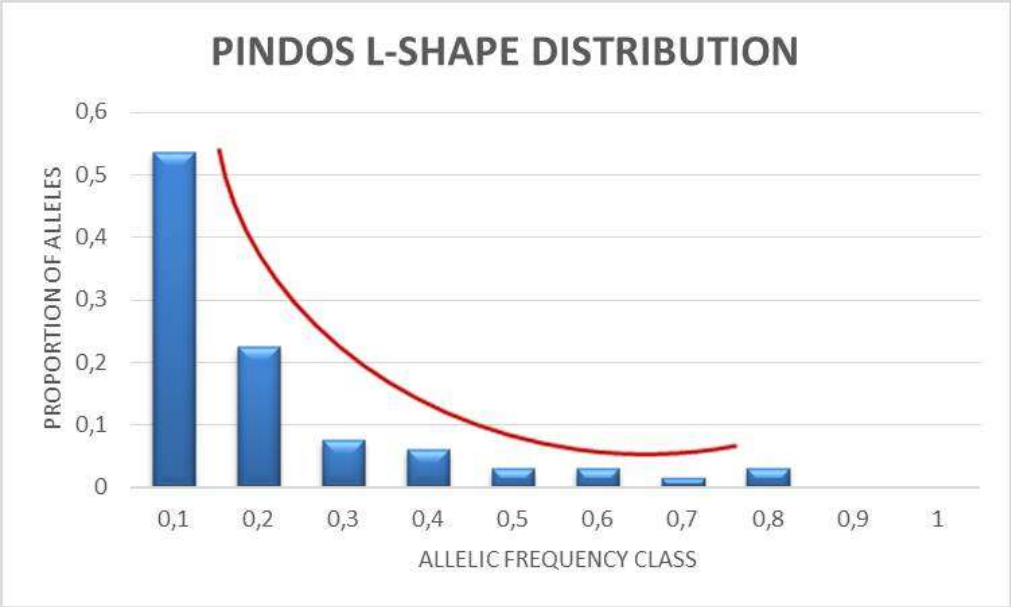


Figure 5. L-shaped distributions of alleles' frequencies.

1.2.5 Total population size (Nc) and effective population size (Ne)

For the estimation of the effective population size (Ne) we used linkage disequilibrium method. Therefore, regarding Pindos we found that Ne=118 (95% confidence interval is from 66 to 371 individuals). Moreover, using the Capwire program we estimate the average of population size (N_C), we found that Nc=202 individuals (95% confidence interval is from 175 to 300 individuals. Moreover, the mean arrest / sample ratio was 1.18 since 56 of the 77 samples were obtained only from one capture. On the contrary, 6 of the 77 samples were captured twice and 3 samples were captured 3 times (Table 14).

Table 14. Sample Recaptures for Pindos NP.

Sample Code	***1st capture (date)***	***2nd capture (date)***	***3rd capture (date)***	***Gender***	***Max Distance between captures (km)***
318	2/7/21	2/7/21		Female	0
326	2/7/21	2/7/21		Male	0
335	9/7/21	9/7/21	9/7/21	Male	0
343	3/7/21	3/7/21		Male	0
349	30/6/21	30/6/21	30/6/21	Male	0
353	7/7/21	7/7/21		Male	0
406	31/7/2021	4/9/2021		Female	0
415	9/8/21	9/8/21		Male	0
421	30/7/21	30/7/21	30/7/21	Male	0

1.3 Rodopi Mountain Range National Park

1.3.1 Samples that were analyzed

In the present study, UTH laboratory received 256 hair samples of brown bears from Rodopi. An attempt was made to isolate DNA from all samples, followed by the application of PCR protocols for the 10 microsatellite loci as well as and ZFX and SRY genes. Regarding hair samples, the quality and quantity of the hair roots determine the outcome of the microsatellite loci amplification. Finally, 6 or more genetic loci were successfully amplified in 152 of the 256 samples (59.4%).

1.3.2 Unique genotypes and sex ratio

A total of 121 unique individuals were identified based on their complex genotype for the 10 microsatellite sites. Moreover, regarding the sex ratio, males were 17,5 times more than the females (103 males / 18 females).

1.3.3 Genetic diversity

Microsatellite data of 10 microsatellite loci revealed abundant genetic diversity in the population "Rodopi". Table 15 shows the number of homozygotes and heterozygotes that are present at each locus of all samples. The number of alleles ranges from 4 (for G10X) to 16 (for G10C) (Table 15). The allele with the highest frequency (Figure 6) in locus G10H is the 257, in Mu26 is the allele 192, in G1D is the allele 184, in G10X is the allele 146, in G1A is the allele 193, in the G10P is the allele 160, in G10C is the allele 97, in Mu59 is the allele 231, in G10L is the allele 168 and in Mu50 is the allele 116.

Table 15. Number of homozygotes, heterozygotes and alleles that are present at each locus.

Locus	Individuals	Heterozygotes	Homozygotes	Number of alleles
G10H	90	23	67	13
Mu26	107	63	44	7
G1D	79	11	68	7
G10X	44	21	23	4
G1A	103	64	39	6
G10P	102	62	40	10
G10C	117	88	29	16
Mu59	83	30	53	9
G10L	114	83	31	9
Mu50	121	101	20	11

Figure 6. Allele freqeuncy of each miscrosatellite locus of the Brown Bear population in Rodopi NP.

The average *Ho* was 0.5366 (range 0.139-0.835), and the average *He* was 0.7271 (range 0.534-0.907). The *PIC* at each microsatellite locus was larger than 0.5 (range 0.449 to 0.895), a threshold value considered to be highly informative for the evaluation of genetic variance. Regarding the Hardy-Weinberg equilibrium, we can see that only the locus **G10X,** is on HWE equilibrium (p<0.001) (Table 16). Regarding the Fis marker for the inbreeding existence (Weir & Cockerham 1984), only 2 loci (G10L and Mu50) have a Fis value smaller than 0.15. The presence of loci with high Fis values, declares a considerable degree of inbreeding.

Table 16. Genetic Information about the population of Brown Bear from Rodopi NP. Number of alleles (A), Allele size per bp (R), Observed Heterozygosity (Ho), Expected Heterozygosity (He), P-value for Hardy-Weinberg Equilibrium (pHW), Inbreeding Marker (Fis), Prorabilities of Indetity (PID-sib) Frequency of null Alleles (Fnull), Polymorphic Information Content (PIC).

locus	*A*	*R (bp)*	*He*	*Ho*	*pHW*	*Fis*	$P_{ID\text{-}sib}$	*Fnull*	*PIC*
G10H	13	234-260	0.833	0.256	0.0000	0.6944	3.481e-01	0.5355	0.808
MU26	7	188-200	0.725	0.589	0.0000	0.1883	1.455e-01	0.1002	0.685
G1D	7	172-190	0.612	0.139	0.0000	0.7736	7.168e-02	0.6386	0.575
G10X	4	136-154	0.608	0.477	0.1111	0.2168	3.645e-02	0.1202	0.524
G1A	6	177-193	0.769	0.621	0.0000	0.1931	**1.420e-02**	0.1127	0.731
G10P	10	140-160	0.847	0.608	0.0000	0.2832	**4.818e-03**	0.1658	0.825
G10C	16	97-126	0.907	0.752	0.0000	0.1695	**1.464e-03**	0.0933	0.895
MU59	9	231-251	0.677	0.361	0.0000	0.4680	**6.600e-04**	0.3120	0.630
G10L	8	160-170	0.534	0.728	0.0000	-0.3657	**3.694e-04**	-0.1692	0.449
MU50	11	111-128	0.759	0.835	0.0000	-0.1000	**1.462e-04**	-0.0557	0.721
Mean	9.2		0.73	0.54		0.3			0.69

1.3.4 Bottleneck

We used the stepwise mutation model (SMM), which is more suitable for microsatellite data and our analysis shows that the population has not been recently decreased, because the p-value in Wilcoxon test is equal to 0. 94727. Therefore, in the Rodopi National Park population no signature of significant bottleneck was detected and L-shaped distributions of allele's frequencies are shown in Figure 7.

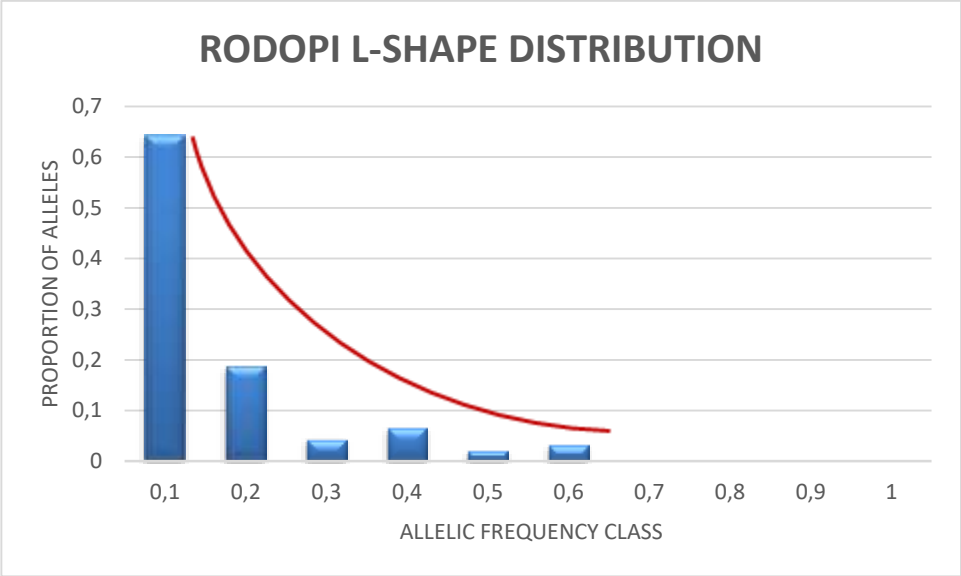


Figure 7. L-shaped distributions of allele's frequencies.

1.3.5 Total population size (Nc) and effective population size (Ne)

For the estimation of Ne we used linkage disequilibrium method. Therefore, regarding Rodopi we found that Ne=57 (95% confidence interval is from 48 to 69 individuals). Moreover, using the Capwire program we found that the average population size Nc=197 individuals (95% confidence interval is 180-255 individuals).
Moreover, the mean arrest / sample ratio was 1.26 since 93 of the 152 samples were captured once, 26 were captured twice, 1 was captured three times and 1 sample was captured four times (Table 17).

Table 17. Sample Recaptures for Rodopi NP.

Sample Code	*1st capture (date)*	*2nd capture (date)*	*3rd capture (date)*	*4thcapture (date)*	*Gender*	*Max Distance between captures (km)*
47	7/11/17	7/11/17			Male	0
58	10/6/20	10/6/20			**Male**	**1 km**
66	14/5/20	14/5/20			Male	0
68	27/5/20	27/5/20	27/5/20	27/5/20	Male	0
74	3/6/20	3/6/20			Male	0
76	27/5/20	27/5/20			Male	0
92	7/5/20	7/5/20			Male	0
94	19/5/20	19/5/20			Male	0
96	11/6/21	11/6/21			Male	0
98	26/5/20	26/5/20			Male	0
108	27/7/20	27/7/20			Male	0
110	27/7/20	27/7/20			Male	0
116	7/7/20	7/7/20			Male	0
121	13/7/20	13/7/20	13/7/20		Male	0
125	13/7/20	13/7/20			Male	0
126	13/7/20	13/7/20			Male	0
128	13/7/20	13/7/20			Male	0
131	6/7/20	30/7/20			Male	0
140	29/7/20	29/7/20			Male	0
143	29/5/19	29/5/19			**Male**	**454 m**
210	3/6/20	3/6/20			Male	0

Sample Code	*1st capture (date)*	*2nd capture (date)*	*3rd capture (date)*	*4thcapture (date)*	*Gender*	*Max Distance between captures (km)*
215	28/5/20	28/5/20			Male	0
217	25/5/20	25/5/20			Male	0
222	19/5/20	19/5/20			Male	0
230	14/5/20	14/5/20			Male	0
276	28/7/20	27/8/20			Female	0
298	30/7/20	30/7/20			Male	0
306	6/7/20	6/7/20			**Male**	**9 km**

2. Camera trapping – Brown bear relative abundance

Pindos National Park

As shown in the Table 18, in PINDNP, the camera trapping effort resulted in **3.529** total trapping days and **290.480** frames. A large amount of frames was not used due to false negatives, technical errors and incorrect intervals between photo captures. In the first cycle, nineteen cameras were used, due to other cameras being unavailable at the time. In the second cycle, twenty-six (26) cameras were used but one was stolen, resulting in loss of data and less total trapping days.
A total of sixteen (16) wildlife and domestic species were identified as follows: brown bear (*Ursus arctos*), wolf (*Canis lupus*), marten (*Marten sp.*), red fox (*Vulpes vulpes*), European badger (*Meles meles*), chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), hare (*Lepus europaeus*), wildcat (*Felis silvestris*), hedgehog (*Erinaceus europaeus*) and feral horse (*Equus ferus caballus*), human (*Homo sapiens*), domesticated or feral dog (*Canis familiaris*), cattle (*Bos taurus*) and smaller livestock (*Oves aries* and *Capra aegagrus hircus*).

Table 18. Overall cameras sampling results in PINDNP.

Northern Pindos National Park	Cycle A	Cycle B	Cycle C	Total
Time period	14.04 - 11.07	05.07 - 22.08	17.08 - 03.10	14.04.21 - 03.10.21
Cameras	19	25	26	70
Trapping days	1561	950	1018	3529
Photographs	132767	78274	79439	290480
Brown bear Events	29	14	82	125
Human Events	1126	872	1598	3596
Bear RAI	1.86	1.47	8.06	3.54
Human RAI	72.13	91.79	156.97	101.90

MB Prespa National Park

The total camera trapping effort was **2.843** trapping days and **68.634** photographs (Table 19). In the first, second and third cycles, eighteen (18) cameras were used, but one camera had technical problems, which resulted in the camera not capturing any photographs. In the fourth cycle, six (6) cameras were stolen resulting in loss of data and less total trapping days.

Table 19. Overall cameras sampling results in MBPNP

Prespes National Park	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Total
Time period	01.04 - 30.05	24.05 - 12.07	09.07 - 03.09	23.09 - 19.10	01.04.21 - 19.10.21
Cameras	17	17	17	12	63
Trapping days	942	779	856	266	2843
Photographs	5064	41796	15322	6452	68634
Brown bear Evets	43	89	30	28	190
Human Events	836	1326	1253	75	3490
Bear RAI	4.56	11.42	3.50	10.53	6.68
Human RAI	88.75	170.22	146.38	28.20	122.76

Rodopi National Park

The total camera trapping effort resulted in **5.743** trapping days and **184.781**photographs (Table 20).

Table 20. Overall cameras sampling results in RMNP.

Rodopi National Park	Cycle 1	Cycle 2	Cycle 3	Total
Time period	01.07.20 - 30.09.20	27.08.20 - 16.12.20	02.03.21 - 27.10.21	01.07.20 - 27.10.21
Cameras	25	27	30	82
Trapping days	1190	1635	2918	5743
Photographs	40487	37107	107187	184781
Brown bear Events	36	37	98	171
Human Events	326	428	498	1252
Bear RAI	3.03	2.26	3.36	2.98
Human RAI	27.39	26.18	17.07	21.80

The following photos (15-19) show some characteristic frames/captures with brown bears.

Photos (15-19): frames with brown bears from PINDNP (15,16), MBPNP (17-18) and RMNP (19) project sub-areas respectively.

In PINDNP: the mean probability of detecting any individual across sites was p = 0.04 (SE = 0.007) and mean relative abundance between sites was N = 2.57 (SE = 1.24) individuals. By

multiplying the relative abundance index by (26) = number of grid cells we obtain an approximate figure on total bear population size that can be compared to outcome from the genetic analyses.
The variation in detection probability caused by the abundance at each camera site is shown in the Figure 8. While the detection probability in each site is dependent on the variables that were selected and the presence/absence data in each site for each trapping day, it is also determined by the abundance in each camera site, where as expected, a higher abundance in a site will increase the detection probability as well.

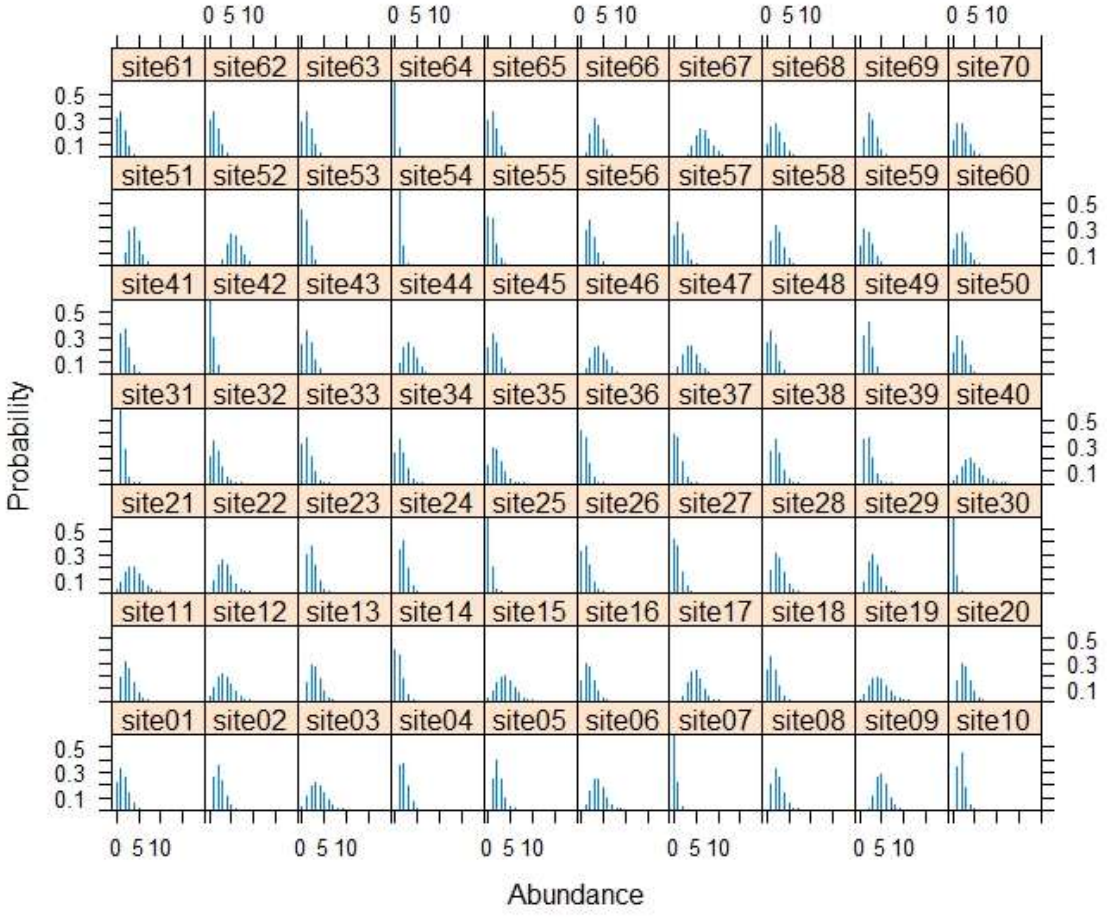
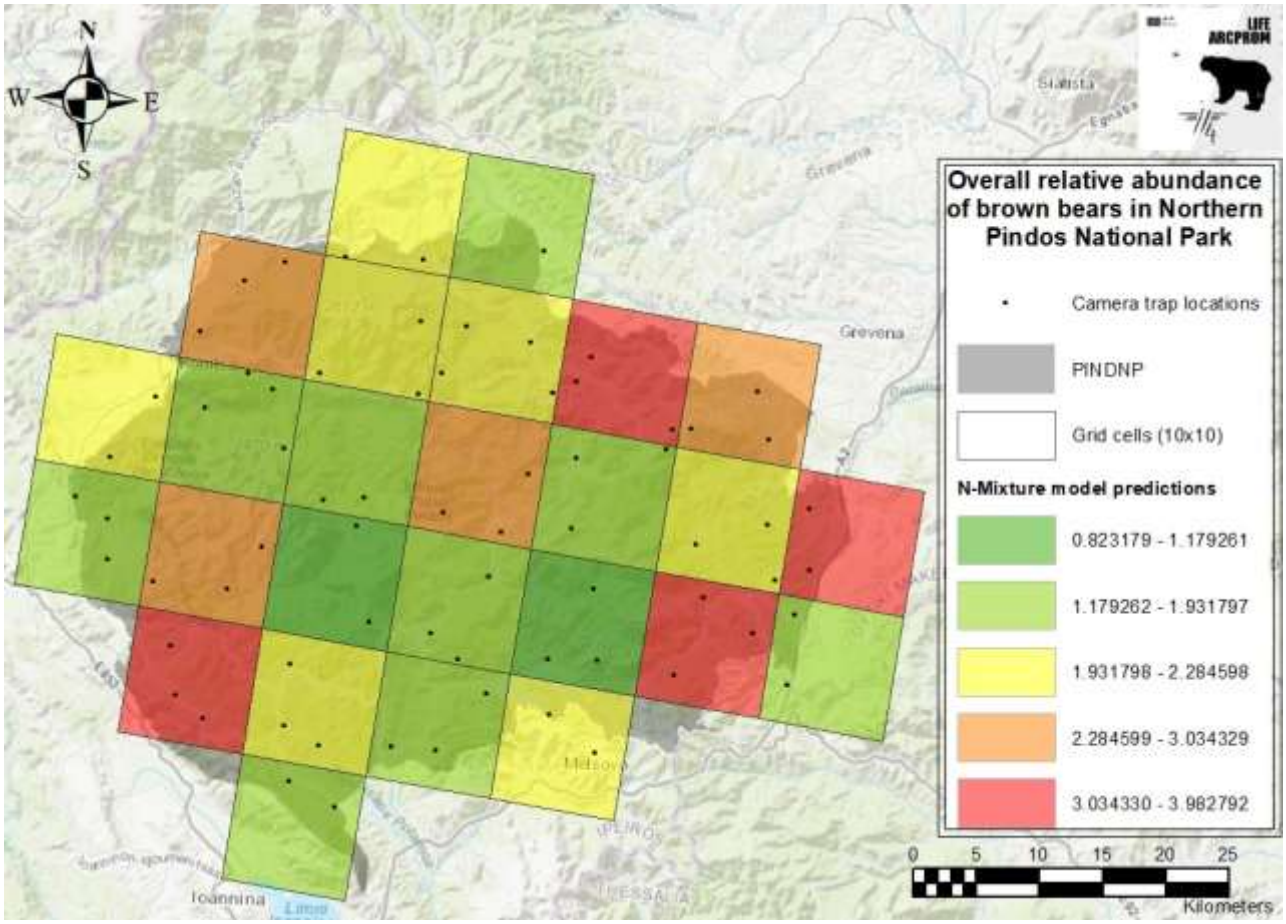
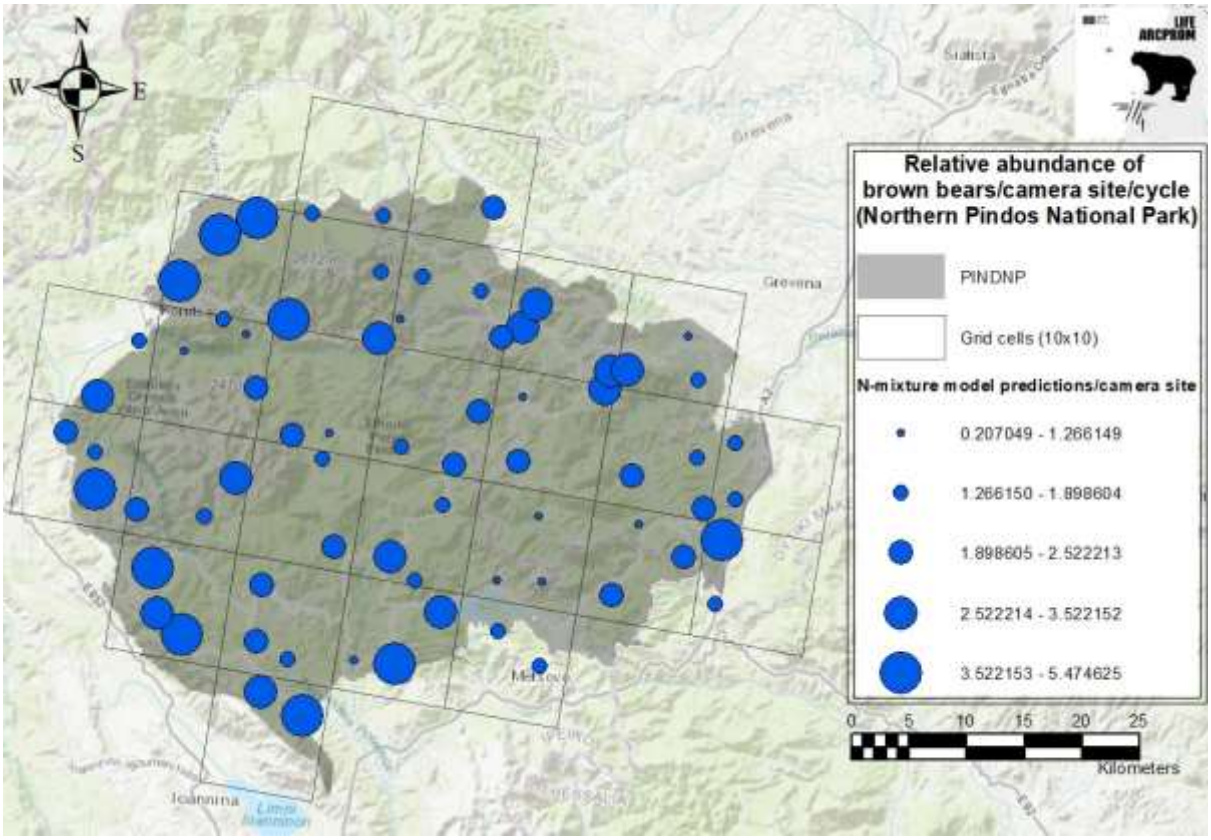


