Protocole de suivi du projet de réintroduction de la Cistude *Emys orbicularis* aux Réserves Naturelles Nationales de l'Estagnol et du Bagnas (Languedoc-Roussillon)

Document préparé par Albert Bertolero
Laboratoire de Biogéographie et Ecologie des Vertébrés EPHE

Document préparé pour le Conservatoire des Espaces Naturels Languedoc-Roussillon

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Introduction

Le travail de suivi doit commencer à partir du moment où les cistudes ont été lâchées des enclos d'acclimatation, car sans suivi la détermination du succès ou de l'échec du programme de réintroduction s'avère impossible.

Comme le projet de réintroduction est réalisé simultanément dans les Réserves Naturelles Nationales de l'Estagnol et du Bagnas les informations seront développées de façon générale pour les deux réserves.

1.- Planning du processus de lâcher

A partir des informations du document initial du projet de réintroduction de la Cistude aux RNN de l'Estagnol et du Bagnas le planning du processus d'acclimatation et de lâcher est résumé à la figure 1. Il est possible aussi de prélever dans la nature quelques adultes de plus et de les lâcher selon ce schéma les prochaines années. D'un autre côté, le nombre d'œufs qui devra être prélevé dans la nature la prochaine année est d'environ 80 (Figure 1). Néanmoins, il faudra encore déterminer si des prélèvements supplémentaires seront nécessaires pour les années suivantes.

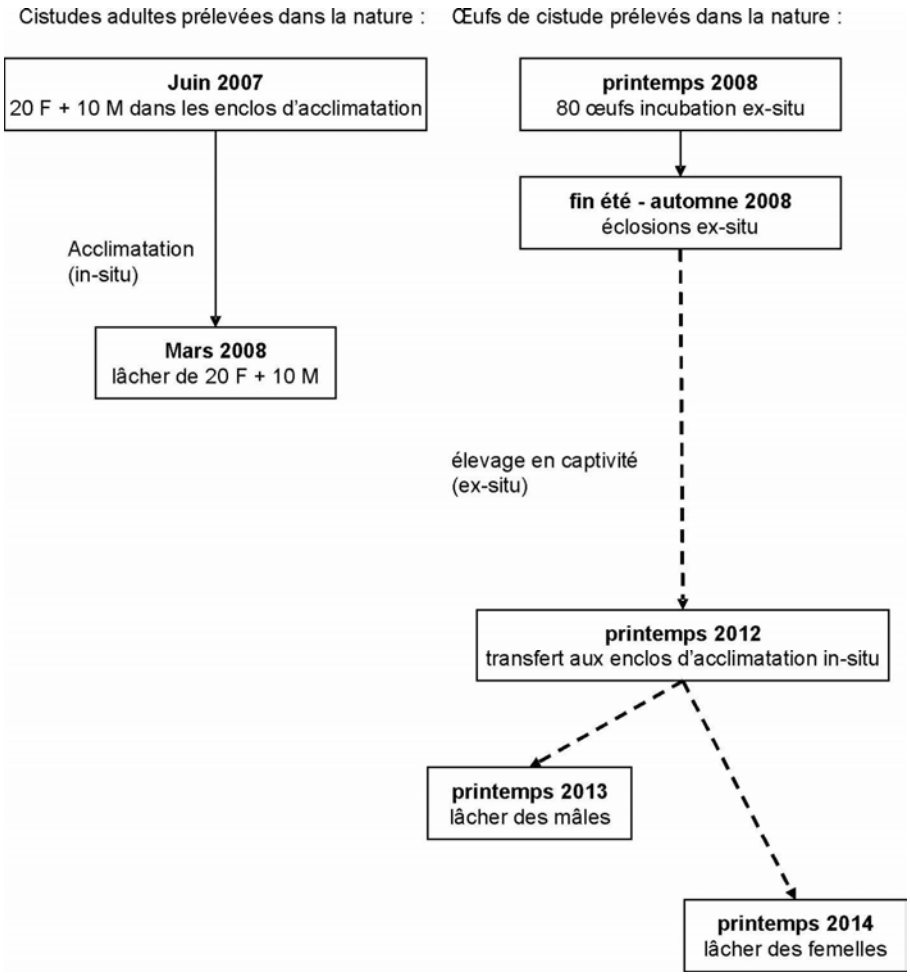

Figure 1 : Planning du processus de lâcher selon le document de réintroduction.
M = mâles ; F = femelles.

2.- Considérations avant le lâcher (dans les enclos d'acclimatation)

Les cistudes seront marquées par des encoches à la carapace lors de leur entrée initiale à l'enclos d'acclimatation (juin 2007, ainsi que chaque fois que des nouvelles cistudes rentreront dans les enclos d'acclimatation). Aussi elles pourront être marquées avec des transpondeurs placés en sous-cutané. Au Delta de l'Ebre on place le transpondeur à la base du cou, tandis que dans d'autres études on propose la mise dans le membre postérieur droit (Cadi et Faverot, 2004).

Il doit avoir une fiche des marques utilisées où doivent apparaître la date de marquage, l'origine de la cistude, le sexe, l'âge, la longueur de la carapace, le poids, si le nombre des écailles est correct et les observations (exemple de fiche : annexe 1).

L'âge peut être réel, si on connaît l'année de naissance (cistude issue de l'élevage en captivité ou moins de 10 stries de croissance). Sinon, l'âge peut être classé par catégories selon les critères suivants :

1) *Juvénile* : sexe pas reconnaissable à partir des caractères sexuels secondaires. Normalement moins de trois stries de croissance principales.
2) *Subadulte* : sexe reconnaissable à partir des caractères sexuels secondaires et toutes les stries de croissances larges (Figure 2).
3) *Adulte* : sexe reconnaissable à partir des caractères sexuels secondaires, deux types de stries de croissance (stries larges et stries fines) ou pas de stries de croissance discernables à la carapace (Figure 2).