Figure 8. variation in bear detection probability in PINDNP related to the abundance at each camera site.

In PINDNP, all variables were fitted and compared to the null model (Akaike Information Criterion = 418.34). The best model in PINDNP (AIC = 386.2) passed three goodness of fit (GoF) tests, namely Sum of Squared Errors (p = 0.71), Pearson's Chi-Squared (p = 0.1) and Freeman-Tukey Chi-squared (p = 0.52). From the beta coefficients of the best model, the relative abundance of bears is significantly higher near settlements but gets lower near rivers and mixed forests. The detection probability was significantly affected by the calendar date, where brown bears were more likely to be detected in the third cycle, which was in Autumn. The results of the relative abundance analysis (overall and by camera-cycle) are presented in maps (9 & 10).

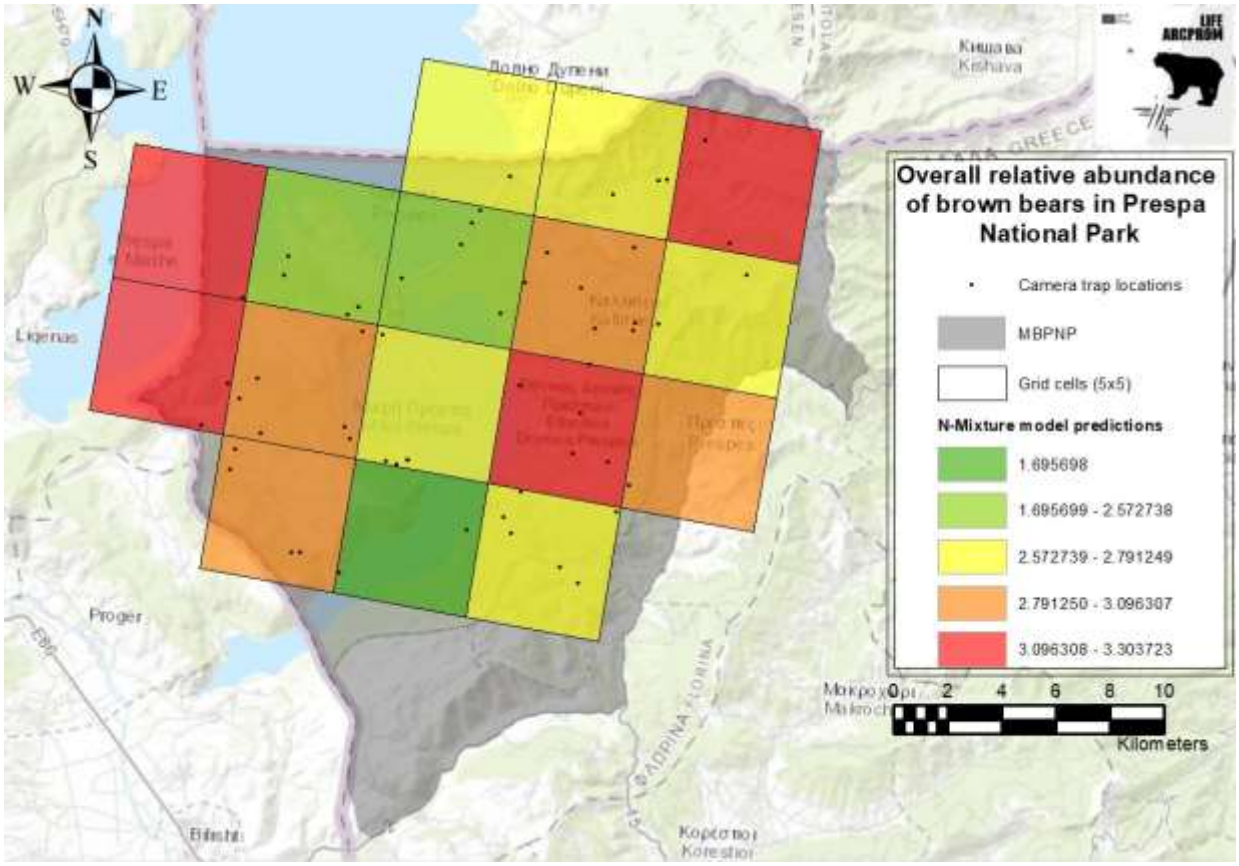


Map (9): Overall brown bear relative abundance in PINDNP as obtained from IR camera traps.

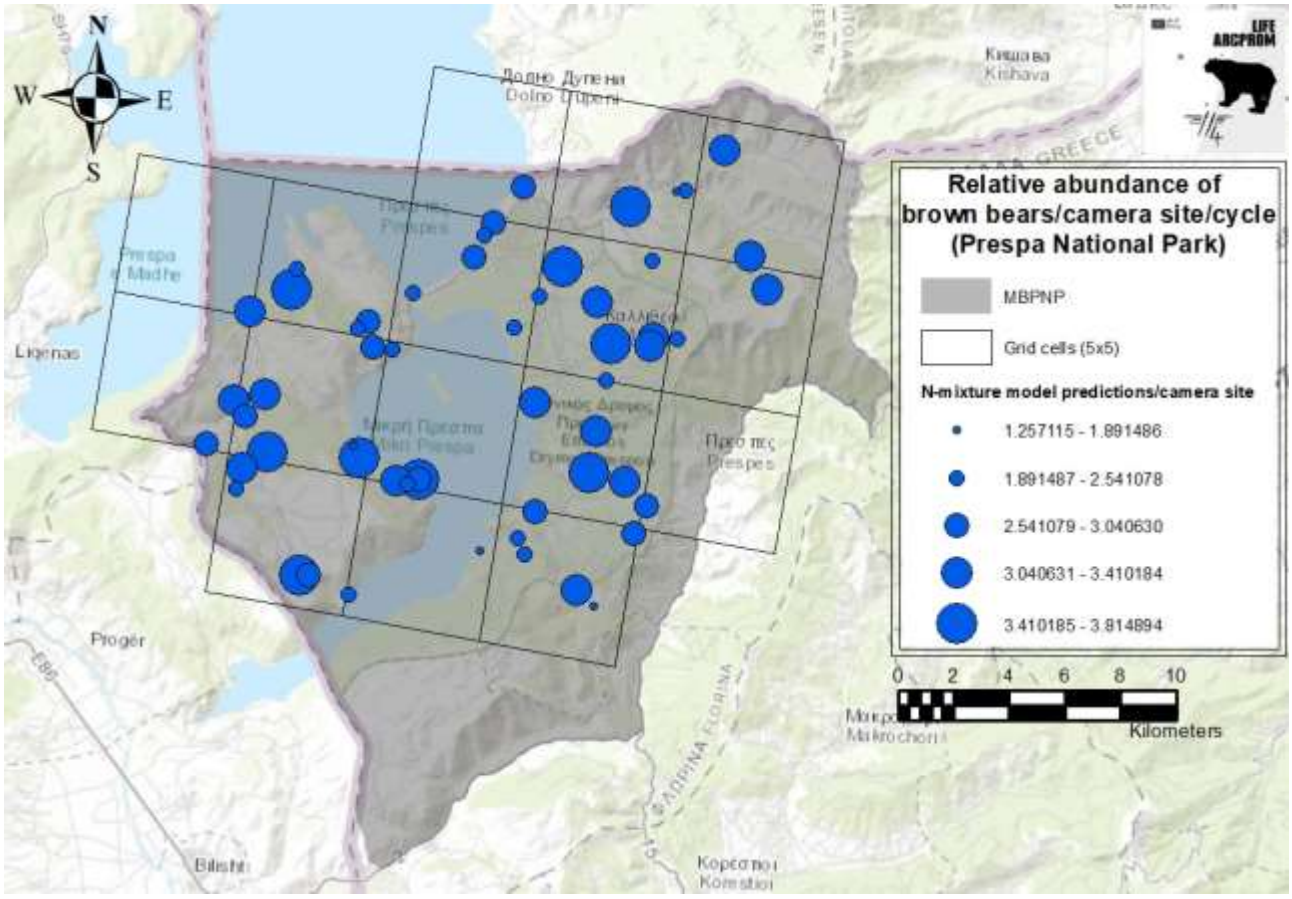


Map (10): Brown bear relative abundance in PINDNP by camera location and sampling cycle.

In MBPNP: the mean probability of detecting any individual across sites was p = 0.1 (SE = 0.03) and mean relative abundance between sites was N = 2.56 (SE = 1.04) individuals. The variation in detection probability caused by the abundance in each camera site is shown in Figure 9.
While the detection probability in each site is dependent on the variables that were selected and the presence/absence data in each site for each trapping day, it is also determined by the abundance in each camera site, where as expected, a higher abundance in a site will increase the detection probability as well.
In MBPNP, all variables were fitted and compared to the null model (Akaike Information Criterion = 561.53). The best model for MBPNP (AIC = 560.1) passed three goodness of fit (GoF) tests, namely Sum of Squared Errors (p = 0.43), Pearson's Chi-Squared (p = 0.48) and Freeman-Tukey Chi-squared (p = 0.38). From the beta coefficients of the best model, the relative abundance of bears is higher near agricultural areas and when the slope is lower. The detection probability was slightly affected by the calendar date, where brown bears were more likely to be detected in the first two cycles, which was in late Spring and early Summer. The results of the relative abundance analysis are presented in maps (11 & 12).



Map (11): Overall brown bear relative abundance in MBPNP as obtained from IR camera traps.



Map (12) Brown bear relative abundance in MBPNP by camera location and sampling cycle.

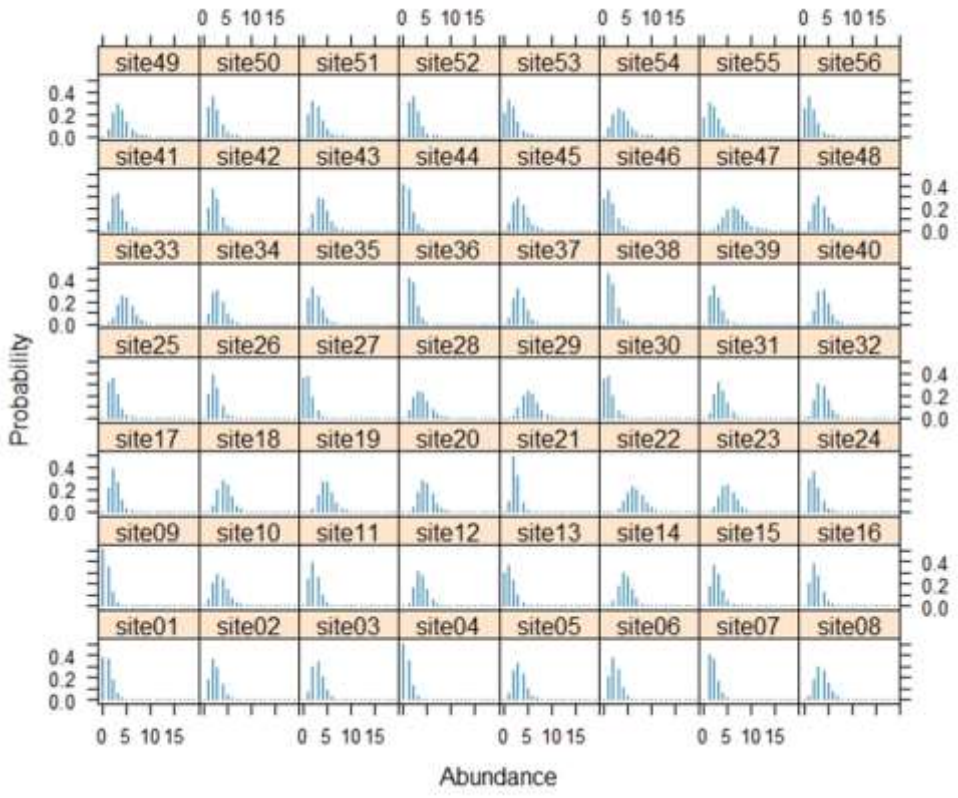


Figure 9. variation in bear detection probability in PINDNP related to the abundance at each camera site.

For RMNP: the mean probability of detecting any individual across sites was p = 0.07 (SE = 0.02) and mean relative abundance between sites was N = 1.76 (SE = 0.54) individuals. The variation in detection probability generated by the abundance at each camera site is shown in the Figure 10. While the detection probability in each site is dependent on the variables that were selected and the presence/absence data in each site for each trapping day, it is also determined by the abundance in each camera site, where as expected, a higher abundance in a site will increase the detection probability as well.