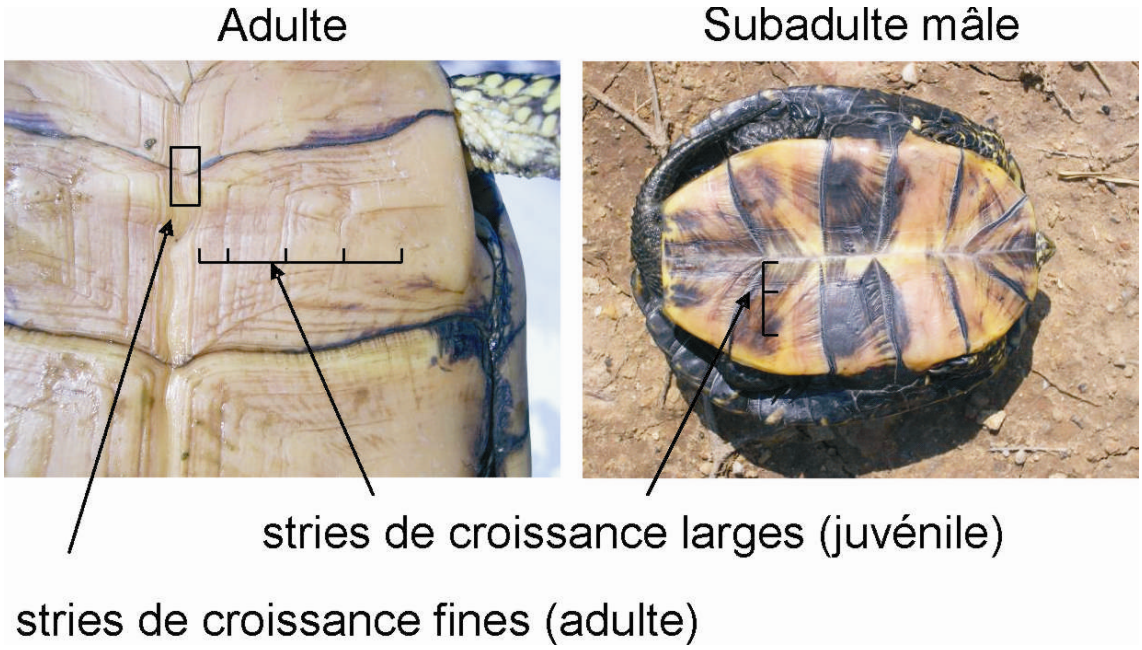

Figure 2. Exemples de plastron des cistudes pour déterminer la catégorie d'âge. A) Adulte : stries de croissance larges (croissance juvénile) et fines (croissance adulte). B) Subadulte : uniquement stries de croissance larges. Cistudes sauvages de la population du delta de l'Ebre (photos : A. Bertolero).

Si le nombre d'écailles est normal on notera sur la fiche « 1 » ; s'il n'est pas normal on notera « 0 » (Figure 3) ; et, dans les observations, quelle est la malformation d'écaillure présente (p.ex. « seulement 10 écailles marginales au côté droit » ou « 6 écailles vertébrales »). De cette façon on disposera d'un caractère supplémentaire d'identification pour certains individus.

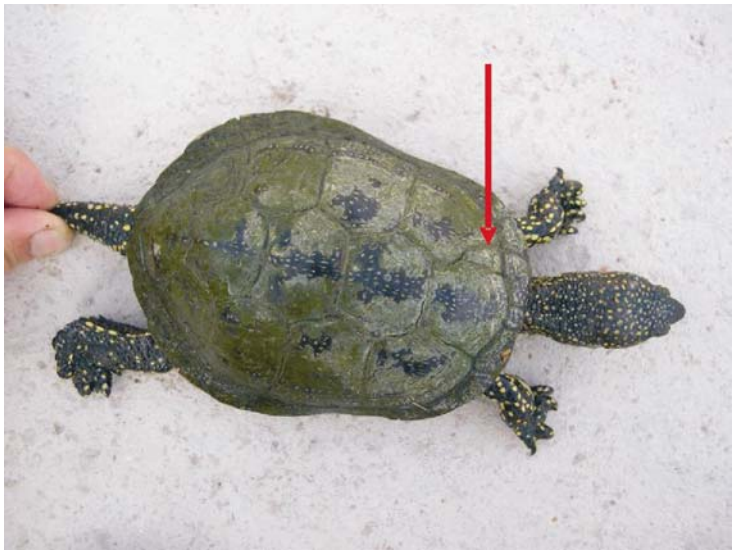

Figure 3. Exemple de malformation dans le nombre d'écailles. La flèche marque une petite plaque supplémentaire entre la première écaille vertébrale et la première écaille costale gauche. Cistude sauvage de la population du delta de l'Ebre (photo : A. Bertolero).

Dans les observations il est important aussi de registrer les possibles blessures que présentent les cistudes. De cette façon, on peu suivre l'évolution des blessures (si elles guérissent) et les utiliser comme caractère supplémentaire d'identification (Figure 4).

Figure 4. Carapace d'une cistude avec des blessures occasionnées par les morsures des rats. Cistude sauvage de la population du delta de l'Ebre (photo A. Bertolero).

3.- Considérations lors du lâcher des adultes (prélevés dans la nature) et des subadultes (issus de l'élevage en captivité)

Le jour du lâcher toutes les cistudes devront être examinées pour déterminer leur état de santé. Il faudra vérifier le marquage avec les encoches (et transpondeurs), prendre les mesures corporelles et photographier les individus selon le protocole exposé à continuation. Toutes les données seront rassemblées sur une fiche des observations de terrain (annexe 2) et entrées postérieurement dans une base de données.

Les données biométriques minimales à prendre sont marquées par un * ; les autres sont optionnelles (Figure 5) :

1) LC* : longueur de la carapace (mm) ;
2) LP : longueur du plastron (mm) ;
3) LMC : largeur maximale de la carapace, entre les plaques marginales 7 et 8 (mm) ;
4) HC : hauteur maximale de la carapace (mm) ;
5) LPC : longueur plastron-cloaque (mm) ;
6) P* : poids (g).

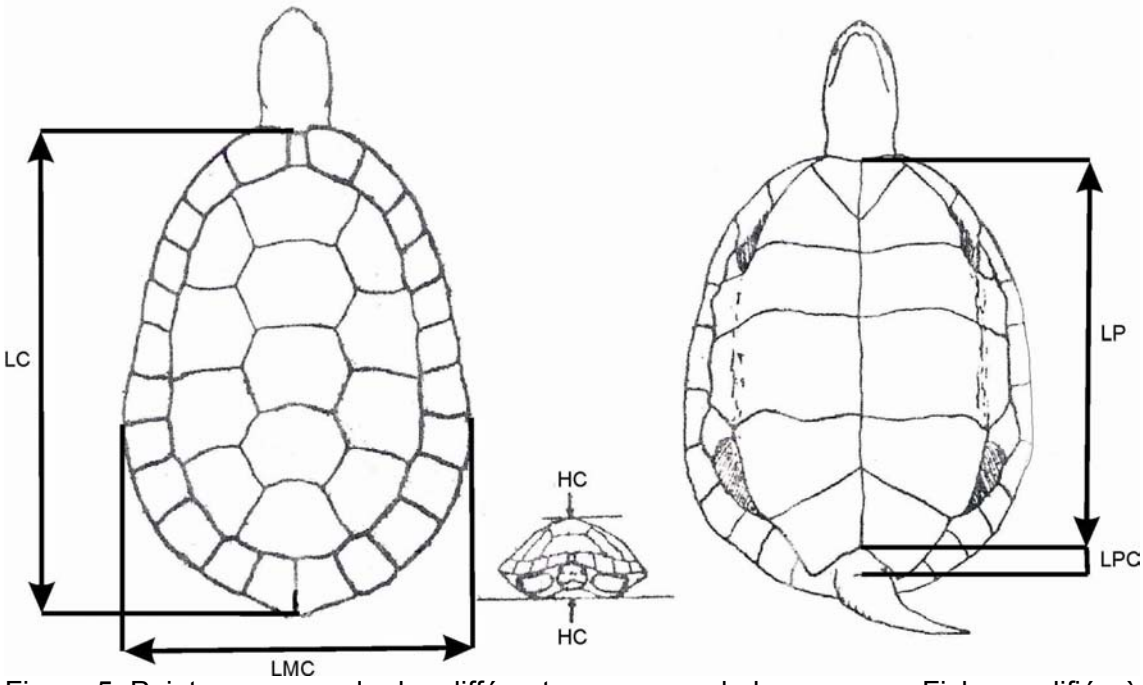

Figure 5. Points pour prendre les différentes mesures de la carapace. Fiche modifiée à partir de la fiche de terrain d'Eduard Filella.

Les données de la longueur, de la largeur et de la hauteur seront prises avec un pied à coulisse (de préférence digital) et le poids, avec une balance de précision (ou des dynamomètres).

Les photographies digitales devront être prises en vue dorsale et abdominale, sur fond de couleur uniforme (Figure 6). Pour identifier la cistude on devra écrire directement sur la carapace le nombre d'identification de la tortue avec un feutre ou bien sur un papier à côté (Figure 6). Les photographies devront être stockées de façon à être facilement retrouvables (p.ex. Cistude/Dossier photo/Dossier Localité/ Dossier date jour de lâcher ou date jour de terrain).

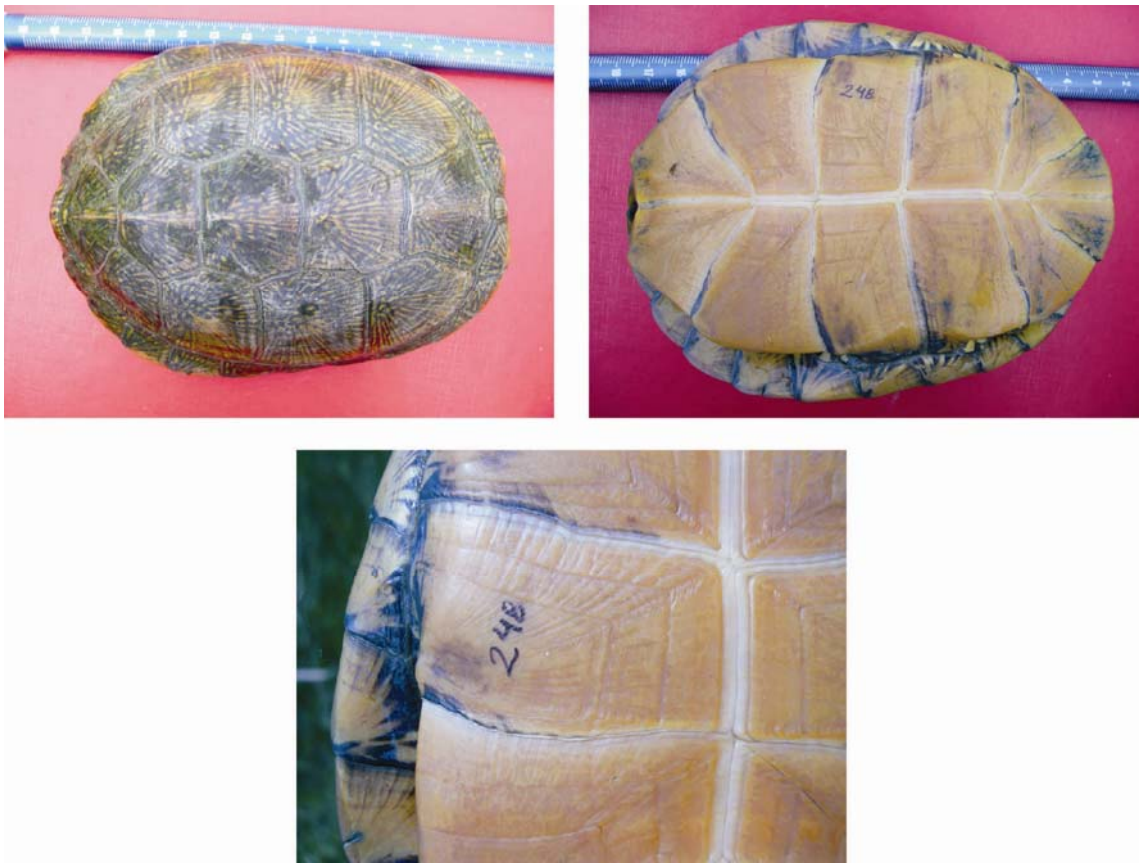

Figure 6. Exemples de photographies pour l'identification des cistudes. Sur le plastron on indique le numéro d'identification de la cistude (nº 248). Le fond contraste clairement avec la cistude. Cistude sauvage de la population du delta de l'Ebre (photos : A. Bertolero).

4.- Suivi des individus lâchés et nés en liberté

Après que les cistudes sont lâchées, commence leur suivi selon deux méthodes complémentaires : le radiopistage et le piégeage.

Le radiopistage permet :
1) de savoir si les cistudes restent fixées dans le site (si l'acclimatation est réussie) ;
2) d'obtenir des taux de survie pendant la première année (s'il y a un coût de lâcher) ;
3) de connaître les mouvements et estimer les domaines vitaux ;
4) de faire un suivi de la reproduction (localisation des sites de pontes) ;
5) de déterminer la sélection d'habitats pendant la période d'activité et l'hivernation.

Le piégeage permet :
1) d'obtenir des taux de survie à long terme (plusieurs années) ;
2) de faire un suivi des données biométriques (croissance, condition corporelle).
3) de connaître les mouvements et estimations des domaines vitaux (avec moins de précision que le radiopistage) ;
4) de faire un suivi de la reproduction par palpation des femelles.

4.1.- Radiopistage

4.1.1.- Nombre de cistudes à suivre

Pendant la première année (printemps 2008, lâcher des cistudes prélevées dans la nature l'année 2007), la situation idéale, c'est d'équiper toutes les cistudes lâchées avec des émetteurs (voir devis approximatif annexe 3). Si cela n'est pas possible à cause de contraintes budgétaires, la priorité, c'est d'équiper toutes les femelles (20 femelles par site). En tout cas, le nombre minimum de femelles à équiper doit être de 10 (50 % des femelles lâchées).
Quant aux tortues nées en captivité, les premiers lâchers sont prévus pour les années 2013 et 2014 (cistudes âgées de 5 et 6 ans), selon le planning initial (Figure 1). Comme dans la situation précédente, l'idéal, c'est d'équiper toutes les cistudes lâchées avec des émetteurs, et, si cela n'est pas possible, au moins le 50 % des cistudes lâchées (il faut équiper prioritairement les femelles).

4.1.2.- Caractéristiques des émetteurs

Même si pour un suivi dans un milieu aquatique il est recommandé d'utiliser des fréquences basses (p.e. entre 40 – 104 MHz pour des suivis des poissons ; Kenward, 1987), les fréquences les plus habituelles pour des suivis de faune sauvage se trouvent entre 138 – 174 MHz (Kenward, 1987). A titre d'exemple, les fréquences utilisées en France pour les suivis des cistudes ont été : en Isère, fréquences entre 148 – 149 MHz (Thienpont, 2005) ; au Lac du Bourget, entre 148 – 150 MHz (Emilie, 2002) ; et en Corse, 150 – 151 MHz (Levadoux, 2004).