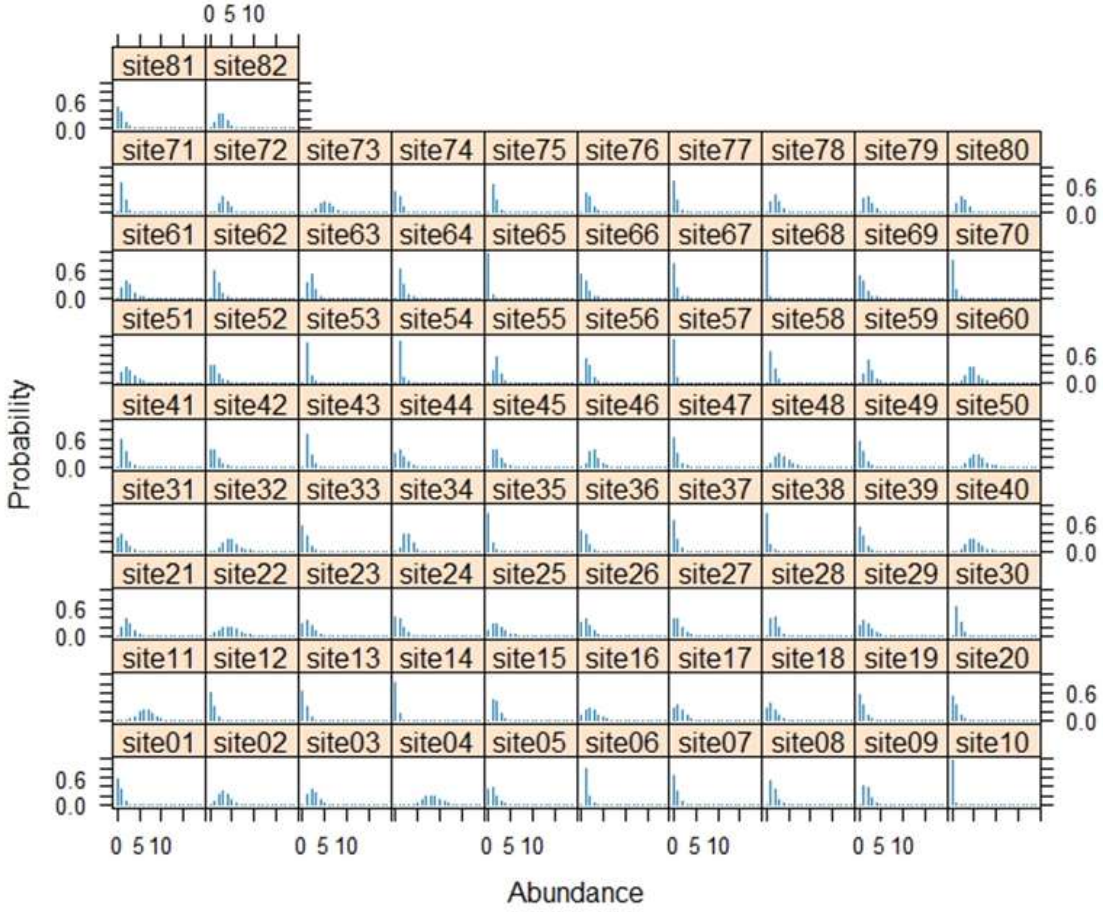
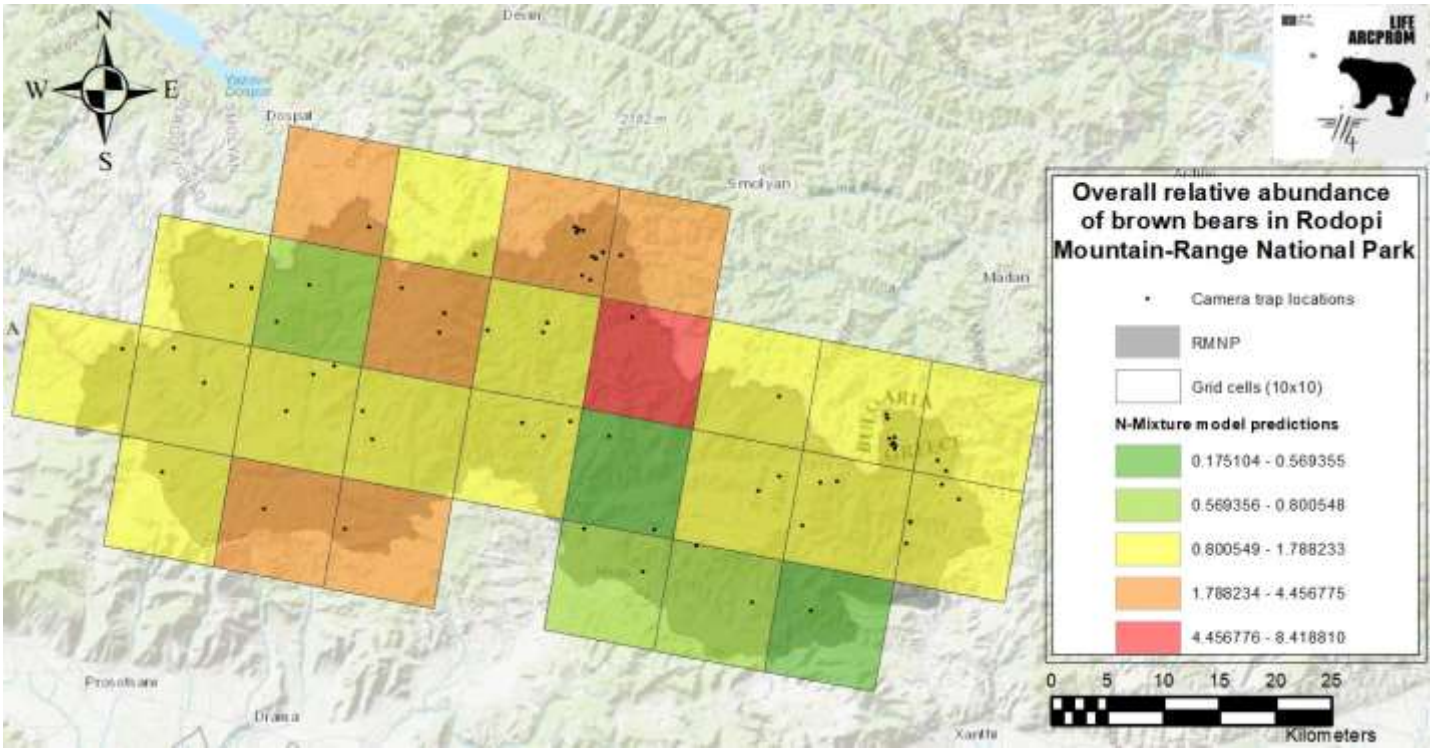
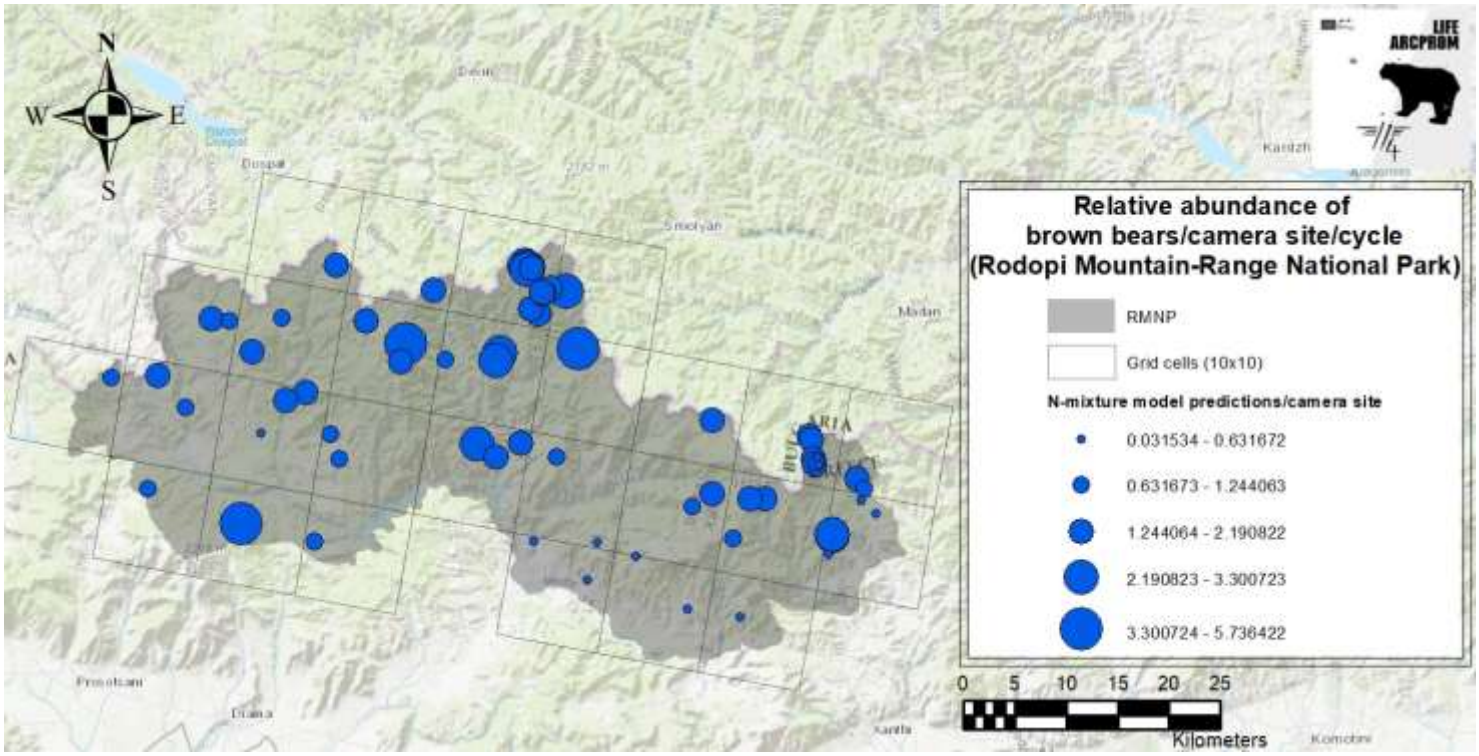


Figure 10. Variation in bear detection probability in RMNP related to the abundance at each camera site.

In RMNP, all variables were fitted and compared to the null model (AIC = 722.02). The best model for RMNP (AIC = 703.45) passed two goodness of fit (GoF) tests, namely Sum of Squared Errors (p = 0.48) and Freeman-Tukey Chi-squared (p = 0.38). From the beta coefficients of the best model, the relative abundance of bears is higher near Natura 2000 areas and significantly higher in areas with a lower road density. It is also slightly lower near shrub lands and agricultural areas. The detection probability was slightly affected by the average temperature of each camera trapping site at the time a camera was active there, showing that the detection probability of an individual was higher in warmer weather. The results of the relative abundance analysis are presented in maps 13 & 14.



Map (13): Overall brown bear relative abundance in RMNP as obtained from IR camera traps.



Map (14): Brown bear relative abundance in RMNP by camera location and sampling cycle.

3. Bear Biosigns and intensity of presence and activity

During the application of this complementary method to form an image regarding the distribution and presence of the bear in the project area and in the wider area, a sample network of routes was scanned in the forest road network with a total length of circa 847 km. In RMNP a total of 102 bear biosigns were recorded. Their spatial distribution is shown on map (15). In MBPNP a total of 141 bear biosigns were recorded. Their spatial distribution is shown on map (16). The frequency of the different biosign categories is shown on Figure 11. Bears intesity of presence and activity using Kernel Density Estimator is shown on maps 17 and 18.

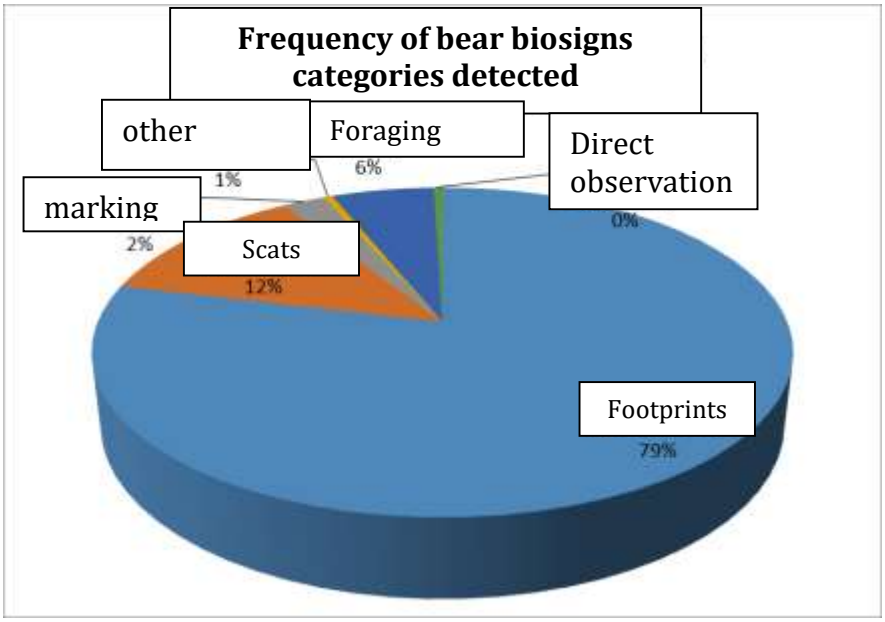
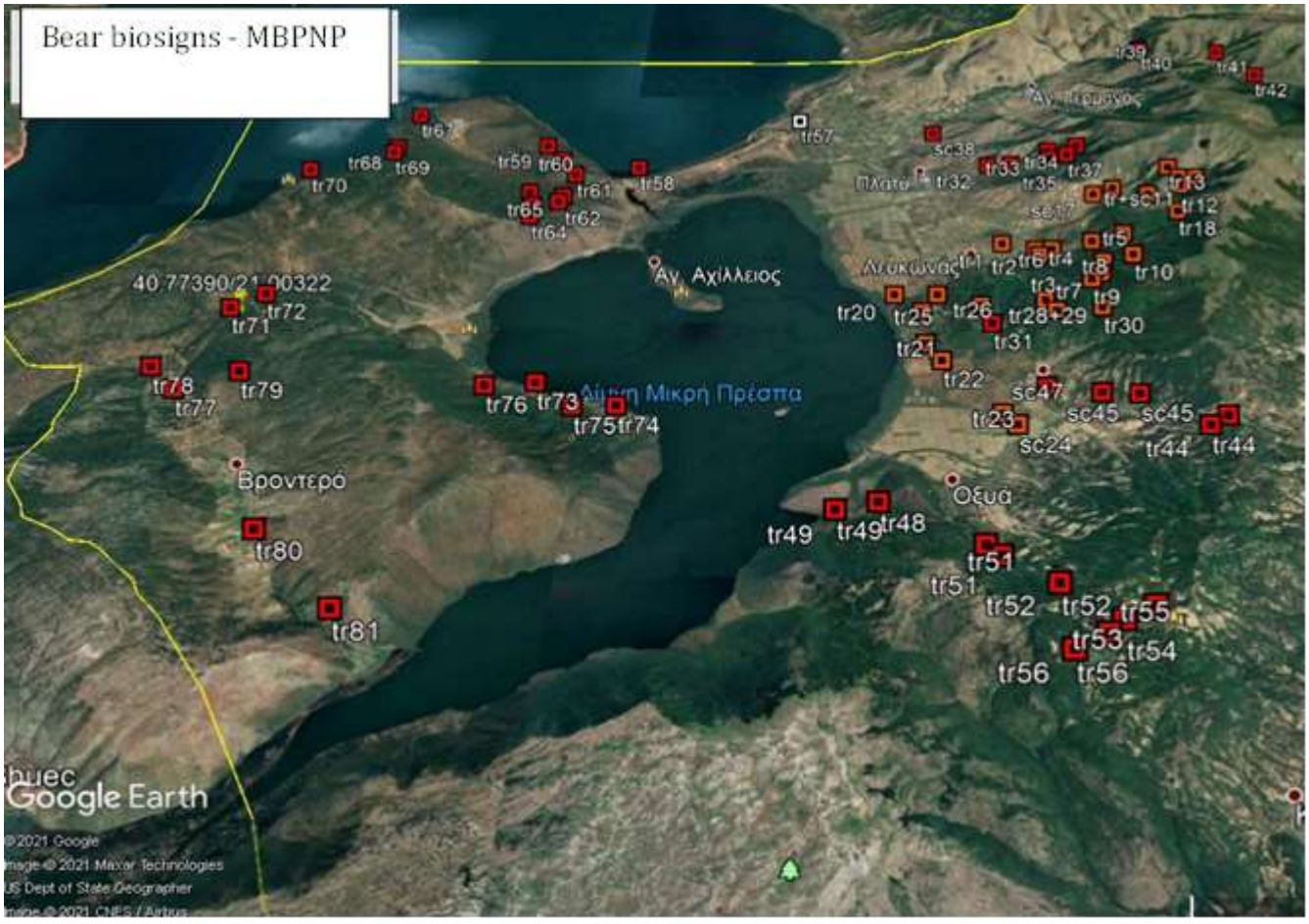


Figure 11. Frequency of the different biosign categories detected.

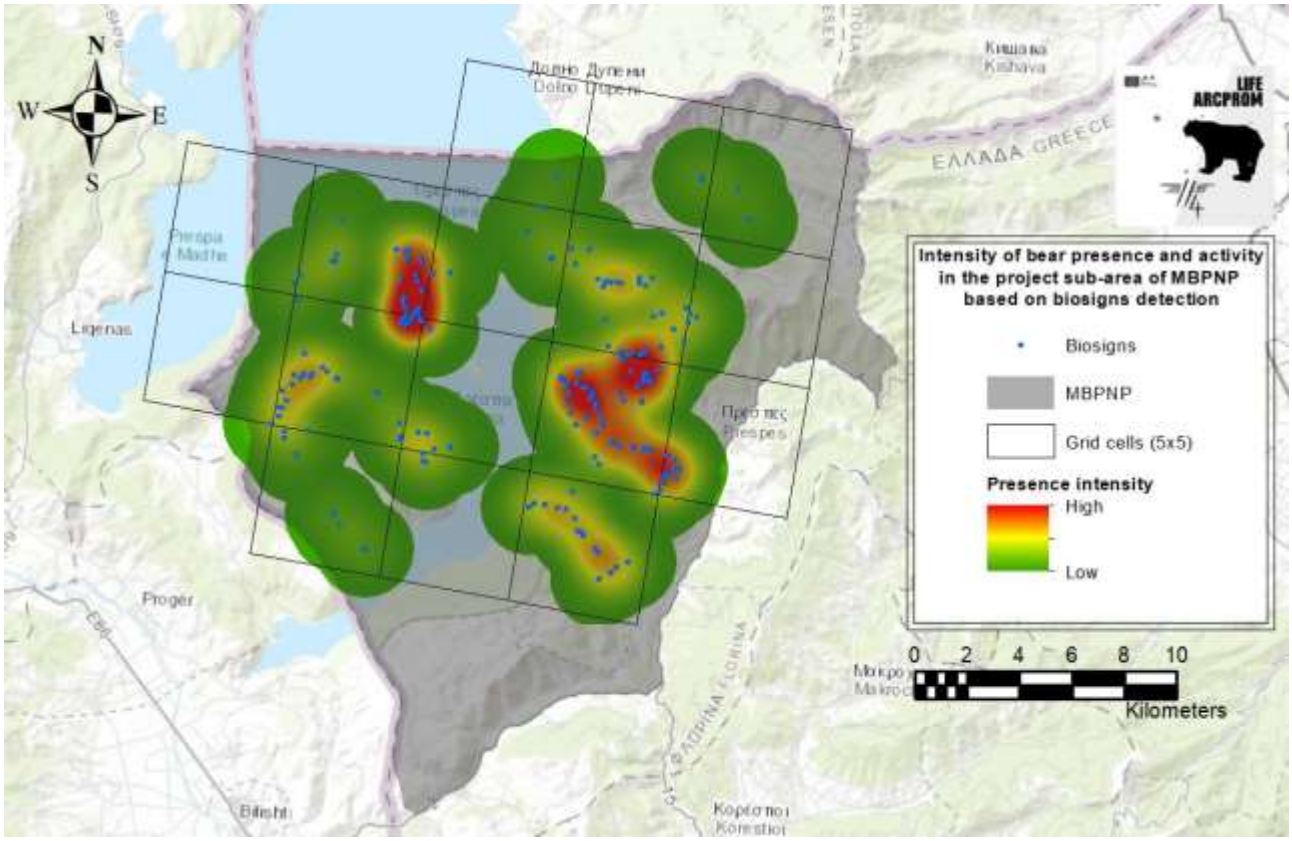
Map 15: Spatial dtistribution of bear biosigns (green dots) in RMNP (n=102).

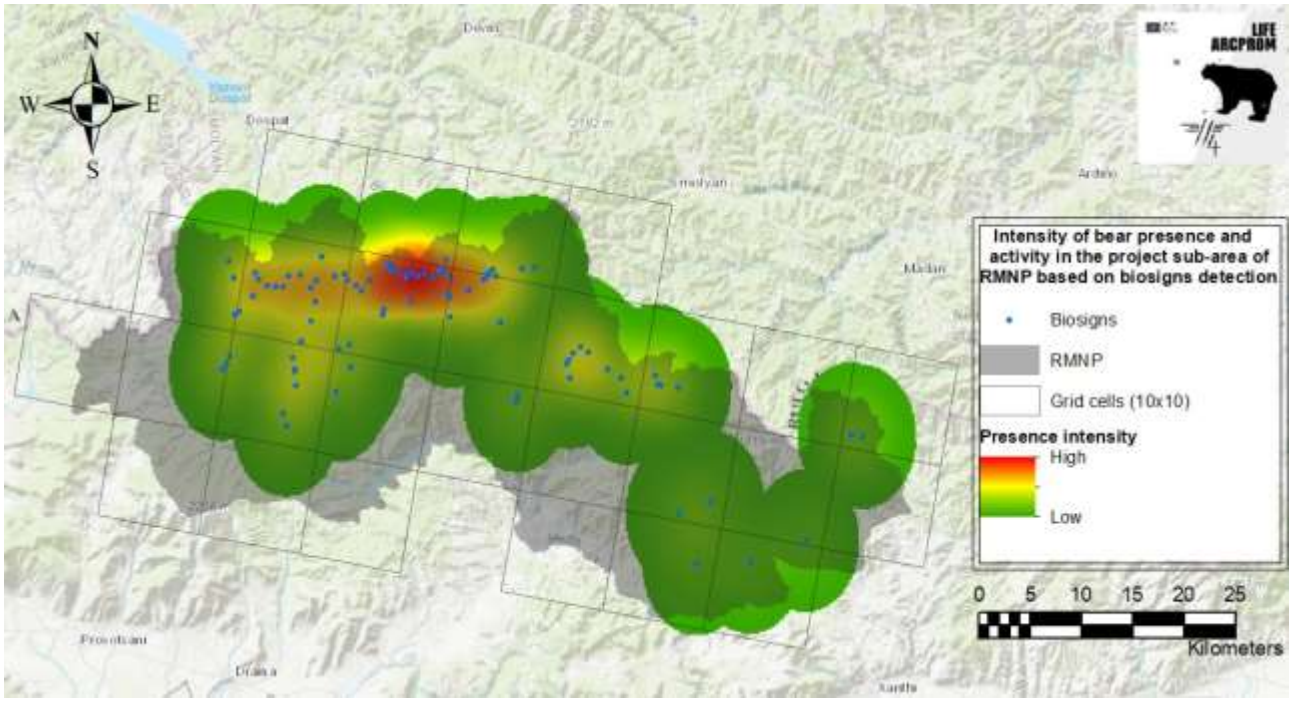


Map 16: Spatial dtistribution of bear biosigns (red squares) in MBPNP (n=141).

Regarding **MBPNP** (map 17) we observe (2) distinct nuclei of high bear presence and activity (high intensity scoring). These (2) nuclei are located on both sides of the mountain-lake ecosystem showing two very important facts: a) the lake of Mikri Prespa does not seem to function as an absolute barrier between the two population groups occupying the surrounding mountains b) the presence of a high bear presence and activity nucleus in the western part of Mikri Prespa Lake basin is of outstanding biogeographic interest given the fact of the poor or totally unsuitable surrounding habitat conditions at a larger scale: N and W delineated by water (lake of Megali Prespa) and NW delineated by a large part of unsuitable/degraded habitat entering the Albanian territories.
Moreover, we observe that the high bear presence and activity nucleus located in the eastern part of lake Mikri Prespa is larger in surface and subdivided in smaller but connected nuclei

Regarding RMNP: (map 18): we observe one single bear presence and activity nucleus with the highest intensity scoring located nearby the border area with Bulgaria in a dense and very productive coniferous ecosystem. The importance of this sector had also been evidenced with telemetry data in the frame of previous LIFE project (LIFE99NAT/GR/006498) (Mertzanis et al. 2005). It also is worth noting (in terms of conservation measures orientation) that compared to the large surface of the entire NP there is only one such nucleus. However, regarding the existence of other such nuclei in the NP a certain degree of underestimation bias has to be mentioned related to the fact that certain sectors are roadless and thus hardly accessible by 4X4 car for sampling.





Maps (17 & 18): Intensity of bear presence and activity based on biosigns and KDE analyses in MBPNP &. RMNP respectively.