En général, il est recommandé que le poids des émetteurs (plus la fixation) représente moins du 5% du poids de l'animal. Dans le cas des Cistudes adultes (400 – 700 g), on peut utiliser des émetteurs de jusqu'à 20 - 30 g, et avec une durée minimum de 18 mois. Cette durée supérieure à un an est très importante pour déterminer les premiers résultats de la réintroduction. Pour la fixation des émetteurs à la carapace il y a au moins deux possibilités : 1) coller les émetteurs directement sur la carapace avec une résine époxy (p.ex. PC-7 Protective Coating Co., Allentown, Pennsylvania, USA; Belzer & Reese,

1995 ; Boarman et al., 1998 ; Wilson et al., 2003 [copie des articles à l'annexe 3]) ; 2) les fixer avec des boulons sur les plaques marginales (p.ex. suivis au lac de Bourget et en Corse).

4.1.3.- Caractéristiques générales du suivi

Pour localiser les cistudes il faudra un récepteur (voir devis approximatif annexe 3) avec une antenne Yagi (antenne à trois éléments ; prévoir un câble de connexion de 4 – 6 m et un mât pliant de 3 – 4 m de hauteur pour faciliter la réception des signaux), un GPS et une boussole. Ces deux derniers permettront de déterminer chaque localisation par triangulation. Normalement pour déterminer la position d'une cistude avec une seule personne il faudra :

a) localiser la cistude avec le récepteur ;
b) déterminer avec le GPS la position où se trouve l'observateur avec le récepteur ;
c) déterminer la direction du signal avec la boussole ;
d) changer de position de façon rapide (± 5 – 10 minutes) et rétablir le contact avec la même cistude, de façon idéale en formant un angle approximatif de 90º avec la première localisation ;
e) déterminer avec le GPS la position du nouvel emplacement où se trouve le récepteur ;
f) déterminer à nouveau la direction du signal avec la boussole.

Dans le cas où deux personnes travailleront avec deux récepteurs, elles pourront localiser simultanément chaque cistude, mais il faudra qu'elles soient en contact par le moyen d'appareils de radio pour pouvoir coordonner le travail

Avec les deux positions du récepteur et les angles, et par trigonométrie, on peut calculer la position où se trouve la cistude. Mais, il faut transformer préalablement les azimuts (angles déterminés avec la boussole) en angles mathématiques (pour plus de détail, voir White & Garrot, 1990). Le logiciel gratuit LOCATE III (Nams, 2006) calcule directement la position de l'émetteur de façon rapide avec l'information du GPS et la boussole. Toutes les données seront rentrées dans une fiche spécifique (annexe 4) et puis rentrées dans une base des données.

Normalement il y aura des sites privilégiés pour localiser les cistudes. On peut considérer ces sites comme des stations fixes de radiopistage, et c'est important de dresser une liste de ces sites avec leurs coordonnées. De cette façon, il n'est pas nécessaire de prendre chaque fois les coordonnées de ces sites avec le GPS et on gagne du temps pendant le suivi.

Chaque localisation se trouve dans un polygone qui est associé à l'erreur produite lors de la détermination de l'angle. Si on calcule l'ecart-type de l'angle, on peut construire son intervalle de confidence (p.ex, au 95%) et calculer la taille du polygone d'erreur des localisations (pour plus de détaille voire chapitre 5 en White & Garrot, 1990). Pour cela il faut faire des tests des localisations des émetteurs avant de les fixer aux cistudes. Par exemple, chaque émetteur est localisé jusqu'à cinq fois (dans des conditions les plus proches à la réalité) ; dès que les positions de l'émetteur et du récepteur sont connues, on peut calculer l'angle réel et, postérieurement, la différence entre l'angle réel et l'angle estimé avec la boussole (pour plus de détail, voir chapitre 5, équations 5.1 à 5.3 dans White & Garrot, 1990).

D'autre part, faire un test du matériel de radiopistage sur le site dans des conditions réelles permet de déterminer quelle est la distance réelle de détection des émetteurs et à quelle distance on doit se positionner pour être sûr (ou presque) de pouvoir localiser une cistude équipée.

4.1.4.- Calendrier des localisations

A partir du lâcher, les localisations seront journalières pendant les deux premiers mois (avril et mai) et, après, elles pourront être hebdomadaires jusqu'à la fin de la vie des émetteurs. De toute façon, et si les ressources humaines et le budget le permettent, après les deux mois de suivi intensif, il est conseillable de faire de trois à quatre localisations chaque semaine (une localisation tous les deux jours) pendant la période d'activité des cistudes (juin – octobre et mars – septembre de l'année suivante). Pendant l'hivernation (novembre – février) les localisations pourront être hebdomadaires ou tous les 10 jours.

En cas d'envisager un suivi spécifique de la reproduction des femelles, les localisations se feront deux fois par jour, le matin et en fin de journée pendant les mois de juin à mi-juillet.

4.2.- Suivi par capture-recapture (piégeage)

Pendant la première année, si le suivi par radiopistage de toutes les cistudes s'avère impossible, il faudra aussi piéger suivant un protocole de capture-recapture pour établir les résultats du programme de réintroduction. De toute façon, après la première année de radiopistage il faudra envisager de planifier un suivi à long terme de capture-recapture par piégeage. Selon Dodd & Seigel (1991), pour déterminer les résultats (réussite ou échec) d'un projet de réintroduction de chéloniens il faudrait des suivis de plus de 20 ans. Toutefois, à partir des travaux de Bertolero (2002, 2003, 2006), il est possible de faire des évaluations par étapes pour déterminer plusieurs types de résultats qui indiqueront la situation du projet et s'il faut le reconduire (annexe 5). Normalement, pour une connaissance sûre de la réussite du projet il faudra attendre que la première génération de femelles nées en liberté soit aussi reproductive (pour les cistudes à l'âge de 7 – 8 ans environ). Selon les conditions de chaque projet, on peut obtenir aussi des résultats définitifs dès la sixième ou septième année de suivi à partir du taux de croissance de la population (ce critère a été utiliseé pour évaluer la situation de la réintroduction de la cistude et de l'emyde lépreuse au Delta de l'Ebre ; Bertolero, 2006). On propose, donc, un minimum de 7 ans de suivi par capture-recapture pour déterminer la situation des projets dans les Réserves de l'Estagnol et du Bagnas. Selon les résultats des premières années, le suivi pourra continuer 5 – 7 ans.

4.2.1.- Piégeage

Le piégeage plus effectif se fait par des verveux ou des nasses (rigides ou pliables ; Figure 7). Les pièges sont appâtés avec des poissons ou des poussins (que lon peut obtenir des fermes d'élevage industriel ; ce type d'appât est habituellement utilisé lors des suivis dans le Delta de l'Ebre). Le nombre de pièges à utiliser dépend de la taille du site, mais aussi de sa topographie (présence de canaux et d'étangs). En France, Cadi & l'Faverot (2004) indiquent des distances d'environ 50 m entre pièges consécutifs, tandis qu'au Delta de l'Ebre la distance est d'environ 100 m. Dans cette dernière localité, si l'on considère la longueur total du système de canaux de chaque site échantillonné (trois sites), il y a un piège tous les 200 m linéaires de canal. Ainsi, on recommande pour les deux réserves d'utiliser suffisamment de pièges pour maintenir ce rapport minimum (1

piège/200 m linéaire de canal). Dans les étangs, il ne faudra pas piéger dans la partie d'eaux libres, et les pièges ne seront placés que sur les berges.

Les verveux sont très efficaces pour capturer les cistudes (et d'autres tortues aquatiques) et, étant pliables, leur transport devient facile (en voiture et à pied). Dans les canaux pas très profonds (moins 110 cm de profondeur, vase comprise), l'installation se fait aisément et assez rapidement. Mais, si les canaux sont profonds (plus de 120 cm ou avec beaucoup de vase), la tâche devient plus compliquée et on pourrait avoir besoin d'un petit bateau ou de se plonger totalement dans l'eau pour sa correcte mise en place, ce qui n'est pas très pratique. Un autre problème des verveux, c'est que, dès qu'il faut qu'ils soient fixés, si le niveau de l'eau change ils peuvent rester au sec ou totalement submergés. Dans ce dernier cas, le risque de noyade des cistudes peut augmenter d'une façon importante. Ainsi, il faut toujours prévoir quel peut être le niveau maximum de l'eau et laisser la partie arrière du verveux suffisamment en dehors de l'eau (Figure 7).

La mise en place des nasses (munies de flotteurs) se fait rapidement. Aussi, elles s'adaptent au changement du niveau de l'eau. Il y en a qui sont pliantes, ce qui permet d'en transporter plusieurs dans une voiture ou à pied. On les trouve dans les commerces de pêche, quoique normalement trop petites (42 cm de longueur et 24 cm de diamètre). Un modèle beaucoup plus grand, mais pas pliant (100 cm de longueur et 63 cm de diamètre), a été mis en place au Delta de l'Ebre (Figure 7). Ce modèle est effectif pour les captures, mais sa grande taille rend son transport difficile. Seulement dans le cas où on peut les laisser en place sur les sites pendant tout le suivi (pas de risque de vol), leur utilisation s'avère pratique. Un autre inconvénient, c'est qu'on doit les construire (3 – 4 nasses par jour/personne ; avec un rouleau de 25 m de long et 1 m de large, on construit 7 nasses).

Figure 7. Type de piège. En haut, des nasses rigides (100 cm de longueur et 63 cm de diamètre) ; en bas, des verveux (photos : A. Bertolero).

Une stratégie à adopter est de mettre une plus grande densité de pièges dans les zones plus favorables ou fréquentées (déterminées par radiopistage), et moins de pièges dans les zones d'expansion possible de la population. Le processus de colonisation de chaque réserve devra être suivi : on prévoit, ainsi, que si la population de cistude augmente elle occupera une plus grande partie de la réserve et il faudra augmenter l'effort de piégeage. Le point où chaque piège est placé doit être enregistré à l'aide d'un GPS et numéroté (p. ex. « piège 1 »), de façon à pouvoir le repérer dans le temps (suivis intrannuels et interannuels). Aussi, sa localisation permettra de savoir toujours le lieu exact de capture de chaque cistude.

L'effort de piégeage doit être toujours enregistré, en indiquant le nombre de pièges, leur type et le temps de travail (exemple de fiche à l'annexe 6).

L'expérience acquise au delta de l'Ebre sur des petites populations de cistudes (moins de 40 exemplaires par population) a montré qu'il est inutile de surveiller les pièges chaque jour, mais qu'une surveillance toutes les 48 heures est largement suffisante. Dans les sites à grande densité de cistudes il faut surveiller les pièges chaque jour, voire même deux fois par jour. Dans tous les cas, il est indispensable que les pièges aient une bonne partie émergée pour éviter les noyades, même si le niveau de l'eau augmente subitement (p.ex. nasses munies de flotteurs, verveux avec de hauts piquets).

Dans les petites populations (cas des réintroductions) un piégeage prolongé dans une saison ne donne pas de meilleurs résultats que des séances courtes espacées dans la même saison. Par exemple, piéger chaque jour pendant trois mois n'est pas plus efficace que piéger une semaine par mois. L'effort est plus petit, mais ça dérange moins les cistudes (celles-ci peuvent éviter les recaptures) et, en même temps, la faune du site, et permet qu'une même équipe de personnes échantillonne plus de sites. Cela permet aussi l'échantillonnage séquentiel des différents secteurs dans les sites de grande taille.

Pour faire le suivi par captures-recaptures de la réintroduction des cistudes dans les Réserves du Bagnas et de l'Estagnol on propose le même protocole du delta de l'Ebre : faire des séances de piégeage de 5 jours, avec une surveillance des pièges toutes les 48 heures (±). Il est également très important de déterminer le nombre de sessions à faire pendant l'année, dans ce cas, un minimum de quatre sessions mensuelles d'avril à juillet. S'il est possible d'augmenter l'effort de piégeage, celui-ci sera mensuel de mars à octobre. Avec la totalité des séances de captures-recapture on pourra calculer la taille de la population annuelle (voir ci-dessous) et aussi d'autres paramètres de l'évaluation par étapes (p.ex. taux de persistance des cistudes lâchées ; le recrutement ; la condition corporelle ; etc.).

5.- Critères d'évaluation des résultats

Dans tout projet de réintroduction le but est d'obtenir des populations viables et autosuffisantes. De cette façon, un projet ne réussira que si dans le long terme on obtient une population viable et autosuffisante. Mais avant de déterminer la réussite à long terme, il y a plusieurs critères d'évaluation des résultats à court et moyen terme qui permettent d'évaluer la situation du projet. Si les résultats des évaluations à court ou moyen terme ne sont pas favorables, on peut toujours prendre des mesures pour reconduire le projet ou, en mettant les choses au pire, l'arrêter.

Pour les deux projets de réintroduction des cistudes au Languedoc on propose les critères d'évaluation suivants (modifiés d'après Bertolero 2002, 2003, 2004).

5.1.- Condition corporelle

La relation poids/longueur de la carapace doit être stable après le lâcher des cistudes. Une mauvaise condition corporelle de toutes (ou de la plupart) des cistudes indique que le site n'est pas de très bonne qualité pour maintenir une population de cistudes. Si la mauvaise condition corporelle n'est présente que chez quelques individus, il s'agirait plutôt d'une mauvaise adaptation de ces individus au site.

Les évaluations des projets de réintroduction ont montré que la qualité du site de réintroduction est très importante, c'est à dire que dans les sites de bonne ou de très bonne qualité les probabilités de réussite augmentent (Griffith, 1989).

Résultats à court terme ; données prélevées grâce au piégeage.

5.2.- Survie après lâcher (coût du lâcher)

Dans la première année en liberté il peut y avoir une plus grande mortalité due aux problèmes d'adaptation des cistudes ou à leur déplacement (émigration). Dans les deux cas, cela se traduit par une baisse de la survie apparente, ce qui peut être considéré comme un coût du lâcher. A partir de la deuxième année ce coût devrait disparaître, sinon on pourrait considérer que les conditions du site ne sont pas favorables pour la survie des cistudes. Le radiopistage pendant les premiers 12 mois permettra de déterminer le taux de survie et le taux d'émigration. La somme de ces deux taux devra être supérieure au 60% (taux de survie apparente > 60%).

Résultats à court terme ; calculé à partir des données du radiopistage.