Discussion

1. Genetic analyses results

1.1 Prespa National Park

To our knowledge this is the first study that includes bears from the overall area of Prespa NP, but there are studies that have conducted genetic analysis of bears from regions close or part of MBPNP (e.g. Peristeri, Kastoria, Amyntaio) (Pylidis et al. 2021, Tsaparis et al. 2015, Mertzanis 2018).
Based on the genetic data received from this study we found that in the Prespes population the microsatellite loci are informative for the estimation of the population size and we can use them for the analysis of genetic variance (PIC>0.5). Regarding the genetic diversity, the average population size (Nc) and the estimated population (Ne) our findings are in agreement with some previous studies (Tsaparis et al. 2015, Mertzanis 2018).

Table 21. Comparative table with studies carried out in Prespes NP and in areas close to Prespa [number of alleles (A), expected Heterozygosity (He), observed Heterozygosity (Ho),average of population size (NC), effective population size (Ne), Inbreeding Marker (Fis)].

Area of Population	Samples	A	He	Ho	Nc	Ne	Fis	Reference
Prespes	53	7	0.73	0.42	191	35	0.28	*This study*
Kastoria	82	5.8	0.548	0.584	219	39.5-49	0.07	*Tsaparis et al., 2014*
Peristeri	30	5.64	0.69	0.65	109	59.1	0.047	*Pylidis, 2021*
Amyntaio	75	6.8	0.582	0.412	154	44	0.25	*Mertzanis et al.,2018 LIFE15NAT/GR/001108*

As shown in Table 21, the inbreeding status of bears in Prespes shows high values of Fis (Fis>0.15). FIS is the proportion of the variance in the subpopulation contained in an individual (inbreeding coefficient) (Weir & Cockerham 1984). The increased inbreeding in combination with the low effective size makes the population of Prespes vulnerable. Moreover, the average of population size of brown bears in Prespes is 5.45 times larger than the effective population size.
These values were calculated through $Ne_{Estimator}$ 1.3 with the option "linkage disequilibrium method". The developers of the program (Botstein et al. 1980, Weir & Cockerham 1984, Boulanger et al. 2001) claim that accurate estimates are obtained with 2 or more captures/ bear. Regarding Prespes the mean arrest / sample ratio was 1.11, therefore this result should be treated with caution. Moreover, false positive estimation of the population size can happen when the bears live is a larger area than the area of our study (Botstein et al. 1980, Weir et al. 1984, Boulanger et al. 2001).
Therefore, population movements may affect positively the number of unique captures and negatively the number of re-captures (Kendall 1999). Regarding the sex ratio, in Prespes we found that males were 2,5 times more than the females. This fact may be explained by the fact that "rubbing behavior" on poles is more common in males than females (Green & Mattson 2003, Karamanlidis et al. 2010).

1.2 Pindos National park

By using genetic data from hair samples collected exclusively from power poles, we obtained precise population estimates despite fairly low capture and recapture rates (in Pindos the mean arrest / sample ratio was 1.18). Based on this data we found that complex genotypes of the 10 microsatellite loci are informative (PIC>0.5) and they show high genetic diversity of brown bears in Pindos NP.
Regarding the 65 unique bears found in Pindos, our findings show that they are characterized with relatively high genetic diversity and low values for Fis in the majority of loci. This declares a low inbreeding level of the population, which is in agreement (Table 23) with some of the previous studies (Karamanlidis et al. 2010, Karamanlidis et al. 2015, Karamanlidis et al. 2018, Pylidis et al. 2021) that are shown in Table 22. Therefore, high genetic diversity combined low inbreeding rates and relatively high effective population size (Ne) shows that the bear population in Pindos NP is not currently threatened by genetic instability.

Table 22. Comparative table with studies carried out in Pindos [number of alleles (A), expected Heterozygosity (He), observed Heterozygosity (Ho),average of population size (N_C), effective population size (Ne), Inbreeding Marker (Fis)].

Area of Population	Samples	A	He	Ho	Nc	Ne	Fis	Reference
Pindos	65	6.7	0.65	0.6	202	118	0.13	*This study*
Pindos	159	6.42	-	0.70	-	182.3	-	*Karamanlidis,2015*
Pindos	47	5-8	0.76	0.77	76		-	*Karamanlidis, 2010*
North Pindos	65	5.471,	0.658,	0.676,	-	65-149.8	-	*Karamanlidis, 2018*
South-Central Pindos	99	5.765	0.680	0.681	-	80.5-148.7	-	*Karamanlidis, 2018*
Pindos	97	5.27	0.64	0.61	299	97.4	0.042	*Pylidis, 2021*

Moreover, our study showed that the average of population size (Nc) of brown bears in Pindos is only 1.7 times larger than the effective population size and that male bears were 2 times more than the females. Regarding the sex ratio, in Pindos we found that males were 2 times more than the females. As referred, this may be explained by the fact that males rub on poles more frequently than females (Green & Mattson 2003, Karamanlidis et al. 2010).

1.3 Rodopi Mountain range National Park

Based on the genetic data received from this study we found that every microsatellite locus in the Rodopi population can be used for the analysis of genetic variance (PIC>0.5). Table 23 compares our data with findings from previous studies conducted in the same area but with a smaller number of samples.

Table 23. Comparative table with studies carried out in Rodopi [number of alleles (A), expected Heterozygosity (He), observed Heterozygosity (Ho), average of population size (N_C), effective population size (Ne), Inbreeding Marker (Fis)]

Area of Population	*Samples*	*A*	*He*	*Ho*	*Nc*	*Ne*	*Fis*	*Reference*
Rodopi	121	9.2	0.73	0.54	197	57	0.3	*This study*
Rodopi	22	6.09	0.73	0.71	91	42.2	0.021	Pylidis, 2021

Rodopi	15	6.529	0.745	0.808	-	-	-	*Karamanlidis, 2018*

The genetic diversity of 121 bear samples analyzed in our study is relatively high. Regarding inbreeding values high Fis (>0.15) implies a considerable degree of inbreeding. The increased inbreeding in combination with the low effective size (Ne) makes the population of Rodopi instable. Moreover, the average of population size of brown bears in Rodopi is 3.46 times larger than the effective population size.
As referred, "linkage disequilibrium method" requires 2 or more captures/ bear in order to be accurate. Regarding Rodopi the mean arrest / sample ratio was 1.26. Moreover, false positive estimation of the population size can happen when the bears live is a larger area than the area of non-invasive sampling (Botstein et al. 1980, Weir & Cockerham 1984, Boulanger et al. 2001, Boulanger et al. 2004).
Moreover, only in the Rodopi population we found such a great difference between the number of male and female bears. Most of the bears detected were males (103 males vs 18 females) indicating heterogeneity in the likelihood of their conception. Male bears are more likely to rub on trees and electricity poles in order to mark the area, especially during the breeding season. This behavior leads to unequal hair sampling between the two genders (Green & Mattson 2003, Karamanlidis et al. 2010) causing at a certain degree a sampling bias.. The combination of hair and another type of bear sampling (like feaces) can prevent the false devaluation/underestimation of the female bear number in similar studies.

1.4 Comparison of genetic data from the three project areas

Non-invasive sampling has many advantages regarding the collection of genetic material from large carnivores. On the contrary, invasive methods can be dangerous, time consuming, costly and can injure and upset the animal. Among the disadvantages regarding the samples received non-invasively (usually hairs and faeces) is the small amount of genetic material that is selected and the probability of contamination by the environment.
These factors make the amplification of genetic markers through PCR difficult, while specific genetic markers may be amplified more easily than others. Based on the aforementioned, in some microsatellite loci non-invasive sampling leads to genotypic errors such as zero and/or false alleles (Waits & Paetkau 2005). In the present study, non-invasive genetic sampling was used in order to collect samples of brown bears that live in the areas of Prespa, Pindos and Rodopi NP. In total, 522 hair samples were collected and 298 samples (57%) were genotyped at 6-10 microsatellite loci. The percentage of samples genotyped successfully in similar studies is about 50% (Prez et al. 2010, Sawaya et al. 2012, Tsaparis et al. 2015).
Regarding the highest allele frequencies of the 10 microsatellite loci in the three project areas (Table 24) it is obvious that some of them are evolutionally conserved between the brown bear populations. The most common alleles between the three subpopulations are found in loci Mu26, G1D, G10X, G1A, G10P, since obviously the three subpopulations are genetically mixed. But it is also notable that some of the loci seem to have mutual most frequent alleles among specific subpopulations: between Prespes-Pindos in only one locus (G10H), between Prespes-Rodopi in three loci (Mu59, G10L, Mu50) and between Pindos-Rodopi only in one microsatellite locus (G10C).

Table 24. Size of the microsatellite loci with the highest allele frequencies in the three project areas.

	G10H	Mu26	G1D	G10X	G1A	G10P	G10C	Mu59	G10L	Mu50
Prespes	**260**	192	184	146	193	160	112,122	**231**	**168**	**116**
Pindos	**260**	192	184	146	193	160	**97**	239	170	111
Rodopi	257	192	184	146	193	160	**97**	**231**	**168**	**116**

The growing interest in microsatellite genotyping, combined with non-invasive genetic sampling has led to the necessity of managing these data. Post analysis of our genetic data showed genetic diversity in all populations, high levels of inbreeding in Prespes and Rodopi and lower values of inbreeding in Pindos NP. Moreover, no signature of significant bottleneck was detected in any of our populations.

2. IR Camera trapping

The results agree with other studies and show that bear presence and activity was higher inside forests (Trolliet et al. 2014) and protected (Natura 2000) areas (Karamanlidis et al., 2015). Brown bears were also found to prefer mostly broad-leaved forests, mixed forests, heterogenous agricultural areas and shrublands, showing similar results with Posillico et al. (2004), Mertzanis et al. (2008) and Sanchez et al. (2014).
However, camera trapping data of unmarked individuals in this study could not be used for estimating population size. Instead, it is a reliable measure of relative abundance, representing the spatial variation between sites. Furthermore, in areas with increased human activity, the probability of detecting bears tends to be lower (Oberosler et al., 2017), suggesting that these areas might already have fewer bears due to human disturbance. According to previous studies (Kyriakidis 2021), the estimations of bear relative abundance and habitat suitability are not always in total agreement. Higher bear numbers can be found in areas with lower habitat suitability and a high suitability was found in areas with lower bear relative abundance estimations.
The method of the relative abundance analysis offers some advantages and disadvantages. Camera trapping data consisted of data from possibly all the available individuals in the study areas, however it might have been biased by the placement of the cameras, which were mostly near dirty (forest) roads and usually near bio-signs too, meaning that the more remote, rough and roadless areas were not completely included in the camera trapping sampling protocol. For this reason, the variable "distance to roads" was excluded from the analysis.
In many cases (such as in PINDNP) validation with telemetry data which are not affected by any direct human-induced bias can be performed given the unbiased nature of this method, focusing however on a rather small sample size (few bear individuals are usually radiotagged) for drawing population-level conclusions in the long-term However individual differences did occur (Mertzanis et al., 2008). Likewise, McLoughlin et al. (2002), mentioned that habitat selection at larger scales might be different from habitat selection at smaller scales.
The relative density estimation can be combined to habitat selection and habitat suitability analyses and could be extrapolated in the nearby areas and NPNP because of similar attributes (i.e. altitudes, road networks and land covers). However, for larger scales, it might be insufficient because of landscape changes and small sample size of the available telemetry data.

3. Biosigns surveys

This approach has its own specific and complementary role in the identification of bear presence and activity with certain limitations and assumptions regarding the extrapolation possibilities. The sampling accuracy is influenced by several factors such as: field crew observation capacities, transects homogeneous distribution strongly depending on forest road network coverage and sampling sectors accessibility, nature of the substratum etc. This being said, it is important to note that the recorded bear biosigns are reflecting the presence and activity of an entire population portion and thus are more representative as regards to the overall presence and activity status of a given sub-population in a given area.

REPORT OF ACTIVITIES IN ITALY

Since bear presence started to be more tangible in the Maiella National Park (2010-2012), the management body started to actively monitor this presence with the aim to know where bears roamed, the number of bears and if females were present. Monitoring strategy has always been mainly opportunistic as systematic monitoring, despite requiring high efforts and field work, gave poor if none results. Between 2010 and 2012 bear presence reporting out of the population core range started to augment in other portion of the Abruzzo region as well so that, in 2014 the Maiella National Park (MNP) and the Abruzzo, Lazio e Molise National Park (PNALM) promoted the institution of a Bear Monitoring Network (RMAM) with the aim to make all the different management bodies collect reliable data in the whole bear range following the same protocol. The RMAM was officially established in 2018 so that, starting from that year, all the monitoring activity carried on by MNP merged into the RMAM protocol, drafted by MNP and PNALM and approved by an official technical commission designated by the State and coordinated by the Environmental Ministry (now Ministry for the Ecological Transition). The monitoring strategy of the aforementioned protocol is based on a territory stratification into areas with different bear density (i.e. different probability to detect bear bio-signs) and, in the monitoring area assigned to MNP, two *strata* resulted from the GIS analysis performed (Figure 12): one, the biggest, where an opportunistic strategy has to be implemented (~98,000 Ha), one, considerably smaller, where a systematic strategy is foreseen (~900 Ha).

Figure 12. MNP monitoring area and location of the two *strata* identified with the GIS analysis performed and reported in the Bear Monitoring Network (RMAM) protocol.

Opportunistic strategy means that specific field surveys for bio-signs detection must be implemented during the periods of maximum detection probability (mating season and fall) in the areas where seasonal food sources are available (e.g. low meadows in spring and beech woods in autumn). Systematic strategy means that the stratum must be divided in cells in which at least one genetic trap/camera-trap must be positioned in the habitats where season-specific food sources are present.

In addition to this two active monitoring strategy, the RMAM protocol also foresees one "passive" activity which turned out to be the most important one: field surveys to verify bear presence events reported by people or any of the stakeholders working in the territory.

Monitoring activities implemented in the frame of Action A2 are thus consistent with activities foreseen in the RMAM protocol and, as opposed to Greece, mainly focus on opportunistic monitoring aimed at acquiring basic information on where bears are, how many individuals are present and if females/females with cubs are present.

Methods

1. Field collection of bear biological material

The collection of non-invasive bear biological material for the Apennine brown bear (ABB) refers to the collection of hair and scats with the first being the preferable one. Scats genetic analysis is, in fact, severely constrained by their age so that only very fresh scats (i.e. maximum 12-24 hours old) have a chance to be successfully analyzed, while older scats usually do not contain good quality DNA. On the contrary, hairs with bulbs are a very good source of high quality DNA and their collection/preservation until the day of the analysis is a little easier than scats and more compatible with the constraints of the field work. Finally, even though in other projects bear scat analysis proved to be a very good monitoring method (e.g. LIFE DINALP BEAR), this is not the case for the Apennine brown bear, probably due to the difference in methods used in the laboratories in charge of making the analyses (HTS versus electrophoresis). In MNP, and in other portion of the ABB range as well, scat genetic analysis gave so far little results even when fresh scats were sampled thus resulting in an unsatisfactory costs/benefits ratio. For these reasons, the methods for bear biological material collection in MNP refer to activities aimed at collecting hairs while scat collection has been considered a side-activity to be implemented incidentally when fresh scats were actually found.
Hair samples collection was implemented using two main methods: the search for hairs during field surveys after receiving a bear presence reporting by people and the setting of specific hair traps using barbed wire. The first method consisted in the opportunistic search for hairs in the locations where the bear presence was registered looking to all the surfaces that could possibly hook bear hairs (e.g. barbed wire, rough surfaces, sticky grasses, rosehip bushes, junipers, moss etc.). The second method consisted in actively building hair traps consisting in barbed wire corrals or in rub trees equipped with barbed wire. While the first method has been implemented opportunistically, the installation of the genetic traps followed a systematic-opportunistic mixed strategy. In the *stratum* where a systematic monitoring is foreseen in the RMAM protocol (Figure 12) at least 1 hair trap/year has been installed while in the rest of the territory hair traps have been opportunistically installed basing on *a)* seasonal food sources availability, *b)* bear presence reporting and *c)* bear presence data collected in the previous years.
Following methods reported in the RMAM protocol, barbed wire corrals (**classic hair traps**), have been built according to the following:

- The location of the trap was individuated as a wooded area, relatively flat, with at least four trees in an adequate position to fix the barbed wire and obtain a trap of 20-25m circumference.

- The barbed wire has been fixed to the trees at a height of 47 (± 2) cm, considered the best one to hook ABB hairs.

- In the center of the trap a pile of woods soaked with a scent lure has been created to simulate a carcass. In some particular cases the barbed wire has been built around actual carcasses of wild animals or animals predated by bears.

Rub trees are unfortunately not so easy-to-find in MNP, possibly due to the low number of bears which leads to less individuals who actually rub and maybe also to a reduced need of rubbing as a form of communication to other bears. However, despite the difficulties in

finding rub trees, efforts have been made to use them as hair traps as they are an excellent source of hairs. In fact, they are used by different individuals of all ages and sex even though adult males are the ones who rub significantly more (Ciucci et al. 2015; Tattoni et al. 2015). During the implementation of Action A2 a specific work has been carried out to find rub trees (see § B. Field tracking surveys) but to set hair traps not only "natural" rub trees have been used but also "artificial" rub trees have been created, a method that proved to be effective in some cases in the past. Hair-trap-rub-trees were thus located where actual rub trees were or whenever a possible rub tree (big, evident tree in a strategic point like a pass) was identified in a worth-monitoring area (i.e. an obligatory passage or a food source hot spot). To turn rub trees into hair traps they have been equipped with several pieces of barbed wire about 20cm long and placed from an height of ~50cm to ~ 2-2.5m in order to avoid collecting hairs from animals smaller than bears, especially wild boars. In case the rub tree was an "artificial" one, it has been lured with the same substance used for the classic hair traps or with turpentine oil. Both classic hair traps and rub trees have been checked regularly in order to collect the hairs as soon as possible and store them properly. Particularly, the following checking methods have been applied:

- Classic hair trap: first check after 12-15 days. If the check was positive the subsequent checks were scheduled every 7-10 days, if the check was negative a second check was scheduled after 12-15 days.

- Rub trees: first check after 12-15 days. If the check was positive the subsequent checks were scheduled every 4-5 days, if the check was negative a second check was scheduled after 12-15 days.

Rub trees needed to be checked more than classic hair traps as the probability of collecting a mixed genetic sample is very high given that they are used by several different individuals. To minimize the probability of collecting mixed genetic samples, not only the checking timing was adjusted but also video-traps have been installed to monitor the number of bears visiting the rub tree during the time between two consequent checks.
Once collected, hairs were stored following the guidelines provided by ISPRA, the laboratory officially in charge to analyze all ABB samples collected in its whole range. Hairs were thus put in a paper bag and then in a hermetic plastic bag where a silica gel pack was also present in order to absorb all the possible humidity that would ruin the DNA. If wet hairs were collected, for example after a raining period, they were first drained using absorbing paper and then put in the storage bags. Scats were sampled using a swab and this was put in a test tube provided by the ISPRA laboratory and filled with a preserving liquid. Collected hair and scat samples were sent to the ISPRA laboratory as soon as possible in order to make the storage period the briefest and minimize the probability of DNA degradation.

2. Field tracking surveys

The implementation of field tracking surveys is a method poorly used for ABB presence monitoring in its whole range. This is mainly due to the fact that a considerable effort is necessary, especially in term of trained personnel, but results are poor if none. With the establishment of the monitoring network RMAM a renewed effort has been put in the implementation of this method, also standardizing its development in different areas of ABB range, but the poor results obtained required a modification of the protocol which now foresees the possibility to replace the tracking survey with a video-trap. The only task that is actually worth implementing is the development of specific surveys in food sources hotspots

like areas with a high density of fruit trees and buckthorn (*Rhamnus alpina*) concentration sites.
For these reasons in the frame of Action A2 in MNP mainly these two last tasks have been planned so that field tracking surveys have been implemented in order to verify bear presence (and collect genetic samples) in known food resources hot spots. According to the project proposal, Action A2 was supposed to last from October 2019 until June 2021 meaning that the useful monitoring period was from spring 2020 to Fall 2020 and spring 2021. Since the closure of Action A2 was then postponed to September 2021, thus adding the whole summer 2021 in the useful monitoring period, in MNP we decided to implement an additional task specifically aimed at finding rub trees. These, as already explained, are a very useful data source being sites where different individuals rub (or at least pass or stop) giving a high contribution in the achievement of our monitoring objectives. In the following paragraphs methods for the implementation of field tracking surveys in hot spots areas (classic field tracking surveys) and surveys specifically aimed at finding rub trees (rub trees surveys) are described.

Classic field tracking surveys have been planned in bear recurring presence areas and in hot spots of cherry/apple trees as well as in buckthorn sites. Particular focus has been put in surveys where cherry and apple trees were present as in these cases it is also possible to collect genetic samples (hairs hooked-up by the tree bark). Before implementing any of the planned survey, a first recognition of actual food source presence was made. In fact, apple/cherry production as well as buckthorn production are highly constrained by the weather and, especially in the last years, extreme events like total absence of fruits or exceptional plenty of them have been registered more and more often. The location of the surveys, as well as the length, were thus dependent on the actual distribution of food sources in that given season.
At least one survey/hot spot/season has been planned and it had to be covered by 1-2 trained persons according to the following method:

- during the survey all the possible bear presence signs had to be searched for while walking and specific stops had to be made when any possible food source hot spot was found, meaning that not only the target food source had to be monitored but also every possible source that could help reveal bear presence (e.g. anthill presence/concentration).