5.3.- Survie à partir de la deuxième année

La formation d'une population viable sera possible seulement si le taux de survie après la période d'acclimatation (coût du lâcher) est comparable aux populations naturelles de cistudes. A partir de la deuxième année le taux de survie annuelle apparente devra être supérieure au 80%. Pour chaque population il faut construire l'histoire de vie de chaque individu de façon à obtenir une matrice avec les individus dans la première colonne et les captures dans les colonnes suivantes (exemple de matrice à l'annexe 7). Pour déterminer les taux de survie cette matrice doit être analysée au moyen des logiciels Mark (White & Burnham, 1999) ou M-Surge (Choquet et al., 2005).

Résultats à moyen terme ; calculés à partir des données de capture-recapture, mais il faut un minimum de 5 ans pour obtenir les premiers résultats valables (!).

5.4.- Reproduction
Si les cistudes lâchées ne se reproduisent pas, la formation d'une population viable est impossible. Si la reproduction se fait régulièrement à partir de la première année et les femelles atteignent la maturité sexuelle vers l'âge de 8 ans, la deuxième génération née en liberté devra naître vers 2016. La consolidation de la nouvelle population dépendra de la reproduction de la première et la deuxième génération de cistudes nées en liberté. Tous les nouveaux nés trouvés ou capturés pendant le piégeage devront être lâchés et ne rentreront pas dans le stock de tortues en captivité.

Le suivi de base de la reproduction se réalisera par la palpation des femelles piégées, la trouvaille des nids et la capture de nouvelles jeunes cistudes pendant le piégeage. Pour obtenir des informations plus précises des paramètres reproducteurs (fréquence de pontes, taille de la ponte et taux de femelles gravides) il faudra faire des suivis spécifiques par radiopistage et rayons X (ou ultrason). Il n'est pas très conseillé d'utiliser les rayons X systématiquement chez toutes les femelles ; il est préférable seulement chez celles qui ont présenté lors de la palpation des œufs calcifiés ou chez les femelles en migration terrestre pour faire la ponte (voir discussion de Hinton et al., 1997 et Kuchling 1998). Le suivi pendant plusieurs années (minimum deux ans) de ces paramètres permettra de calculer leur variabilité annuelle.

Résultats à court et moyen terme ; suivi de la reproduction par radiopistage, palpation ou rayons X (ou ultrason) des femelles capturées. Piégeage des cistudes nées en liberté, suivi par capture-recapture.

5.5.- Taille de la population
La taille annuelle de la population est calculée a partir des résultats de capture-recapture (piégeage). Pour ce calcul il y a plusieurs méthodes qui s'appliqueront suivant certaines suppositions et conditions. Si on peut considérer la population fermée (pas de naissances, ni de mortalité, ni de migrations) et il y a un minimum de trois sessions de piégeage annuelles, on peut utiliser la méthode de Schnabel (Krebs, 1989). Avec cette méthode les individus ne sont pas individualisés et les seules catégories à considérer sont « capturé pour la première fois » ou « recapturé ». Aussi par capture et recapture, dans la considération d'une population fermée, les individus étant marqués de façon individuelle et avec un minimum de cinq sessions de piégeage annuelles, on peut utiliser le logiciel CAPTURE (Otis et al., 1978), inclus actuellement dans le logiciel Mark (White & Burnham, 1999). Dans le cas des populations ouvertes et avec un minimum de cinq sessions de piégeage annuelles, on peut utiliser la méthode de Jolly-Seber (Krebs, 1989), inclus aussi actuellement dans le logiciel Mark (White & Burnham, 1999).

Si le projet marche bien, la taille de la population devra être stable ou augmenter. Les valeurs de taille de population devront être calculées avec leur intervalle de confiance pour déterminer s'il y a un changement significatif réel entre années.

Résultats à court et moyen terme ; calculés a partir des données de capture-recapture.

5.6.- Taux de croissance de la population
A partir de la taille annuelle de la population on peut calculer le taux de croissance de la population (Morris & Doak, 2002). Cela est également possible par le moyen d'analyses de viabilité des populations (PVA), mais dans ce cas il faut beaucoup plus

d'informations démographiques de la population (dans le cas le plus simple d'un modèle déterministe d'une population, le minimum est : a) structure de la population selon classes d'âge ou stages, b) âge de la première reproduction, c) taux de reproduction selon classes d'âge ou stages, et d) taux de survie selon classes d'âge ou stages; Beissinger & Westphal, 1998). Si pendant le suivi à long terme on prend ce type de données, on pourra faire un PVA de la population réintroduite.

Dans le cas le plus simple (à partir de la taille annuelle de la population) le taux de croissance de la population (λ) est calculé à partir de la moyenne géométrique de la façon suivante :

$$\lambda = [\prod (N_{t+1}/N_t)]^{1/T}$$

N_t = taille de la population au temps t,
N_{t+1} = taille de la population au temps t+1, et
T = temps passé entre la première estimation de la taille de la population et la dernière.

Si $\lambda > 1$ la population, est en croissance ; si $\lambda = 1$, la population est stable ; et si $\lambda < 1$, la population est en décroissance. Pour déterminer si le taux λ est significativement différent de 1, il faut calculer la pente et son intervalle de confiance par le moyen d'une régression linéaire du $\log(N_t)$ et du temps (voir Morris & Doak [2002] pour les détails du calcul). Il faut un minimum de 6 – 7 ans pour commencer à obtenir une tendance claire (voire même un minimum de 10 ans selon Morris & Doak, 2002).

Résultats à long terme ; calcul de la taille de la population pendant un minimum de 6 – 7 ans.

6.- Remerciements

Je remercie très cordialement Marc Cheylan, Anthony Olivier et Pauline Priol pour toutes les informations facilitées.

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Annexes

Annexe 1 :

exemple de fiche de marquage

Annexe 1 : exemple de fiche de marquage

Code marquage[1]	Numéro identification[2]	Transpondeur[3]	Date M[4]	Origine[5]	Date L[6]	Site L[7]	S[8]	A[9]	LC[10]	P[11]	Ec[12]	Observations[13]
...							...					...
exemples :												
1(14)(34)4	1574		3/4/07	Camargue	15/4/08	Estagnol	M	Ad	135.63	404	non	6 vertébrales
244(14)	2445		15/9/09	Bagnas	15/9/09	Bagnas	I	J	63.57	49	oui	

1) Code marquage : combinaisons des encoches marquées sur la carapace.
2) Numéro d'identification (selon le système de marquage) : numéro d'après la lecture des encoches.
3) Transpondeur : numéro du transpondeur (s'il n'y en a pas, laisser la cellule vide).
4) Date M : jour du marquage.
5) Origine : lieu de provenance de la cistude (site naturel où elle a été prélevée ou centre de reproduction où elle a été élevée).
6) Date L : jour du lâcher (après le séjour d'acclimatation).
7) Site L : site du lâcher (aussi pour les nouveaux nés trouvés dans la nature et issus des adultes lâchés ; dans ce cas, il s'agirait du site de naissance).
8) S : sexe (mâle / femelle / indéterminé).
9) A : catégorie d'âge (juvénile / subadulte / adulte) ou âge réel (année de naissance connue).
10) LC : longueur de la carapace (mm) au moment du marquage. Voir Figure 6.
11) P : poids (g) au moment du marquage.
12) Ec (écailles) : si le nombre des écailles est normal (oui / non). Si c'est « non », la cellule d'Observations doit indiquer les « erreurs ».
13) Observations : toute remarque qui pourrait aider à identifier la cistude (blessures / colorations particulières / etc.).