- If a bear presence sign was found, the area around the sign had to be carefully explored in order to find any possible additional sign and a particular effort had to be put in the research of biological material working as genetic sample.

- All the bear presence signs had to be recorded and UTM WGS84 coordinates had to be taken.

During the surveys, whenever bear signs were actually found, also the possibility to implement side-activity like *ad hoc* observation sessions was evaluated as the observation of animals could help assess information impossible to obtain from genetic samples analysis like the age of the individual.
Once collected, all bear presence signs have been assigned to the reliability values 1, 2 or 3 according to the following criteria reported in the RMAM protocol:

- Reliability value 1: signs that are objectively and unequivocally bear signs (GPS collar locations, bear carcasses, genetic samples, direct sightings by experts, videos, pictures and footprints validated by experts). This category is the one not suffering at all the issue of false positives.

- Reliability value 2: signs that have high probability of being bear signs but they cannot be considered as such objectively and unequivocally (sightings by trained/reliable persons, scats attributed to bear by experts without the genetic analysis, destroyed anthills detected by experts, damages, carcasses with the typical signs of bear consumption). This category includes all the signs having low probability of being false positives.

- Reliability value 3: signs not validated by experts or that are attributed to bears using a high degree of subjective evaluation (sightings by people, old/not clear bear-signs, tipped stones, bites on trunks, beds/dens without bear hairs, digs, destroyed fruit trees without hairs etc.). This category refers to bio-signs having high probability of being false positives.

Bio-signs with reliability 1 and 2 are the only ones actually considered bear bio-signs for any further analysis while bio-signs with reliability 3 are just used as an information on where to implement *ad hoc* monitoring.

To implement **rub trees surveys** a more specific work has been carried out and, considering that this was a brand new activity for MNP, the planning started from a literature review aimed at better understanding where bears rub and which trees they possibly prefer and/or select. The aim of this review work was, in fact, to understand which variables, if any, affected the location of rub trees and consequently plan the surveys in the areas with the highest probability of actually hosting them.
Several papers have been read and evaluated but two of them specifically addressing rub tree use and selection are the ones that gave the information needed to identify the portion of MNP with the highest probability of hosting rub trees, these are Tattoni et al. (2015) developed in Trentino, North of Italy, and Gonzalez-Bernardo et al. (2021) developed in the Cantabrian Mountains, Spain. This last especially, is a work aimed at describing both landscape variables and tree features affecting the use of rub trees by bears and was thus the main reference work for the planning of this activity in MNP. Basing on the results of the above-mentioned works we considered 4 variables as relevant to individuate both the areas with the highest probability of rub tree presence and the paths to be covered to search for them (Table 25).

Table 25. Variables used to perform the GIS analysis and individuate the portions of the MNP monitoring area with the highest probability of hosting rub trees as well as individuate the paths to be covered to search for them.

Variable	Explanation
Aspect: N, NE and S	Rub trees location is significantly correlated with Northern exposure (Gonzalez-Bernardo et al. 2021), NE and S exposures (Tattoni et al. 2015).
Wooded areas with particular attention to conifers	Conifers and birches (absent in MNP) are the only two species selected for rubbing (Gonzalez-Bernardo et al. 2021).
Areas more than 1Km from roads and other human settlements.	Landscape surrounding of RTs is predominantly covered by habitats > 1Km far from human settlements (Gonzalez-Bernardo et al. 2021).
Presence of trails, forest roads etc.	Rub trees are predominantly located along trails, forest road etc. (Gonzalez-Bernardo et al. 2021).

According to the study of Gonzalez-Bernardo et al. (2021) the density of rub trees was not positively correlated with the density of bear signs nor with the distribution of bears in the range. For this reason, in MNP we decided to develop the searching for rub trees in the whole monitoring area (i.e. not only in the areas with the highest density of bio-signs) and to apply a systematic design based on the individuation of at least one path to be covered for each cell of 10x10Km. A GIS analysis was thus performed (Figure 13):

1) First we selected all the wooded areas from the Corine Land Cover layer.

2) Using a Digital Elevation Model, we created a polygon vector layer of the monitoring area divided into 8 aspect categories (N, NE, E, SE, S, SO, O, NO) and then we extracted only the priority aspects (N, NE and S).

3) We created a 1Km buffer around paved roads and we then selected wooded areas to be monitored as the ones falling out of the paved road buffer.

4) We created a grid of 10x10Km cells over the monitoring area.

5) Finally, we projected together the selected wooded areas with the grid, the priority aspect layer and the trail/unpaved road layer to individuate the paths to be covered.

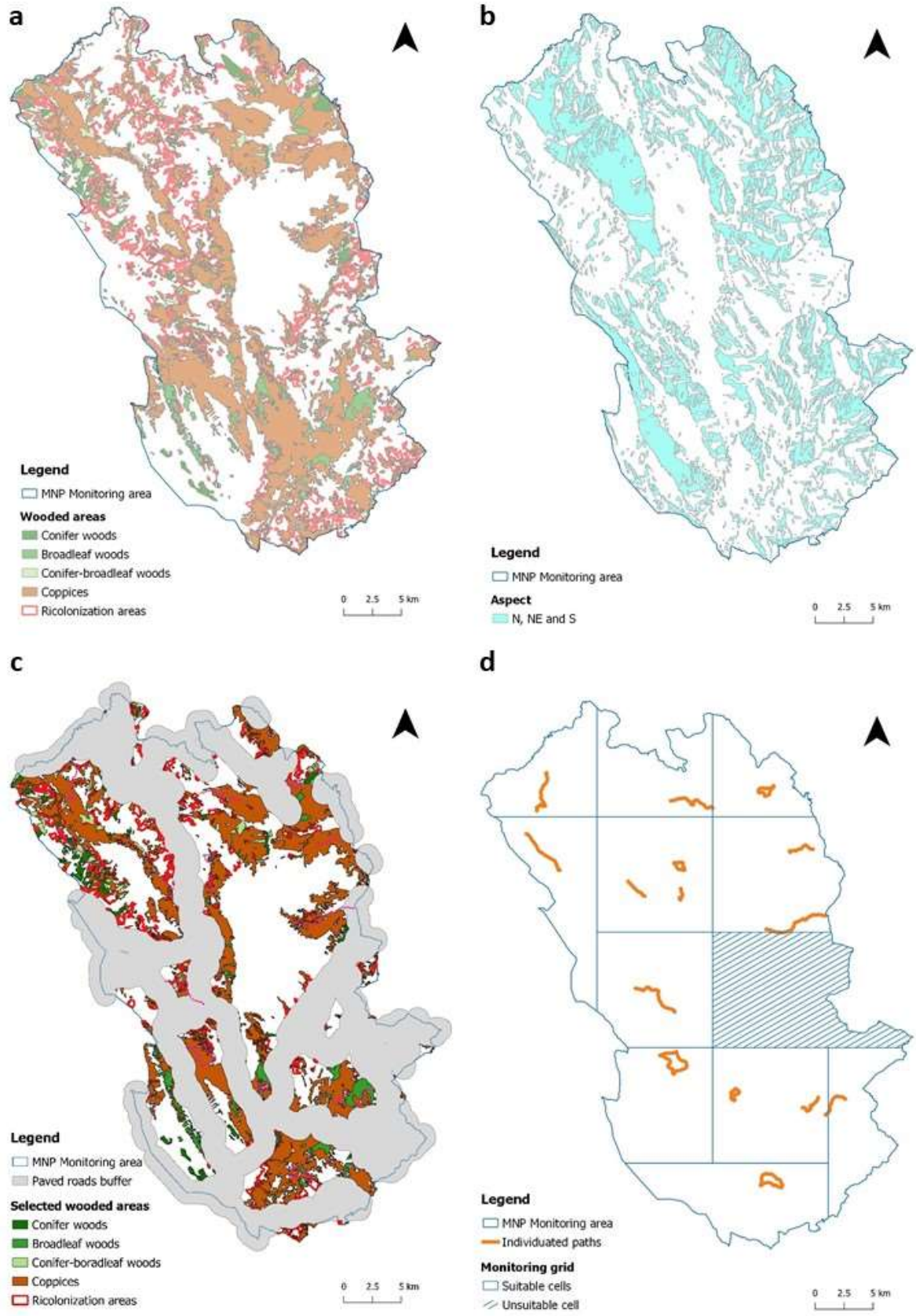


Figure 13. Main steps of the GIS analysis performed to individuate the paths to be covered to search for rub trees in the MNP monitoring area: a. Selection of wooded areas from the Corine Land Cover; b. Creation of a polygon layer of areas with N, NE and S exposure; Selection of wooded areas falling out of the 1Km buffer from paved roads; c. Paths individuated projecting together the selected wooded areas with the monitoring grid, the priority aspects layer and the trail/unpaved road layer.

At least one path/cell was individuated but more than one path is present in those cells with several areas with high probability of hosting rub trees. Particularly, a special attention has been paid to conifer woods: these are not naturally present in MNP but there are plantations which, in some cases, form patches of significant size to be used by bears. Considering that in the Cantabrian Mountains conifers were preferred even though not naturally present there either (Gonzalez-Bernardo et al. 2021), we believed conifer woods to be worth visiting.
After individuating the paths to be covered, also methods to search for rub trees once in the field have been assessed. Basing on the literature review (i.e. considering the variables that were positively correlated with rubbing probability) and on the experience gained so far in the ABB core range, trees to be checked as possible rub trees had to be:

a) Standing, live trees.

b) Trees along the trail/road with a relatively large DBH[1] (i.e. larger than the ones around them).

c) Trees with a higher distance from other trees than the ones around them (i.e. areas of lesser forest density as compared to the surroundings).

d) Trees with evident signs of scratching and/or biting.

e) In broadleaf woods or conifer-broadleaf woods, conifers possibly present had to be checked with priority.

The tree species is an important variable in explaining rubbing probability (Gonzalez-Bernardo at al. 2021), however it is strictly dependent on the habitats actually present in one specific area. Species used in one area could not necessarily be the same used in another one. Actually, Gonzalez-Bernardo et al. (2021) found that distribution of rub trees among the different species reflected their availability except for birches and conifers that were selected for. In MNP the prevalent species is the beechnut (*Fagus sylvatica*) which is not reported in the literature as being particularly attractive for bears. However, this is the main species available so that bears may rub there just for this reason. During field surveys all the trees having the features above-listed in points a-d were thus checked without any regard to the species but, if a conifer was present, this was checked with priority.
The best period to implement the research of rub trees is the mating season, a period during which bears rub significantly more than other periods (Clapham et al. 2013, Tattoni et al. 2015). Unfortunately, in MNP it was not possible to implement this activity during the mating season (May-June) so that it was implemented during summer-fall 2021.

3. IR cameras network operation

Camera-traps are one of the best way for monitoring a rare species as they offer a very convenient costs/benefits ratio. The main problem of using these tools is the theft risk, a risk existing also in MNP. Massive thefts suffered in the past heavily constraints current possibilities to develop this kind of monitoring and special attention must be paid in order to avoid losing camera-traps. According to the RMAM protocol, camera- trap monitoring follows an opportunistic-systematic method, with the first one to be implemented in the majority of

[1] Diameter at Breast Height is the standard for measuring trees and it refers to the tree diameter measured at ~ 1.5 meters form the ground.

the territory while the second one in the small portion where systematic monitoring is foreseen (see Figure 12). The opportunistic strategy mainly consists on the possible activation of camera-traps after a bear presence reporting by people which is usually constrained by the actual suitability of the area where the reporting happened. In the frame of Action A2, given the high potential of the camera trap monitoring, we decided to put in place an extra-effort and implement a systematic monitoring with camera traps in the whole MNP monitoring area. A 10x10Km cell monitoring grid, the same used in 2021 for the implementation of rub tree surveys (see the previous paragraph and Figure 13), was thus created and we planned to place at least one camera-trap/cell. The choice of the exact location inside each cell and the monitoring period was based on the guidelines reported in the RMAM protocol which are basically the same used for the installation of hair traps:

- Locations were chosen basing on where food resources hotspots were present or in possible obliged passages. Areas where recurrent bear presence data have been collected were included as well.

- The (minimum) monitoring period was consistent with the food source availability.

Camera-traps have been set to record videos of 40”-1’ length, setting the interval between videos in the “as fast as possible” mode. All the camera-traps used has the “no glow” led, meaning that the led set has the lowest visibility possible, thus representing the least disturb possible for animals.

Results

Where bears are

Combining results obtained using all the methods (field collection of bear biological material, field surveys and camera traps) a total of 356 bear bio-signs were found in the frame of Action A2 (201 in 2020 and 155 in 2021, Table 26). Among these, 318 have reliability 1 or 2 while only 38 have been assigned to reliability 3.

Table 26. Bear bio-signs and their reliability detected in the MNP monitoring area in the frame of Action A2.

Year	Reliability			*Total*
	1	2	3	
2020	139	40	22	***201***
2021	119	20	16	***155***
Total	***258***	***60***	***38***	***356***

Among the 318 bio-signs with reliability 1 or 2 (from here called just "bear bio-signs"), 14 different types were detected having different distribution in the two years of Action A2 implementation (Figure 14).

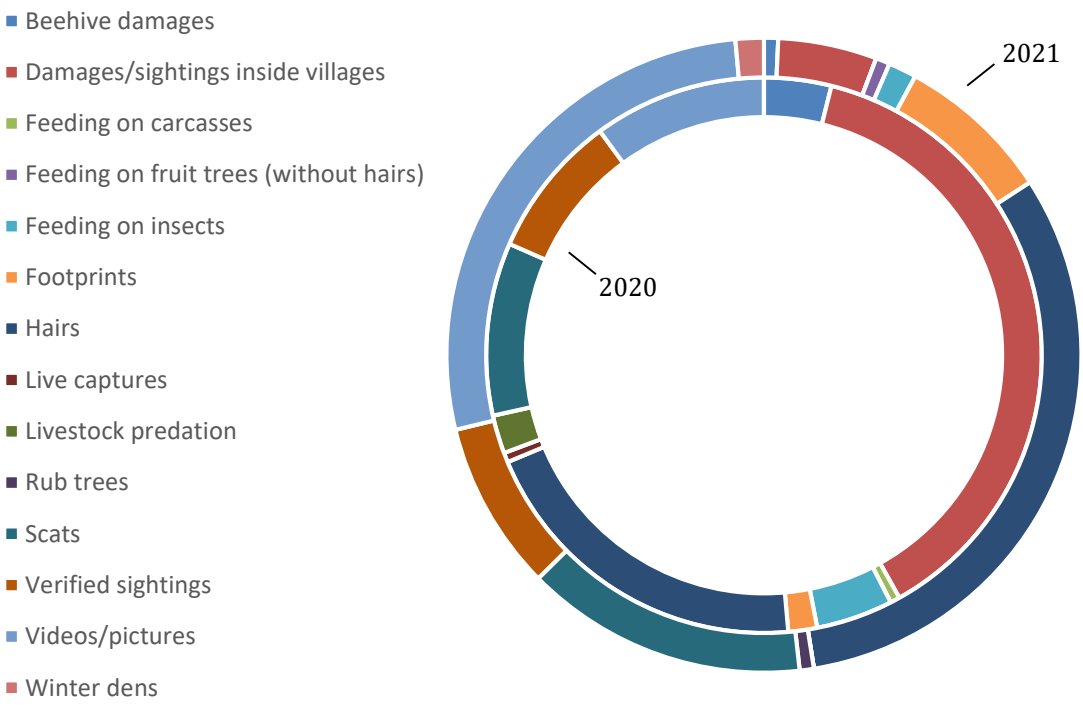


Figure 14. Type of bio-signs with reliability 1 and 2 detected in the MNP monitoring area during the implementation of Action A2 (2020 and 2021).

Bear bio-signs in 2020 were found mainly in the southern portion of the monitoring area while in the northern portion only few bio-signs were found representing the 6% of the total (Figure 15). In 2021 bear signs were found both in the northern and the southern portion of the monitoring area and the most important result is the detection of bear presence signs in the north-eastern portion of the monitoring area where few or no signs at all had been detected in the last 10 years. Particularly: 3 presence signs represented by a picture from a

camera-trap, a genetic sample and footprints on the snow have been detected in a portion of the monitoring area where bear bio-signs had never been detected from 2004 to 2020; 1 scat and footprints on the snow have been detected in a valley frequented by a radio-collared bear in 2015 where few signs had been detected in 2016 (Figure 15).

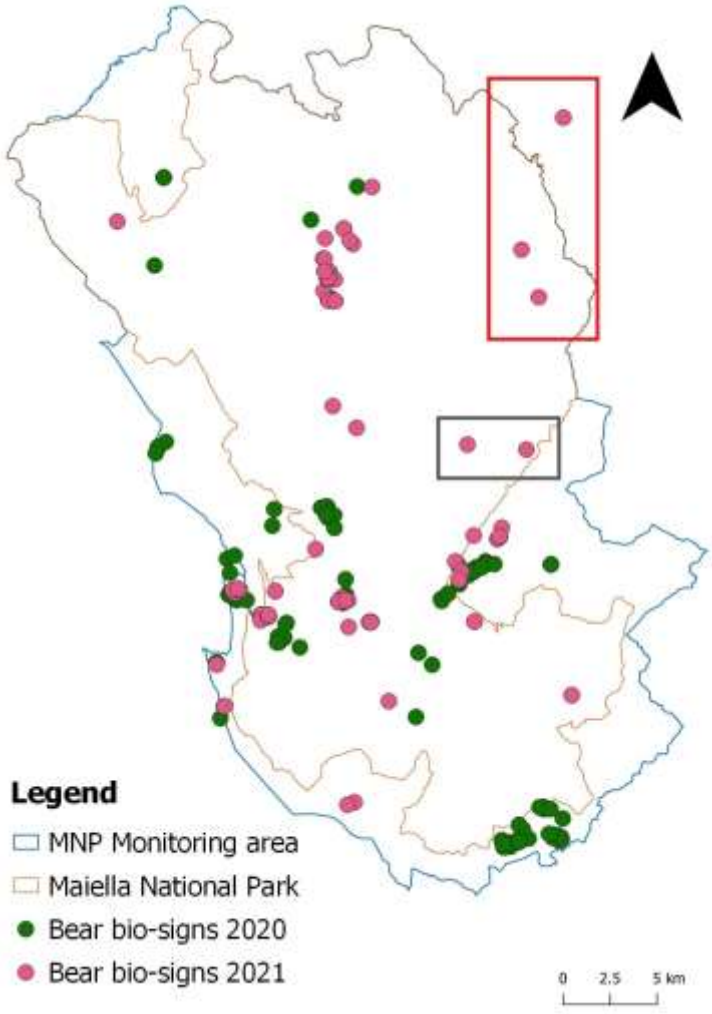


Figure 15. Bear bio-signs detected in the MNP monitoring area during the implementation of Action A2. The red rectangle highlights bio-signs detected in a portion of the monitoring area where bear bio-signs had never been detected before; the grey rectangle highlights bio-signs detected in a valley frequented by a radio-collared bear in 2015 where only few signs had been detected in 2016.

Since MNP is a recolonization area, beyond being affected by methods used, bear bio-signs detection can also be affected by the recolonization dynamics. Additionally, since the main scope of MNP monitoring (in general, not only in the frame of the LIFE ARCPROM project) is to have the best possible information to implement conservation activities, it is important not only to assess where bears actually roam but also to understand if a specific re-colonization pattern is going on. This last issue is crucial to implement proactive conservation measures and thus maximize the effectiveness of the conservation strategy. For these reasons, data collected in 2020 and 2021 have been observed also in light of data collected in the previous years both in term of number of bear bio-signs detected and areas interested by bear presence.

From 2004 to 2021 the number of bear bio-signs augmented passing through peaks and hollows, as expected when a recolonization process is ongoing. However, 2011 seems to have been a turning point after which the number of detected bio-signs increased reaching the

maximum values in 2020 and 2021 (Figure 16). Considering that methods applied so far to search for bio-signs in MNP are mainly opportunistic, it is reasonable to hypothesize that data collected have been affected by the inhomogeneity of methods applied in different years. However, even though absolute numbers/year may actually be biased, it is still clear that the positive trend observed cannot be explained only by a possible different monitoring effort: the number of bio-signs detected passed from a few dozens of the years 2004-1011 to more than 100 in the last ten years.

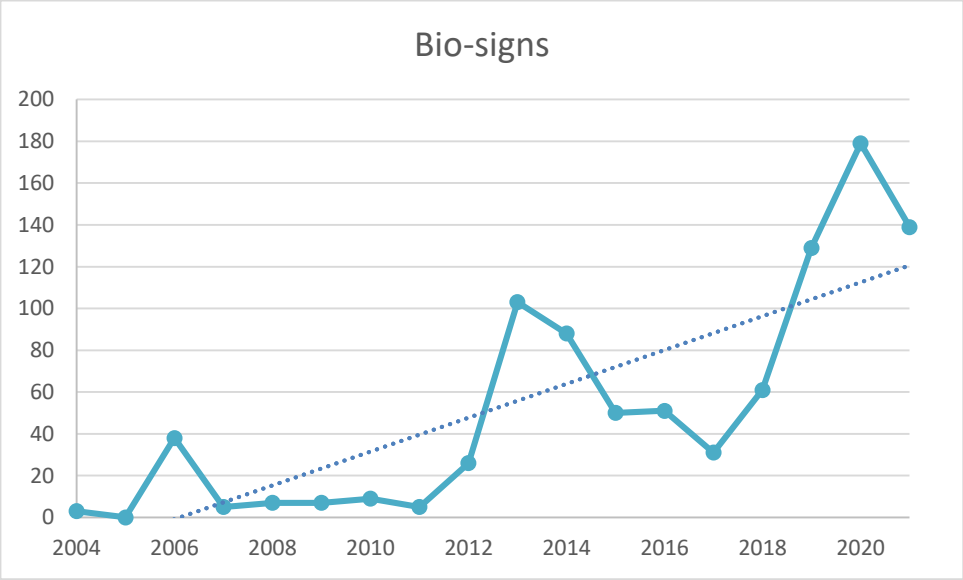


Figure 16. Number of bear bio-signs detected from 2004 to 2021 in the monitoring area of the Maiella National Park. The blue dotted line represents the linear trend of bear bio-signs detection.

These considerations on the increase of the number of bio-signs detected are supported by results of the evaluation of bio-signs distribution. Comparing Minimum Convex Polygons of bio-signs detected in the periods 2004-2010 (when few dozens of bio-signs/year were found), 2011-2019 (from the turning point to beginning of the LIFE ARCPROM) and 2020-2021 (years of implementation of Action A2) a change in their spatial distribution can be observed (Figure 17). While the three MCPs overlap in the southern portion of the monitoring area, things are different in the northern portion. In 2004-2010 only a small cone of the MCP is present in the northern portion, thus suggesting that bears were mostly concentrated in the south and only occasionally frequented the north. The MCP of the period 2011-2019 is more even, thus suggesting that also bear presence was more evenly distributed in the two portions of the monitoring area. The MCP of 2020-2021 not only suggests an even bear distribution in the north and south portions but also reveals that bear presence was detected in a new portion of the monitoring area toward north-east.
This rough analysis of bear bio-signs distribution in the last 17-years period gives the following essential information:

1. Bear presence reached a degree of stability first in the south portion of the monitoring area, which is the one closest to the Abruzzo, Lazio and Molise National Park where the source population lives.

2. The area were bio-signs were detected changed during the years with a progressive inclusion of the north-western and north-eastern portion of the monitoring area.

3. The bear presence in the new area in the north-east was detected in a short time-span monitoring activity (2 years).

This three information suggest that bear presence in MNP is the result of a recolonization process that started almost twenty years ago and never stopped since then making bears use more and more portions of the area every year. This process passed through a starting phase, when changes in bear presence were slow and difficult-to-detect, and now maybe entered an actual recolonization phase corresponding to more tangible changes even in the short term.

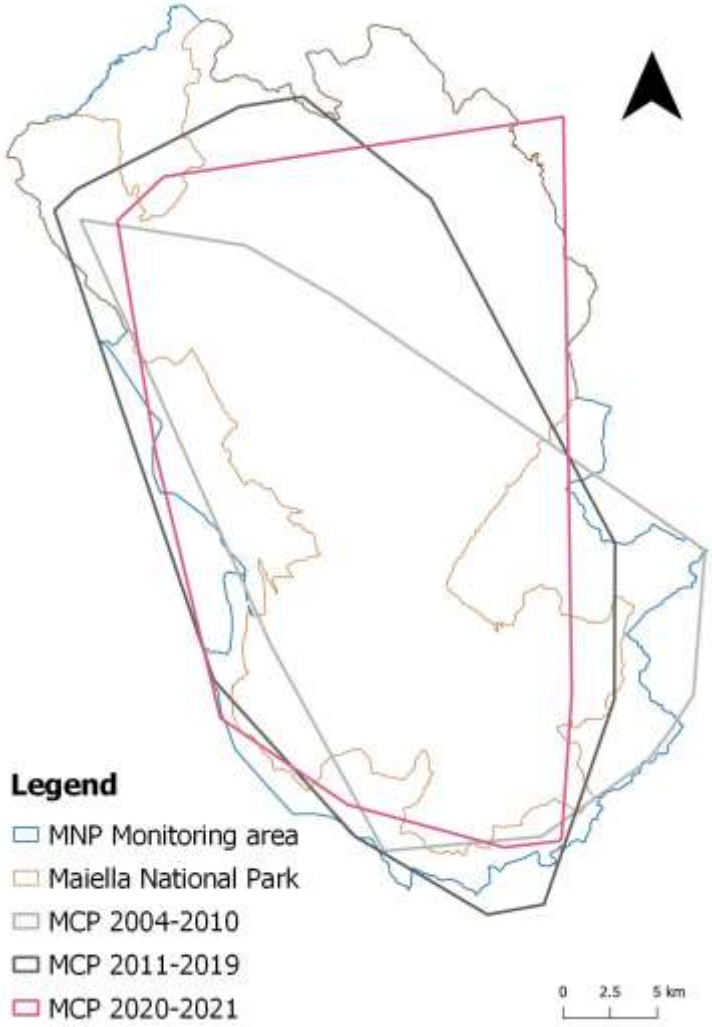


Figure 17. Minimum Convex Polygons (MCP) of bear bio-signs detected in three different periods: 2004-2010 (when few dozens of bio-signs were found each year), 2011-2019 (from when the number of bio-signs started to increase until the beginning of the LIFE ARCPROM) and 2020-2021 (years of implementation of Action A2).

Results obtained with the implementation of Action A2 gave an essential contribution in assessing where bears roam and what's happening in the territory. Years 2020-2021 seem to actually represent a new turning point: from a period when some territories could still be excluded by some conservation activities to a period when all the territories falling in the MNP monitoring area must be included in all the conservation activities. In fact, even though bear presence in the north-eastern portion may not be detected in the next couple of years, still data suggest that bear will eventually use that portion of the area in the short term[2],

[2] If the status of the source population of the Abruzzo, Lazio e Molise National Park doesn't get worse.

meaning that conservation activities need to be implemented immediately in order to guarantee a proactive-approach.

Even though bear radio-collaring is not an activity foreseen in the frame of Action A2, to give a more comprehensive information, we here report that additional bear presence data have been collected in 2020-2021 from the radio-collars of the problematic female F1.99 (radio-collared in the MNP in the frame of Action C5 of the LIFE ARCPROM), the female F1.143 and the problematic male M1.176 both radio-collared in the Abruzzo, Lazio e Molise National Park (Figure 18).

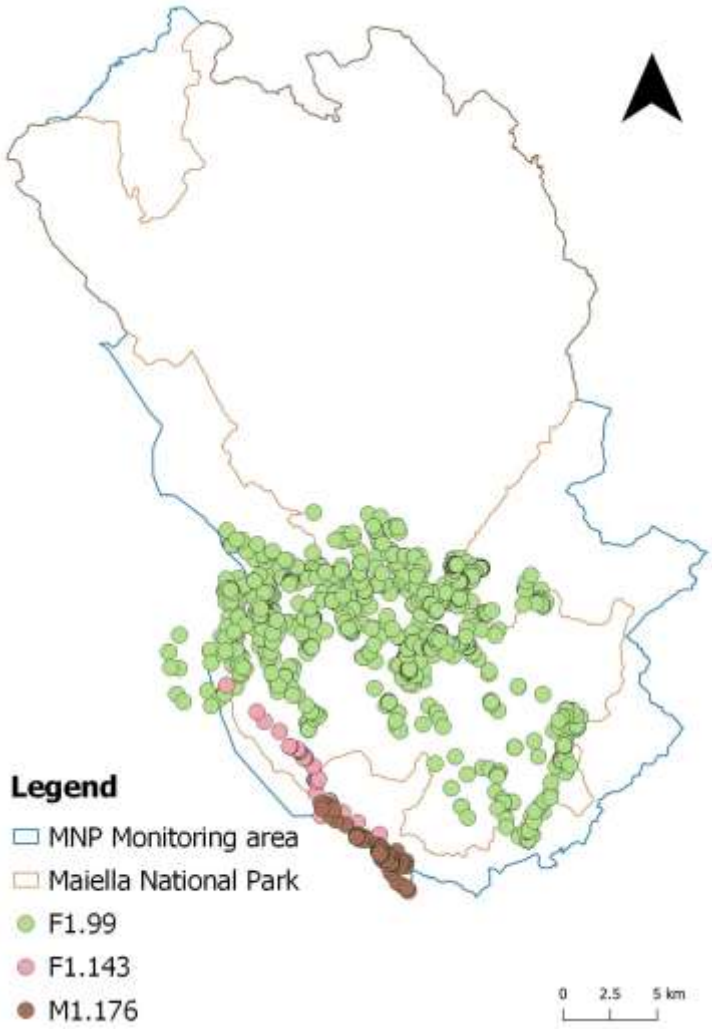


Figure 18. GPS locations acquired by the radio-collars of the female F1.99 (fall 2020-spring 2021), captured in MNP in the frame of Action C5, the female F1.143 (late summer 2020) and the male M1.176 (fall 2021) both captured in the Abruzzo, Lazio e Molise National Park.

F1.99 was equipped with a GPS/GSM radio collar the day 17/11/2020 during a BET intervention and she lost the collar the day 27/06/2021 while feeding on a cherry tree. A total of 2,199 locations were acquired by the collar and two dens used during the winter 2020-2021 were also located hanks to the GPS collar monitoring. The female F1.143 was captured by the Abruzzo, Lazio e Molise National Park (PNALM) staff and roamed in an area between PNALM and MNP. In July-August 2020 she occasionally frequented the MNP to feed on buckthorns so that a total of 55 locations of that period have been acquired inside the MNP monitoring area. Her presence was detected in 2021 as well, when she entered in the village of Rivisondoli in the SW portion of the MNP monitoring area but no GPS locations are available as the radio-collar was broken.

The male M1.176 is one of the four cubs of a problematic female that showed problematic behaviours as well. M.176 with his mother and the other three littermates roamed in a portion of the Special Protected Zone (ZPE) of the Abruzzo, Lazio e Molise National Park. After the family break-up, when he was 1.5 years old, M1.176 started to show problematic behaviours in the same areas but then moved closer to the MNP monitoring area. In September and October 2021 he did two sporadic visits but then, in October-December 2021, he also frequented the village of Rivisondoli and 188 locations were collected inside the MNP monitoring area.

How many individuals are present and who they are

Basing on the genetic analysis of bear biological material, the minimum number of bears present/year in the MNP monitoring area can be assessed. In the frame of Action A2 a total of 104 genetic samples have been collected in the field. Since in some cases several samples were collected from a single event (e.g. when a single bear rubbed to a barbed-wire-equipped rub tree) not all the samples have been labelled. Out of 104 samples, 88 have been assigned to a code while 16 duplicates have been stored for any possible future use. Out of 88 samples 80 were selected to be sent to the ISPRA laboratory and 7 duplicates were sent in a second time for a total of 87 samples analyzed (39 in 2020 and 48 in 2021; Table 27).

Table 27. List of samples of bear biological material sent to the ISPRA laboratory for the implementation of the genetic analyses aimed at assessing the sex and the genotype of the bear.

*** This is a new genotype sequenced only once, it needs to be confirmed before it can be considered an actual new genotype. See text for details.**

****This result is incoherent with results of genetic analysis in other portion of the range. See text for details.**

YEAR	TYPE OF SAMPLE	CODE	SEX	GENOTYPE
2020	Hair	PNM_CG_161	F	172
2020	Hair	PNM_CG_162	F	172
2020	Hair	PNM_CG_163		Bear
2020	Hair	PNM_CG_164		Analysis failed
2020	Hair	PNM_CG_165		Analysis failed
2020	Hair	PNM_CG_166		Bear
2020	Hair	PNM_CG_167	M	171
2020	Hair	PNM_CG_168		Analysis failed
2020	Hair	PNM_CG_169		Bear
2020	Hair	PNM_CG_170		Bear
2020	Hair	PNM_CG_171		Bear
2020	Hair	PNM_CG_172	M	128
2020	Hair	PNM_CG_173		Bear
2020	Hair	PNM_CG_174		Bear
2020	Hair	PNM_CG_175	M	171
2020	Hair	PNM_CG_176	M	171
2020	Hair	PNM_CG_177		Bear
2021	Hair	PNM_CG_205		Bear
2021	Hair	PNM_CG_206		Analysis failed
2021	Hair	PNM_CG_207		Bear
2021	Hair	PNM_CG_208		Bear
2021	Hair	PNM_CG_209	M	120
2021	Hair	PNM_CG_210	M	199
2021	Hair	PNM_CG_211	M	120
2021	Hair	PNM_CG_212	Bear	Bear
2021	Hair	PNM_CG_213	Bear	Bear
2021	Hair	PNM_CG_214	M	In depth analysis ongoing
2021	Hair	PNM_CG_215	F	99
2021	Hair	PNM_CG_216	M	In depth analysis ongoing
2021	*Hair*	*PNM_CG_217*		*Analysis ongoing*
2021	Hair	PNM_CG_218	M	In depth analysis ongoing
2021	Hair	PNM_CG_219	F	99
2021	Hair	PNM_CG_220	M	150
2021	Hair	PNM_CG_221	M	150

YEAR	TYPE OF SAMPLE	CODE	SEX	GENOTYPE
2020	Hair	PNM_CG_178	Analysis failed	
2020	Hair	PNM_CG_179	Analysis failed	
2020	Hair	PNM_CG_180	F	99
2020	Hair	PNM_CG_181	F	99
2020	Hair	PNM_CG_182	Analysis failed	
2020	Hair	PNM_CG_183	Analysis failed	
2020	Scat	PNM_CG_184	Analysis failed	
2020	Scat	PNM_CG_185	Analysis failed	
2020	Hair	PNM_CG_188	F	99
2020	Hair	PNM_CG_189	F	99
2020	Scat	PNM_CG_190	Analysis failed	
2020	Scat	PNM_CG_191	Analysis failed	
2020	Hair	PNM_CG_192	M	120
2020	Hair	PNM_CG_193	M	120
2020	Hair	PNM_CG_194	M	120
2020	Hair	PNM_CG_201	M	171
2020	Hair	PNM_CG_202	F	99
2020	Hair	PNM_CG_203	Analysis failed	
2020	Hair	PNM_CG_164_BIS	M	120
2020	Hair	PNM_CG_165_BIS	M	120
2020	Hair	PNM_CG_182_BIS	M	120
2020	Scat	PNM_CG_204	Bear	

YEAR	TYPE OF SAMPLE	CODE	SEX	GENOTYPE
2021	Hair	PNM_CG_222	F	99
2021	Hair	PNM_CG_223	M	120
2021	Hair	PNM_CG_224	F	99
2021	Hair	PNM_CG_225	Bear	
2021	Hair	PNM_CG_226	M	181
2021	Hair	PNM_CG_227	Bear	
2021	Hair	PNM_CG_228	Bear	
2021	Hair	PNM_CG_229	M	171
2021	Scat	PNM_CG_230	M	175
2021	Hair	PNM_CG_231	M	199
2021	Hair	PNM_CG_232	M	171
2021	Hair	PNM_CG_233	F	172
2021	Hair	PNM_CG_234	F	196
2021	Hair	PNM_CG_235	F	196
2021	Hair	PNM_CG_236	M	198
2021	Hair	PNM_CG_237	F	196
2021	Hair	PNM_CG_238	F	172
2021	Hair	PNM_CG_239	M	197
2021	Scat	PNM_CG_240	F	172
2021	Hair	PNM_CG_241	F	196
2021	Hair	PNM_CG_242	M	198
2021	Hair	PNM_CG_243	M	179
2021	Hair	PNM_CG_244	F	172
2021	Hair	PNM_CG_245	F	172
2021	*Hair*	*PNM_CG_246*	*Analysis ongoing*	
2021	*Scat*	*PNM_CG_247*	*Analysis ongoing*	
2021	*Hair*	*PNM_CG_248*	*Analysis ongoing*	
2021	*Hair*	*PNM_CG_205_BIS*	*Analysis ongoing*	
2021	*Hair*	*PNM_CG_207_BIS*	*Analysis ongoing*	
2021	*Scat*	*PNM_CG_240_BIS*	*Analysis ongoing*	
2021	*Hair*	*PNM_CG_243_BIS*	*Analysis ongoing*	

Out of 39 samples analyzed in 2020, only 18 (46%) have a complete successful analysis, for 9 samples (23%) only the species could be confirmed and, finally, for 12 samples (31%) the genetic analysis completely failed. In 2021, out of 48 samples sent to the laboratory, 28 (58%) resulted in a genotype, in 8 cases (17%) only the species was confirmed, 3 samples (6%) require additional analysis to assess the genotype, 8 samples are still being analyzed while only for 1 sample the analysis completely failed.
In 2020 five different genotypes resulted from the analysis (2 females and 3 males) while in 2021, twelve different genotypes resulted so far (3 females and 9 males). However, two genotypes resulted in 2021 cannot reliably be considered as referring to actual individuals for two different reasons:

- M1.181 is a new genotype resulted for the first time in MNP on a rub tree. Since it has been sequenced only this once, it could be a fake genotype resulting from the combination of different DNAs coming from different bears rubbing on the same tree. In fact, this sample was collected on a natural rub tree not equipped with barbed-wire and thus with high probability of having hairs coming from different individuals. Until

M1.181 will not be sampled again, it will conservatively be considered a fake genotype and thus removed from the count of the minimum number of bears.

- M1.175 resulted from the analysis of a scat collected just outside the MNP monitoring area the day 02/09/2021. The very same day, two samples (1 hair and 1 fresh scat) have been collected at more than 200Km in the Lazio region and both resulted as M1.175. Considering that all of the three samples were fresh, meaning that we know that the biological material had surely be deposed that very morning, this result suggests that M1.175 was in the same time in two places more than 200Km away. Since this is not possible, and since in the Lazio region M1.175 has been sampled several different times, the presence of M1.175 in MNP is conservatively considered not reliable until this genotype will, possibly, be sampled again in the future.

The final number of different genotypes detected for 2021 is thus 10 (3 females and 7 males). To assess the minimum number of bear present, results of the genetic analysis were combined with results coming from other monitoring activities and GPS collars. In 2020 the radio-collared female F1.143 frequented the MNP monitoring area (see Figure 18) but none of the genetic samples analyzed belongs to her so that the minimum number of bear present in 2020 is 6: 5 genotypes detected plus the female F1.143. The high percentage of failed genetic analysis in 2020 probably hid the presence of a seventh individual since, despite the bio-signs detected (including 11 videos from a video-trap), none of the genetic samples collected in the northern portion of the monitoring area had a complete successful analysis (i.e. resulting in a genotype). In 2021 the radio-collared bear M1.176 frequented the MNP monitoring area (see Figure 18) and the marked females F1.129 and F1.143 were also observed and photographed. None of their genotypes resulted from the genetic analyses so that the minimum number of bears present in 2021 is 13: 10 genotypes detected plus M1.176, F1.129 and F1.143.
The minimum number of bears present is a datum that, exactly like bear bio-signs number and distribution (see § *Where bears are*), gives additional important information if observed in a given time-range rather than in a single year.

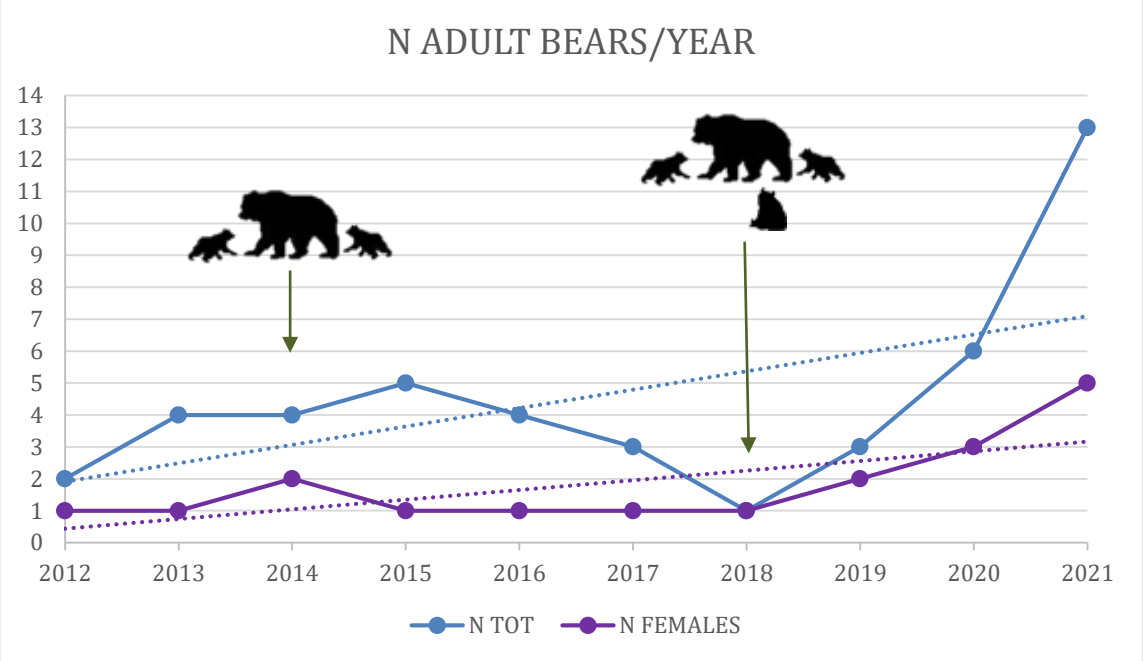


Figure 19. Minimum number of adult bears detected in the MNP monitoring area from 2012 (i.e. form when standardized and comparable analysis of genetic samples are available) to 2021. In 2014 one of the females gave birth to 2 cubs while in 2018 the only female detected gave birth to 3 cubs. Dotted lines represent the linear trend of the total number of bears (blue) and the number of females (purple).