Commentaires des exemples :

a) Cistude mâle adulte capturée en Camargue, marquée le 3 avril 2007 et lâchée à la Réserve de l'Estagnol le 15 avril 2008.

b) Jeune cistude de sexe inconnu capturée pour la première fois à la Réserve du Bagnas (née donc en liberté) le 15 septembre 2009, marquée le même jour et lâchée au même endroit.

Annexe 2 :

exemple de fiche de suivi par capture-recapture (aussi pour le jour du lâcher après le séjour d'acclimatation)

Annexe 2 : exemple de fiche de suivi par capture-recapture (aussi pour le jour du lâcher après le séjour d'acclimatation)

Site[1] :								
Date[2]	Piège[3]	Numéro identification[4]	Transpondeur[5]	S[6]	A[7]	LC[8]	P[9]	Observations[10]

... ...

exemples								
18/05/08	1	1212		F	Ad	149.65	638	émetteur retiré (poids émetteur 15g). Poids sans émetteur
	1	1457		F	Ad	165.66	739	palpation négative
	5	1576	977200001603721	M	Ad	135.63	404	on met le transpondeur
19/05/08	-	2444		I	J	63.57	49	capturé à la main

1) Site : Estagnol / Bagans.
2) Date : jour du piégeage (ou du lâcher).
3) Piège : chaque piège a un numéro d'identification à une place fixe. S'il y a un type de piège différent, il faut l'indiquer aussi (verveux / nasses / etc).
4) Numéro identification (selon le système de marquage) : numéro d'après la lecture des encoches.
5) Transpondeur : numéro du transpondeur (s'il n'y en a pas, laisser la cellule vide).
6) S : sexe (mâle / femelle / indéterminé).
7) A : catégorie d'âge (juvénile / subadulte / adulte) ou âge réel (année de naissance connue).
8) LC : longueur de la carapace (mm) le jour de la capture.
9) P : poids (g) le jour de la capture.
10) Observations : toute autre remarque (p.ex. femelle gravide par palpation).

Commentaires des exemples :
Le premier jour de piégeage, le 18 mai 2008 on capture 2 femelles au piège nº1 et 1 mâle au piège nº5.
On enlève l'émetteur à la femelle 1212, car il ne marche pas ; le poids de la femelle a été enregistré sans l'émetteur.
On fait une palpation à la femelle 1457, mais le résultat est négatif.
On met le transpondeur nº 977200001603721au mâle 1576.

Le 19 mai, lors d'une séance de piégeage, on capture à la main un juvénile. On le marque avec le numéro 2444, et il faut l'enregistrer sur la fiche de marquage.

Annexe 3 :

coût approximatif du matériel pour le radiopistage, informations sur le matériel et articles sur la fixation des émetteurs

Coût approximatif du matériel et informations sur le matériel

Récepteur modèle Sika de Biotrack (Angleterre) : 2400 € (TTC, frais de transport inclus).

Antenne Yagi de Biotrack (Angleterre) : 205 € (TTC, frais de transport inclus).

Emetteurs de Biotrack (Angleterre) : 220 € l'unité (TTC, frais de transport non inclus).

Emetteurs de Sirtrack (Nouvelle Zélande) : 160 € l'unité (plus 37.5 € de douane approximatif pour chaque unité [la taxe douanière est proportionnelle au prix du colis]).

Mât pliant : 25 € (mât en aluminium utilisé pour nettoyer les piscines).

Biotrack '*Sika*' Radio-tracking Receiver

Sika is a truly international radio-tracking receiver. Most tracking receivers cover a 1-2 MHz band only, ***Sika*** covers 30 MHz!

Main Features

- Each Sika covers 90% of all radio-tracking frequencies (138-168 MHz or 144-174 MHz)
- Light & easy to carry (150 x 85 x 55 mm, 800g with shoulder strap)
- Completely waterproof
- Robust aluminium box
- Highly sensitive (Minimum discernible signal –150 dBm on all frequencies)
- Exceptionally directional when tracking at short range
- 256 channel, non-volatile memory (retained even if battery is removed)
- Memory scanning with 1-999 seconds dwell time per channel
- Option to scan selected channels only
- Internal rechargeable '4A' battery pack lasts for 24 hours of continuous use (also runs on external power or 4 internal primary or rechargeable AA cells)
- Sophisticated internal battery charging circuit with thermal safety cut-out
- Dual gain control (keyboard or thumb-wheel)
- Signal strength displayed as a bar graph and number
- Fine tuning in 1 kHz or 0.1 kHz steps
- LCD back-light for tracking at night
- Serial port (RS232) to control Sika from a PC
- Excellent aftercare service from Biotrack (e.g. In the unlikely event that your ***Sika*** breaks down we will lend you a replacement receiver while yours is repaired)

Included free with each Sika

- Adjustable shoulder strap
- Rechargeable battery pack (fitted inside the receiver)
- Battery holder for primary or NiCad/NiMH AA cells
- International Mains Power Supply (110/240V and assorted mains plug adapters), also used for charging the battery
- Power supply lead for use in a vehicle (for cigarette lighter socket)
- Headphones
- Connector dust caps (+ spares)

Sika Specifications

The **Summary Specifications Table** below gives at-a-glance features that make the Biotrack receiver so different from the crowd.
The **Full Specifications Table** overleaf lists the specifications, their values and explains what they mean and why they are important in practical terms for animal radio-tracking. Sentences in ***bold italics*** are the key ways in which you should judge receiver performance.

Summary Specifications Table

Frequency Band	▪ Each receiver covers all frequencies from 138 to 168 MHz or from 144 to 174 MHz (and the band can be changed) ▪ Fine tuning in 1kHz or 0.1kHz steps
Functions and Controls	▪ Direct frequency entry from the membrane keypad (no need to enter MHz part of frequency each time a frequency is set) ▪ 256 user-programmable channels ▪ Scanning of all or selected channels (dwell time 1 to 999 s, settable to 1 s resolution) ▪ Internal speaker ▪ Headphones socket (switches out internal speaker) ▪ LCD Back-light (automatic switch-off after 4 minutes) ▪ Dual gain control (keypad buttons and thumb-wheel) ▪ Control of frequency, channel & gain from a PC serial port (RS232) ▪ Bar chart and numerical display of signal strength
Environmental Specification	▪ *Fully water-proof* (to IP65) ▪ *Operating temperature range:* –20 to +50°C (battery charging temperature range: 5 to 35°C) ▪ *Temperature stability:* +/- 1 kHz over –20 to + 50°C
Electrical & Mechanical Specification	▪ *Minimum Discernible Signal:* -150 dBm over entire frequency band ▪ *Gain Control Range:* >90 dB, typically 95 dB ▪ *Power supply:* internal 4A battery pack, external power supply (DC, 10-15V, >500 mA) or four internal rechargeable or primary (non-rechargeable) AA cells. ▪ *Battery Life:* 24 hours on internal 4A NiMH battery pack ▪ *Weight:* 800g including strap and battery ▪ *Size:* 150 x 85 x 55 mm (6 x 3.25 x 2 inches)