From 2012 (i.e. form when standardized and comparable analysis of genetic samples are available) to 2018 the minimum number of adults varied from 1 to 5 bears while, starting from 2019, a tangible increase seems to be happening (Figure19). The minimum number of bears detected doubled from 2019 to 2020 and again from 2020 to 2021 going from 3 to 12 in only two years. As already discussed for the bio-signs, surely it can be argued that this datum could be biased by the inhomogeneity of the monitoring methods but, again, it is hardly plausible that the change observed from the period 2012-2018 to the period 2019-2021 is the result of a biased monitoring strategy. This consideration is also bolstered by data referring to the minimum number of females: from 2019 to 2021 they passed from 2 to 5 and they have only been detected in the south portion of the monitoring area, closest to the source population of the Abruzzo, Lazio e Molise National Park. The trend observed for the number of bio-signs is confirmed by the trend of the minimum number of bears present in the area and both of them, together with the data concerning number and location of females, are consistent with the hypothesis that the recolonization process entered in an active phase.

In order to better understand the recolonization dynamic, beyond assessing the minimum number of bear present, it is important to investigate if the sampled bears have been already sampled in MNP and if they have been sampled in other portions of the bear range. The combination of these two information allows to assess if (and which) bears are stably present in the MNP monitoring area and how individuals move within the whole recolonization range. A total of 14 individuals have been present in MNP in 2020-2021: 11 different genotypes have been sampled in 2020 and/or 2021 and three additional individuals have been detected with methods different from the collection of bear biological material (Figure 20).

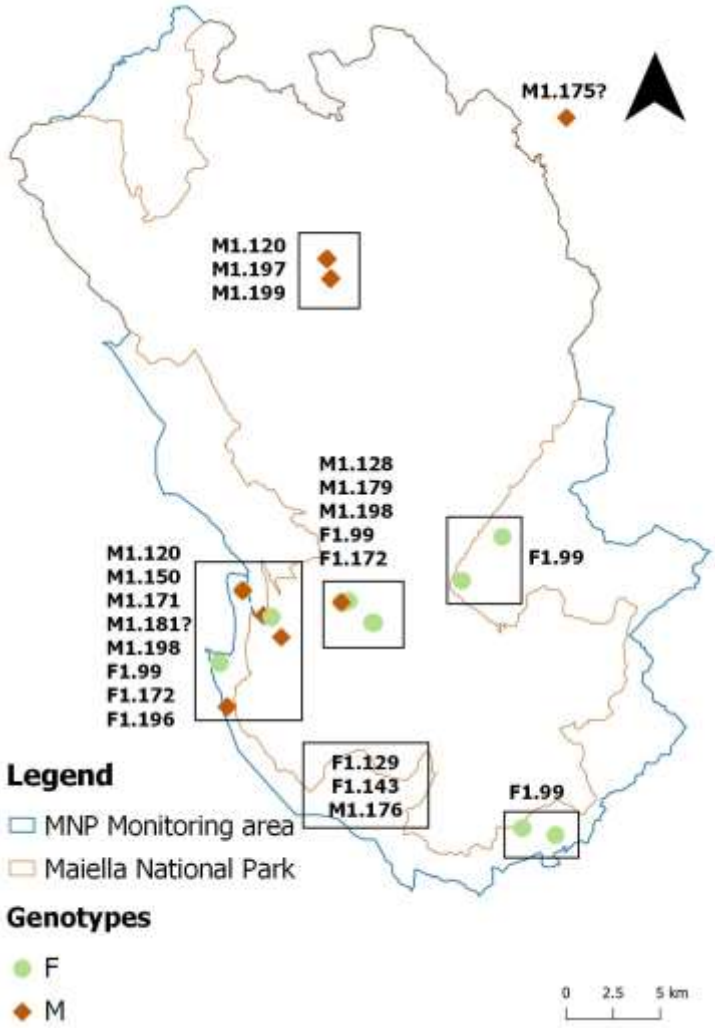


Figure 20. Name and sampling location of the 11 genotypes resulted from the analysis of the bear biological material and of the three additional individuals detected with methods different from the collection of bear biological material. Genotypes marked with "?" need to be confirmed before being reliably considered actual individuals (see text for details).

Eleven out of 14 individuals (79%) have been detected also in other portions of the bear range while 3 individuals (21%) have been detected only in the MNP monitoring area (Table 28). The ones detected in other portions of the bear range fall in the following categories:

- Detected only occasionally in areas adjacent to MNP but stably present in the MNP monitoring area since more than 5 years: F1.99.

- Detected 2-3 years ago in areas between MNP and the Abruzzo, Lazio e Molise National Park and thus possibly "just arrived" from the source population: M1.120, M1.150, F1.129, F1.143, M1.176.

- Born in MNP, detected in other adjacent areas for 1-2 years and sampled the last time in MNP: M1.128 (cub of F1.99).

- Detected in MNP and other adjacent areas in the same time: M1.171, F1.172, M1.179, M1.197.

Table 28. Individuals present in the MNP monitoring area between 2020 and 2021 as compared to their presence in other portion of the bear range. The green rows highlight the three genotypes sampled only in the MNP monitoring area.

Individual	First detection in MNP	Detection in other areas	First detection in other areas	Last detection in MNP	Last detection in other areas
F1.99	2013	Yes	2012	2021	2020
M1.120	2020	Yes	2019	2021	2021
M1.128	2018	Yes	2018	2020	2019
M1.150	2021	Yes	2019	2021	2021
M1.171	2020	Yes	2020	2021	2021
F1.129	2021	Yes	2019	2021	2021
F1.143	2020	Yes	2019	2021	2021
F1.172	2020	Yes	2020	2021	2020
M1.176	2021	Yes	2020	2021	2021
M1.179	2021	Yes	2021	2021	2021
F1.196	2021	No	-	2021	-
M1.197	2021	No	-	2021	-
M1.198	2021	No	-	2021	-
M1.199	2021	No	-	2021	-

The three individuals only detected in MNP have all been sampled in 2021. The female F1.196 and the male M1.198 have been sampled in different sites of the south-western portion of the monitoring range while M1.199 has been sampled in the northern portion. F1.196 and M1.198 could thus be bears coming from the source population sampled for the first time in MNP just out of chance. M1.199 was sampled in the same area of M1.197 where in fall 2021 a video-trap recorded 3 videos of two bears associated having the same physical structure, thus possibly the same age. This datum suggests that M1.197 and M1.199 could be two brothers that, with high probability, were born in the MNP monitoring area.
Information obtained by the analysis of *if* and *where* individuals have been sampled before, led to the conclusion that new individuals are arriving from the source population and that, with high probability, new individuals are also being born in the MNP monitoring area. The genotypes sampled in the areas between MNP and the source population are individuals that made their first appearance in the bear recolonization range in 2019, meaning that a movement of several individuals has certainly been happening in the last 2-3 years. This particular datum is the last confirmation that the trends observed in the number of bio-signs and in the number of bears present is due to an actual increase of bear "migration" form the source population to the recolonization areas like MNP.

Presence of females with cubs

In 2020 and 2021 no females with cubs have been detected in the MNP monitoring area. The last detection of a family group is thus the one of the problematic female F1.99 which, in 2018, gave birth to 3 cubs. Considering that the number of females increased from 2019 to 2021, particular attention will be paid in the next years to detect any possible reproduction event.

Results of the different methods implemented

The above reported results on where bears are, how many bears are present and the presence of females with cubs come from the combination of results obtained with the different methods implemented. Even though the achievement of results through the implementation of different methods was the objective of Action A2, in order to understand the strengths and the weaknesses of the monitoring strategy, it is useful to give a quick look to results obtained for each method applied.
The research for bear bio-signs was implemented both applying the "passive" method of searching for them when bear presence events were reported, and the "active" methods consisting on the use of hair traps, field surveys and camera-traps. Out of 318 bio-signs collected in 2020 and 2021, 206 (65%) were found using the passive method and 112 (35%) were found using the active methods. Among bear bio-signs, 34 (39%) of the 88 bear biological material samples were collected using the passive method while 54 (61%) were collected using the active methods (Table 29).

Table 29. Number of bear bio-signs/year detected in the MNP monitoring area using the passive and active methods. In brackets the number of bear biological material samples is reported.

Year	Passive method (genetic samples)	Active methods (genetic samples)	Total (genetic samples)
2020	143 (18)	36 (26)	179 (44)
2021	63 (16)	76 (28)	139 (44)
Total	***206 (34)***	***112 (54)***	***318 (88)***

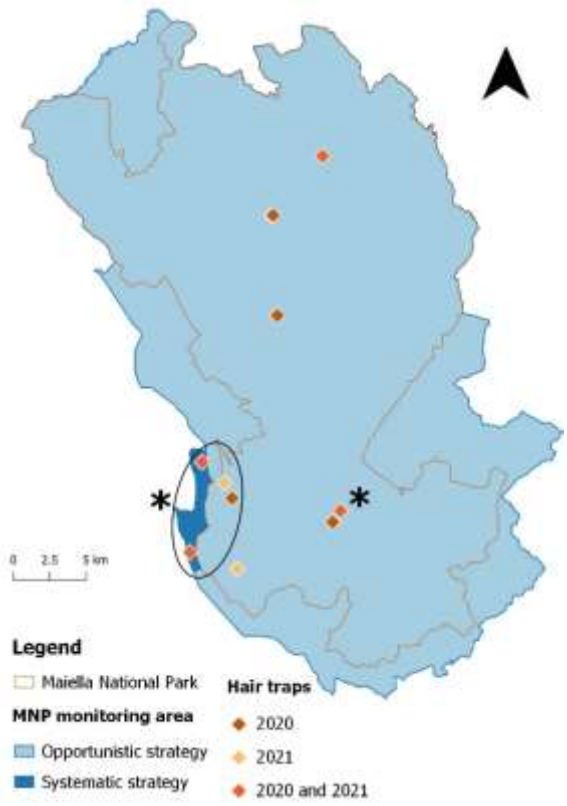
Considering only the "active" methods, results show that bear bio-signs have been collected for each method applied except for the field tracking surveys aimed at finding rub trees (Table 30).

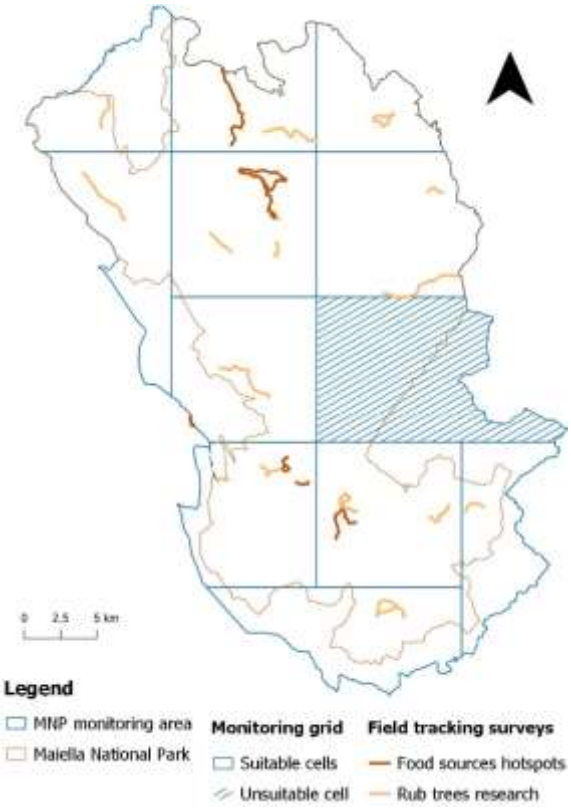
Table 30. Number of bear-bio-signs/year detected in the MNP monitoring area using only the "active" methods and their distribution among the different methods used.

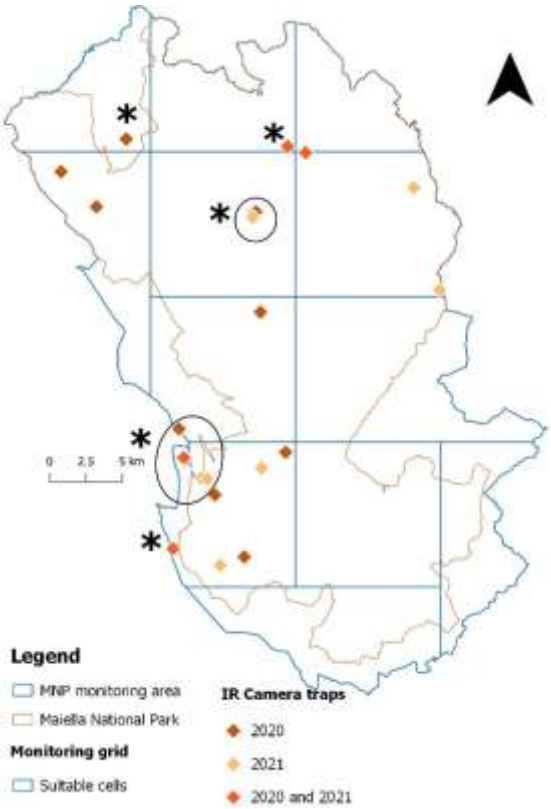
Year	Hair traps	Field tracking surveys (food sources hotspots)	Field tracking surveys (search for rub trees)	IR cameras operation	Observations	Total
2020	19	7	Not implemented	10	0	35
2021	7	34	0	34	1	76
Total	***26***	***41***	***0***	***44***	***1***	***112***

A total of 12 hair traps have been built in 2020-2021, 5 classic and 7 artificial rub trees. Six of them have been used only in 2020, 2 only in 2021 and 4 both in 2020 and 2021 for a total of 10 hair traps working during 2020 and 6 during 2021. In the stratum were a systematic sampling had to be implemented, 3 hair traps have been working in 2020 (2 artificial rub trees and 1 classic trap) and 2 in 2021 (both artificial rub trees). Six (50%) of the 12 traps had a positive outcome (Figure 21), 3 were classic hair traps and 3 were artificial rub trees.
In 2020-2021, 8 field tracking surveys have been implemented in areas of food sources concentration (type 1 surveys) while 16 surveys specifically aimed at natural rub trees individuation (type 2 surveys) have been implemented only in 2021 (Figure 21). At least one "Type 2" field tracking survey has been implemented for each of the 11 suitable cells of the monitoring grid while no surveys have been implemented in one cell where no suitable wooded areas (i.e. accomplishing the selection criteria reported in the method section) were

present (Figure 21). Five of the 8 “Type 1” (62%) surveys had a positive outcome while none of the surveys aimed at natural rub trees individuation had a positive outcome (Table 30).







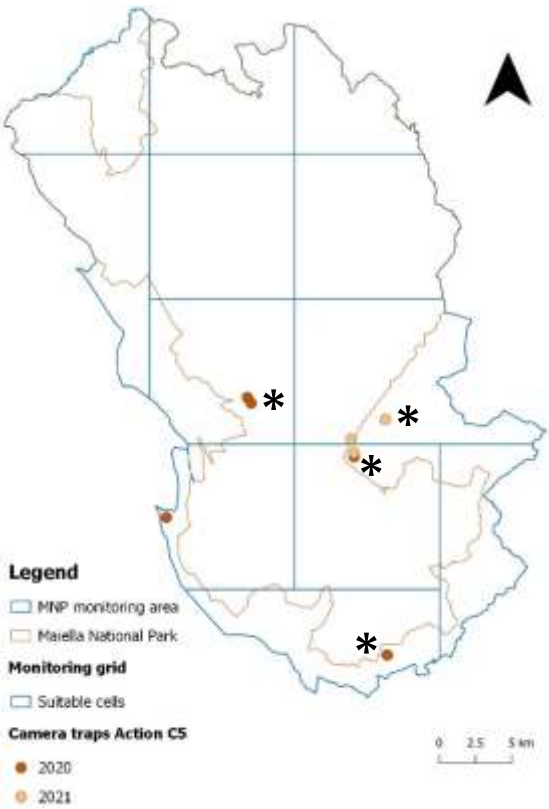


Figure 21. Results of the hair traps and camera traps positioning as well as field tracking surveys implementation in the MNP monitoring area in the frame of Action A2. Black asterisks refer to hair traps and camera traps that had a positive outcome, see text for details.

A total of 25 camera traps have been installed in 2020-2021 in 8 of the 12 monitoring cells (Figure 21). Four cells were excluded because of the high theft risk but, in the frame of Action C5, 5 additional camera traps have been positioned in 3 of the 4 missing cells (Figure 21). Seventeen camera traps have been working during 2020, 11 during 2021 and 5 during both 2020 and 2021 for a total of 22 camera traps working in 2020 and 18 in 2021. Twelve camera traps had a positive outcome leading to the collection of 44 bear presence recordings (corresponding to 199 videos of bears). All the videos recorded refer to single bears except for two videos recorded in the northern portion of the monitoring area where two bears associated have been filmed (see also § How many individuals are present and who they are).

Discussion

Results obtained with Action A2 implementation lead to two main considerations:

1. The combination of different methods and the mixed opportunistic-systematic strategy were both crucial to achieve action A2 objectives.
2. Some methods gave little if none results and need thus to be revised/improved for future development.

The usefulness of developing different monitoring strategies can be argued not only from the good results obtained, but also observing the distribution of bear bio-signs detected. The passive method led to the collection of more bio-signs than the active ones but the active methods allowed the collection of more bear biological material samples. The ratio bear biological material collected/total bio-signs is, in fact, 0.16 for the passive method against 0.45 of the active methods. While the passive method seems thus to be very important to collect bio-signs, active methods seem to be crucial to collect genetic samples which, in turn, are essential to assess the number and the sex of bears. The combination of these two different approaches will thus surely be continued in the next years.
Not all the active methods implemented, however, led to the best possible result. The main problems identified for each method are:

1. The hair traps only worked in the southern portion of the monitoring area.
2. Field tracking surveys aimed at searching for rub trees gave no results.
3. The majority of the camera traps that recorded videos with bears were positioned at a site of attraction (rub tree, cherry tree, apple tree, hair trap etc.).

The negative outcome associated with hair traps positioned in the northern portion of the monitoring area also happened before the starting of the LIFE ARCPROM and it can possibly be associated to a lower bear density. This negative result should thus not discourage from implementing the hair traps setting in this area, on the contrary this task should be continued in the next years in order to assess if something changes. The northern portion is, in fact, an area where bear density seems to have been augmenting in the last 2-3 years so that better and better results are expected to be achieved in the future.
The searching for rub trees through the implementation of *ad hoc* field surveys was a first in the MNP but still the total absence of results was an unexpected outcome. According to the literature, the areas surveyed are the ones with the highest probability of hosting rub trees whose presence should not be affected by the low bear density. However, the incidental

finding of a natural rub tree (during a camera trap monitoring activity) revealed how hard it is to recognize rub trees at first as hairs can be well hidden and visible only using the light of a torch. Additionally, in 2021 this activity was developed during summer while the best period to detect rub trees is the mating season when bears rub the most. For these reasons, and considering how powerful rub trees are to collect bear presence data, this task will be implemented again in 2022 during May-June and the length of the paths to cover will be adjusted considering that a lot of time is required to carefully inspect all the possible rub trees.

The fact that the majority of the camera traps that recorded videos with bears were positioned at a site of attraction implies that data collected so far with cannot be used to develop statistical analysis to assess bear distribution and density such as occupancy models or, let alone, Capture Mark Recapture models. We believe that the difficulty of filming bears with camera traps randomly positioned could also be related to the low bear density but, possibly, it is also due to the biases generated by the theft risk evaluation which affects camera-traps placement. Given the low probability to obtain good results and given that the positioning of camera-traps following a given sampling scheme also requires dedicated personnel, with high probability the positioning of camera traps will still be opportunistic in the next years. However, if results on the bear bio-signs distribution and on the number of individual will be like the ones obtained in 2021 or even better, the implementation of a different design will be considered.

CONCLUSIONS AND CONTINUATION OF THE ACTIVITIES

Greece

Genetic approach

Inbreeding/genetic diversity: Post analysis of our genetic data showed genetic diversity in all populations, high levels of inbreeding in Prespes and Rodopi and lower values of inbreeding in Pindos NP. Moreover, no signature of significant bottleneck was detected in any of our populations. The increased values of inbreeding in combination with the low Ne shows that the sub-populations of **Rodopi and Prespes** are more vulnerable compared to the population of Pindos.

Populations size and connectivity: The average population size was 191, 202 and 207 individuals for Prespes, Pindos and Rodopi NP respectively. Moreover, based on our genetic data we showed that our three populations were successfully distinguished in three clusters with a clear distinction between the eastern (Rodopi) and western (Pindos) population.

Towards this direction are the findings of Pylidis et al. (2021) which determined that the geographical populations of Peristeri (which is a part of the Prespa NP), Pindos, and Rodopi host distinct genetic demes. Based on our First values, the population of Prespes seems to show a tendency of breeding more with the population of Rodopi than with the population of Pindos, while the population of Rodopi seems to be genetically distinguished from Pindos. In brown bears a long-range displacement of males (up to 360 km) has been recorded (Bartoń et al. 2019).

This could mean that the distance which separates Prespes from Rodopi is well within the dispersal capacity of the species. Moreover, the mountainous terrain in Northern Greece may act as either as a limiting barrier or as a facilitating bridge for dispersal and communication between the sub-populations of Pindos and Prespes.

Regarding the continuation of the project's activities, it is clear that they have to be thoroughly implemented in the projects sub-areas and sector where the targeted species exposes more instability and vulnerability features and characteristics. By prioritizing this criterium is obvious that Rodopi Mountain Range National Park is at the highest rank followed by Prespa National Park.

IR camera trapping approach

The results showed that the highest levels of brown bear relative densities (ranging from 2.28 – 3.98 values) vary across the (3) project sub-areas . In terms of surfaces (in km^2) over each NP's total surface they range from 26% in the case Rodopi Mountain Range National Park , to 40% in the case of Northern Pindos National Park and up to 59% of total surface in the case of Prespa National Park. In all (3) cases the sectors with higher bear relative abundance appear to be adjacent, however this is not always the rule. The geographic identification of the sectors with higher relative bear abundance indexes will contribute in a spatially better orientated implementation of the CCA's in the frame of the project.

Italy

Results obtained with Action A2 implementation are crucial for the implementation of all the concrete Actions foreseen in the LIFE ARCPROM and, in general, for the implementation of the whole bear conservation strategy in MNP. The number of bears detected in 2021 correspond to more than 25% of the estimated bear population and is the highest number ever recorded for MNP. In the same time, data concerning the distribution of bio-signs and the number of females point to fact that in the last two years bear presence in MNP changed significantly. Data collected in 2020 and 2021 with Action A2 allowed to understand the current "re-colonization" dynamic thus providing crucial information to implement the needed pro-active strategy. If before 2020 some areas of the Park could be still considered "without bears", thus not vulnerable to bear damages or any other possible source of human-bear conflicts, data collected with Action A2 clearly indicate that the whole MNP monitoring area should now be included in all the conservation actions implemented. Additionally, the fact that the minimum number of bears doubled from 2019 to 2020 and again from 2020 to 2021 also suggests that possible human-bear conflicts are more and more likely to happen given the augmented bear density in the area. Even though the number of bears could drop in the immediate future as a consequence of the normal alternation of peaks and hollows in a re-colonization process, still the trend is toward an augmented bear presence in the mid/long term which requires MNP (and people living in the territory) to be prepared to handle all the possible human-bear interactions. However, bear presence in MNP is strongly dependent not only from variables tied to the Park itself but also from the situation of the source population which needs to be healthy to act as a good source of individuals. Given the uncertainty in what is going to happen in the short term in MNP, and given the need to act in a pro-active way, all the activities implemented in the frame of Action A2 need to be implemented on a yearly basis. The monitoring activity will thus be continued in the next years in MNP, keeping the systematic-opportunistic strategy as the general method to apply but, possibly, improving the efficacy of each single method. The research for rub trees, for example, will surely be repeated but it will be implemented during the mating season in order to maximize the probability to actually find marked trees. Monitoring methods will thus be evaluated on a yearly basis in order to implement every year a better and better strategy in term of cost/benefit ratio.
Regarding the genetic analysis, we foresee to have some little improvement as well in the next years. In this report we saw that in Italy it was impossible to develop detailed genetic analyses. In fact, while in Greece genetic robustness and variability has been assessed basing on genetic samples collected, in Italy this was not possible due to the high levels of inbreeding and the low genetic variability. The most recent analysis of Apennine brown bear genome (Benazzo et al. 2017) reveals that there is no variation at all in the mitochondrial genome and that the nuclear genome has long stretches with none (or almost none) variation alternated with levels of variation similar to other bears, a situation possibly caused by the inbreeding. In fact, the estimated inbreeding coverage *F* for the Apennine brown bear (ABB) reveals that individuals are highly inbred: its value is between 0.69 and 0.77 with the Spanish bear as the next most inbred (F =0.57) while all other brown bears analyzed in the study show F values < 0.29 (Benazzo et al. 2017). However, in 2019-2020 MNP, together with other members of the Apennine Brown Bear Monitoring Network (RMAM), financed a specific study aimed at investigating at least the possibility to apply the SNPs technique to assess kinship among Apennine brown bear individuals. The study, carried out by ISPRA, the Italian reference laboratory for ABB genetic analyses, had promising results opening the possibility to use SNPs to at least test some specific kinship hypothesis but the work is still ongoing thus impeding the inclusion of kinship analysis in the frame of Action A2.

Basing on the final results of the above mentioned study, in the next years MNP together with all the other bodies involved in ABB monitoring, will thus possibly improve the number and type of information gathered from the analyses of bear biological material.

BIBLIOGRAPHY

Arrendal J, Vilà C, Björklund M. 2007. Reliability of noninvasive genetic census of otters compared to field censuses. Conserv Genet . 2007 Sep ;8(5):1097–107.

Bartoń KA, Zwijacz-Kozica T, Zięba F, Sergiel A, Selva N. 2019. Bears without borders: Long-distance movement in human-dominated landscapes. Glob Ecol Conserv. 2019 Jan 1;17:e00541.

Barton, K. 2020. MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn.

Belant JL. A. 2003. Hair snare for forest carnivores. Wildl Soc Bull. 2003;31:482–5.

Bellemain E and Taberlet P. 2004. Improved noninvasive genotyping method: application to brown bear (Ursus arctos) faeces. Mol Ecol Notes . 2004 Sep 1 ;4(3):519–22.

Benazzo A., Trucchi E., Cahil J.A., (24 more authors). 2017.Survival and divergence in a small group: The extrahordinary genomic history of the endangered Apennine brown bear stragglers. Proceedings of the National Academy of Siences 114(45): E9589-E9597.

Blankenheim, D. 2018. A camera trapping study about the seasonal habitat use and circadian activity of brown bears (Ursus arctos) regarding the human factor in northern Pindos, Greece. MSc thesis, University of Natural Resources and Life Sciences, Vienna.

Boitani L. and R. A. Powell (2013) Carnivore Ecology and Conservation: A Handbook of Techniques, Published to Oxford Scholarship Online: December 2013, Print ISBN-13: 9780199558520 - DOI:10.1093/acprof:oso/9780199558520.001.0001.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet . 1980 ;32(3):314.

Boulanger J, McLellan B. 2001. Closure violation in DNA-based mark-recapture estimation of grizzly bear populations. Can J Zool . 2001 ;79(4):642–51.

Boulanger J, Mclellan BN, Woods JG, Proctor MF. 2004. Sampling design and bias in dna-based capture-mark-recapture population and density estimates of grizzly bears. J Wildl Manage. 2004;68(3):457–69.

Camarra J.J.1982. Typologie des indices de presence ursine. Rapport Technique, Office National de la Chasse. (ONC - SNPN), 22pp (inedit).

Ciucci P., Altea T., Antonucci A., (13 more authors). 2017. Distribution of the brown bear (*Ursus arctos marsicanus*) in the Central Apennines, Italy, 2005-2014. Hystrix It. J. Mamm. 2017; 28(1): 86–91.

Ciucci P., Gervasi V., Boulanjer J., Altea T., Boitani L., Gentile D., Sulli C., Tosoni E. 2015. Ex post non invasive survey of the core Apennine bear population (*Ursus arctos marsicanus*) in 2014. Action E5 Project LIFE09 NAT/IT/160 ARCTOS.

Clapham M., Nevin O.T., Ramsay A.D., Rosell F., 2013. The function of strategic tree selectivity in the chemical signalling of brown bears. Animal Behaviour 85(6): 1351—1357.

Denes, F. V., Silveira, L. F., Beissinger, S. R. 2015. Estimating abundance of unmarked animal populations: accounting for imperfect detection and other sources of zero inflation. Methods in Ecology and Evolution, 6(5), 543-556.

Esri Inc. 2020. ArcMap (Version 10.7.1). Esri Inc. https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol . 2005 Jul ;14(8):2611–20.

Fiske, I., Chandler R. 2011. unmarked: An R Package for Fitting Hierarchical Models of Wildlife Occurrence and Abundance. Journal of Statistical Software, 43(10), 1-23. URL http://www.jstatsoft.org/v43/i10/.

González-Bernardo E., Bagnasco C., Bombieri G., (9 more authors). 2021. Rubbing behaviour of European brown bears: factors affecting rub tree selectivity and density. Journal of Mammalogy 102(2): 468-480.

Green GI & Mattson DJ. 2003. Tree rubbing by Yellowstone grizzly bears Ursus arctos. Wildlife Biol . 2003 Mar 1 ;9(1):1–9.

Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, et al. 2011. Current trends in microsatellite genotyping. Mol Ecol Resour . 2011 Jul ;11(4):591–611. Available from: https://pubmed.ncbi.nlm.nih.gov/21565126/.

Harris, R.B. 1986. Reliability of trend lines obtained from variable counts. - Journal of Wildlife Management 50: 165–171. Google Scholar.

Kaczensky P, Chapron G, von Arx M, Huber D, Andrén H, Linnell J, et al. 2013. Status, management and distribution of large carnivores-bear, lynx, wolf & wolverine-in Europe. DOI: 10.13140/RG.2.2.11382.88645.

Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol . 2007 Mar ;16(5):1099–106.

Karamanlidis AA, De Gabriel Hernando M, Krambokoukis L, Gimenez O. 2015. Evidence of a large carnivore population recovery: Counting bears in Greece. J Nat Conserv . 2015 ;27:10–7.

Karamanlidis AA, Drosopoulou E, de Hernando MG, Georgiadis L, Krambokoukis L, Pllaha S, et al. 2010. Noninvasive genetic studies of brown bears using power poles. Eur J Wildl Res. 2010 Feb 23 ;56(5):693–702.

Karamanlidis AA, Skrbinšek T, De Gabriel Hernando M, Krambokoukis L, Munoz-Fuentes V, Bailey Z, et al. 2018. History-driven population structure and asymmetric gene flow in a recovering large carnivore at the rear-edge of its European range. Heredity (Edinb) . 2018 Dec 11 ;120(2):168–82.

Karamanlidis AA, Skrbinšek T, de Gabriel Hernando M, Krambokoukis L, Munoz-Fuentes V, Bailey Z, Nowak C, Stronen AV. 2018. History-driven population structure and assymetric gene flow in a recovering large carnivore at the rear-edge of its European range. Heredity 120:168-182.

Karamanlidis AA, Youlatos D, Sgardelis S, Scouras Z. 2007. Using sign at power poles to document presence of bears in Greece. Ursus. 2007;18(1):54–61.

Karamanlidis AA. 2011. Genetic estimation of bear population in Greece. Final Report in Greek; Arcturos. Hell Minist Rural Dev Food Hell Minist Environ Energy Clim Chang. 2011.

Kelly MJ, Betsch J, Wultsch C, Mesa B, Mills LS. 2012. Noninvasive sampling for carnivores. Carniv Ecol Conserv. 2012 Dec 18;47–69.

Kendall WL. 1999. Robustness of Closed Capture-Recapture Methods to Violations of the Closure Assumption. Ecology. 1999 Dec;80(8):2517.

Kyriakidis, S.I. 2021. Mapping brown bear (Ursus arctos) habitat preference from camera trapping and telemetry data: implications for wind farm development in Vasilitsa mountain, Greece. MSc thesis, Linköping University, Sweden.

McKelvey KS, Schwartz MK. 2005. DROPOUT: A program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Mol Ecol Notes . 2005 Sep ;5(3):716–8.

McLoughlin, P. D., Case, R. L., Gau, R. J., Cluff, D. H., Mulders, R., Messier, F. 2002. Hierarchical habitat selection by barren-ground grizzly bears in the central Canadian Arctic. Oecologia, 132(1), 102-108.

Mertzanis G, Giannakopoulos A, Pylidis C. 2009. Status of the brown bear Ursus arctos (Linnaeus, 1758) in Greece. In: Legakis A, Maragou P, editors. Red data book of threatened vertebrates of Greece. Hellenic Zoological Society; 2009. p. 385–7.

Mertzanis G, Isaak I, Mavridis Avr., Nikolaou Ol., Riegler S. , Riegler Ar., Tragos Ath. 2005. Movements, activity patterns and home range of the brown bear (Ursus arctos, L .) in Rodopi mountain range, Greece. Belg. J. of Zool., 135(2): 217-221.

Mertzanis G. 2018. Assessment of the distribution, population size and activity of the brown bear (Ursus arctos, L.) subpopulation with the use of IR cameras, genetic analyses and

biosigns in the area of Amyndaio, & Florina – NW Greece. Technical Report A2. Thessaloniki; 2018.

Mertzanis G., Psaroudas Sp., Karamanlidis AA (CALLISTO / ARCTUROS). 2021. LIFE-IP 4 NATURA: Integrated actions for the conservation and management of Natura 2000 network areas, species, habitats and ecosystems in Greece - Action Plan for the brown bear (Ursus arctos), Ministry of Environment and Energy. Athens, p. 146.

Mertzanis, Y., Kallimanis, A., S., Kanellopoulos, M., Sgardelis, S., P., Tragos A., Aravidis I. 2008. Brown bear (Ursus arctos) habitat use patterns in two regions of northern Pindos, Greece – management implications, Journal of Natural History, 42:5-8, pp. 301-315.

Mertzanis, Y., Karaiskou, N., Petridou, M., Patronidis, P., Psaralexi, M., Blankenheim, D., 2018. Estimation of the presence, population size and activity of the brown bear using photo traps, genetics and biomarkers in the area of Florina. Technical report, project LIFE15NAT / GR / 001108, (action A2), 86 pages.

Miller CR, Joyce P, Waits LP. 2005. A new method for estimating the size of small populations from genetic mark-recapture data. Mol Ecol . 2005 Jun ;14(7):1991–2005.

Moeller, A. K., Lukacs, P. M., Horne, J. S. 2018. Three novel methods to estimate abundance of unmarked animals using remote cameras. Ecosphere, 9 (8), e02331.

Muhly, T. B., Semeniuk, C., Massolo, A., Hickman, L., Musiani, M. 2011. Human Activity Helps Prey Win the Predator-Prey Space Race. PLOS ONE 6 (3): e17050.

O'Brien, T. G. 2011. Abundance, density and relative abundance: a conceptual framework. In *Camera traps in animal ecology* (pp. 71-96). Springer, Tokyo.

O'Connell AF, Nichols JD, Karanth KU. 2010. Camera traps in animal ecology: Methods and analyses. Camera Traps Anim Ecol Methods Anal.1–271.

Oberosler, V., Groff, C., Iemma, A., Pedrini, P., Rovero, F. 2017. The influence of human disturbance on occupancy and activity patterns of mammals in the Italian Alps from systematic camera trapping. *Mammalian Biology*, *87*(1), 50-61.

O'Brien, T. G., Kinnaird, M. F. Wibisono, H. T. 2003. Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. Anim Cons, 6: 131–139.

Paetkau D, Strobeck C. Ecological genetic studies of bears using microsatellite analysis. 1998. Ursus 10: 299–306.

Pagès M, Maudet C, Bellemain E, Taberlet P, Hughes S, Hänni C. 2009. A system for sex determination from degraded DNA: a useful tool for palaeogenetics and conservation genetics of ursids. Conserv Genet . 2009 Aug ;4(10):897–907.

Papamichael, G., Arapis, T., Petkidi, K., Fytou I., and Hatzirvasanis V.2015. Deliverable 8, C - Phase of Study 7: "Monitoring and evaluation of conservation status mammal species of Community interest in Greece." Ministry of Environment, Energy and Climatic Change, Athens, scholars Partnership and consultancy firms' ARAPIS THOMAS EYAGGELOY, GEOANALYSI S.A. and PAPACHARISI ALEXANDRA THEODORA ", Athens. pp. 47.

Peel D, Peel S, Ovenden JR.2003. NeEstimator: Software for Estimating Effective Population Size. Molecular Fisheries Laboratory, Department of Primary Industries; 2003.

Pennell MW, Stansbury CR, Waits LP, Miller CR. 2013. Capwire: a R package for estimating population census size from non-invasive genetic sampling. Mol Ecol Resour . 2013 Jan;13(1):154–7.

Piggott MP, Taylor AC. 2003. Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. Wildl Res . 2003 ;30(1):1–13.

Piry S, Luikart G, Cornuet JM. 1999. Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. J Hered . 1999 Jul 1 ;90(4):502–3.

Poole KG, Mowat G, Fear DA. DNA-based population estimate for grizzly bears Ursus arctos in northeastern British Columbia, Canada. https://doi.org/102981/wlb2001014 . 2001 Jun 1 ;7(3):105–15.

Posillico M., Al. Meriggi, El. Pagnin, S. Lovari, L. Russo .2004. A habitat model for brown bear conservation and land use planning in the central Apennines. Biological Conservation 118 (2004) 141–150.

Prez T, Naves J, Vzquez JF, Seijas J, Corao A, Albornoz J, et al. 2010. Evidence for improved connectivity between Cantabrian brown bear subpopulations. Ursus. 2010 Jan 1;21(1):104–8.

Puechmaille SJ, Petit EJ. 2007. Empirical evaluation of non-invasive capture–mark–recapture estimation of population size based on a single sampling session. J Appl Ecol . 2007 Aug 1 ;44(4):843–52.

Pylidis C, Anijalg P, Saarma U, Dawson DA, Karaiskou N, Butlin R, et al. 2021. Multisource noninvasive genetics of brown bears (Ursus arctos) in Greece reveals a highly structured population and a new matrilineal contact zone in southern Europe. Ecol Evol . 2021 Jun 1 ;11(11):6427–43.

Pylidis, C., Mertzanis, Y., Anijalg, P., Saarma, U., Giannakopoulos, A., Iliopoulos, Y., Krupa, A., Harris, S., & Burke, T. A. 2015. The genetics of the brown bear (Ursus arctos) in Greece: summarizing a comprehensive study. In A. A. Karamanlidis (Ed.), 23rd International Conference on Bear Research and Management "Bears and humans in the 21st century: challenges and solutions for a peaceful coexistence". pp. 181.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rondinini C, Battistoni A., Peronace V., Teofili C. (compilatori). 2013. Lista Rossa IUCN dei Vertebrati Italiani. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma.

Rousset F. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour . 2008 Jan ;8(1):103–6.

Royle, J.A., Nichols, J.D., 2003. Estimating abundance from repeated presence – absence data or point counts. Ecology 84, 777–790.

Sawaya MA, Stetz JB, Clevenger AP, Gibeau ML, Kalinowski ST. 2012. Estimating Grizzly and Black Bear Population Abundance and Trend in Banff National Park Using Noninvasive Genetic Sampling. PLoS One . 2012 May 2 ;7(5):e34777.

Scarpulla E, Boattini A, Cozzo M, Giangregorio P, Ciucci P, Mucci N, et al. First core microsatellite panel identification in Apennine brown bears (Ursus arctos marsicanus): a collaborative approach. BMC Genomics . 2021 Dec 1 ;22(1):1–18.

Schwartz MK, Luikart G, Waples RS. 2006. Genetic monitoring as a promising tool for conservation and management. Trends Ecol Evol . 2006 Jan ;22(1):25–33.

Sollmann, R., Mohamed, A., Samejima, H., Wilting, A. 2013. Risky business or simple solution–Relative abundance indices from camera-trapping. *Biological Conservation*, *159*, 405-412.

Taberlet P, Camarra JJ, Griffin S, Uhrès E, Hanotte O, Waits LP, et al. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Mol Ecol . 1997 Sep 1 ; 6(9):869–76.

Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, et al.1996. Reliable Genotyping of Samples with Very Low DNA Quantities Using PCR. Nucleic Acids Res . 1996 Aug 1 ;24(16):3189–94.

Tattoni C., Bragalanti N., Groff C., Rovero F. 2015. Patterns in the use of rub trees by the Eurasian brown bear. Hystrix It. J. Mamm. 2015; 26(2): 118–124.

Tessier C, David J, This P, Boursiquot JM, Charrier A. 1999. Optimization of the choice of molecular markers for varietal identification in Vitis vinifera L. Theor Appl Genet . 1999 ;98(1):171–7.

Trolliet F., M.-C Huynen, C.Vermeulen, Al. Hambuckers. 2014. Use of camera traps for wildlife studies. A review. Biotechnol. Agron. Soc. Environ. 2014 18(3), 446-454.

Tsaparis D, Karaiskou N, Mertzanis Y, Triantafyllidis A. 2015. Non-invasive genetic study and population monitoring of the brown bear (Ursus arctos) (Mammalia: Ursidae) in Kastoria region – Greece. J Nat Hist . 2015 Feb 24 ;49(5–8):393–410.

Valière N. gimlet: a computer program for analysing genetic individual identification data. 2002. Mol Ecol Notes . 2002 Sep 1 ;2(3):377–9.

Waits LP & Paetkau D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J Wildl Manage. 2005 Oct 1;69(4):1419–33.

Wei, T., & Simko, V. 2017. R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). Available from https://github.com/taiyun/corrplot.

Weir BS, Cockerham CC. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution (N Y). 1984 Nov;38(6):1358.