Full Specifications Table

Specification	Value	Explanation
Frequency Band	138-168 MHz or 144-174 MHz	Most tracking receivers cover a 1-2 MHz band only. Sika covers 30 MHz! The same receiver can be adjusted to either band. The main benefits of such a wide band are: ▪ ***Sika can be used almost anywhere*** ▪ ***Sika is 'future-proof' against changes in frequency allocation***
Sensitivity MDS	-150 dBm	***The more sensitive a receiver, the better the chances of you hearing very weak signals***. MDS means 'Minimum Discernible Signal' and is the weakest signal that can be heard on the receiver. ***The more negative the MDS, the better the sensitivity (e.g. –150 is better than –145)***.
Selectivity 6 dB 90 dB	± 3.75 kHz ± 12 kHz	***The more selective the receiver, the less chance you will hear 'interference' from adjacent frequencies*** (including radio tags and signals from other radio users). However, beware selectivity that is too narrow (e.g. < 2 kHz at 6dB). This will make tuning more critical and increases the risk of missing a tag that has shifted frequency slightly (*e.g.* due to a change in temperature).
Gain Control Range Receiver gain	90 dB	When tracking powerful tags at close range you have to be able to reduce the gain to very low levels, otherwise the signal will no longer appear to be directional. ***The greater the Gain Control Range, the less likely you are to encounter problems with close range tracking***. Receivers with inadequate gain control range need attenuator switches.
Channels Number of channels (user programmable)	256	***Having channels makes the receiver easier to use in the field and enables memory scanning for lost animals.***
Scanning Min. – Max. dwell time	1 – 999 secs	The Scanning function steps through the channels on your receiver and stops on each one for a user-defined 'dwell' time. ***Scanning automates the process of frequency changing when searching for a number of tags at once.*** It is especially useful during searches from vehicles.
Frequency Stability Over –20 to +50 C	< 1 kHz	If a receiver frequency changes with temperature there is a chance that you will miss tags because the receiver is no longer tuned to the best frequency on which to hear them. ***The more stable the frequency of the receiver over temperature and time, the less the risk you will miss a tag because of frequency shift.***
External power supplies	10 – 15 V	***Powering a receiver from an external power supply saves internal battery life and allows the receiver to run from a vehicle or for long periods with a data logger.***
Waterproofing Method Rating	Neoprene and silicone seals. IP65	The waterproof rating code 'IP65' is from a standard dust and water resistance scale. It means the device is dust-tight and impenetrable to water spray from all directions. Sika has a waterproof seal on box lid and battery compartment . The speaker is fully covered by the membrane keypad and the gain control is hermetically sealed. All connectors are sealed on inside of box and external covers are supplied for connectors when not in use. ***Water-proofing to IP65 is an essential feature of any modern radio-tracking receiver.***

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Specialists in Animal Radio Monitoring

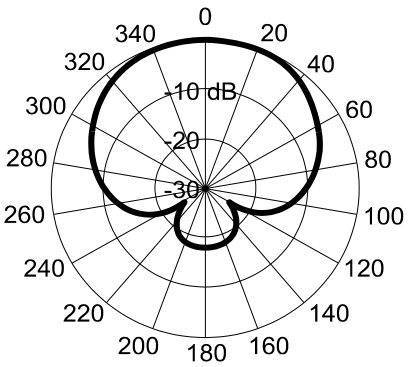
Yagi antennas

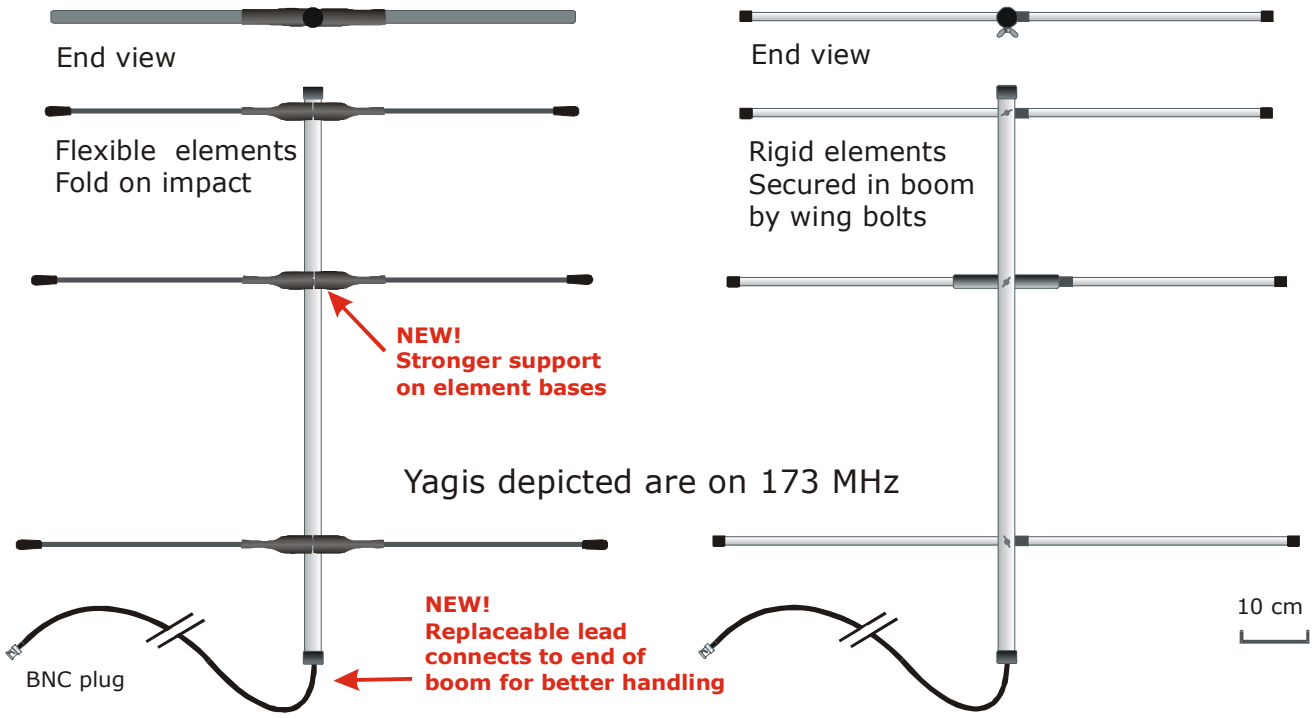
Biotrack supplies two models of Yagi antenna, developed for us by Lintec Antennas Ltd - a specialist antenna manufacturer. Both antennas give excellent performance, and are light-weight and robust. They have a number of novel features that set them apart from other designs. One Yagi has conventional rigid elements, and the other has flexible elements that fold on impact and automatically spring back into position afterwards. The antenna with rigid elements can be dismantled easily for transportation and storage. In common with most good Yagi designs, these antennas are 'matched' to the standard 50 ohms input impedance of radio-tracking receivers. However, the matching is achieved by 'inductive coupling' via internal windings that are sealed within the central element. This avoids the need for a more cumbersome external device such as a gamma-match. Both antennas have user-replaceable leads.

Specifications

	Flexible Yagi	Rigid Yagi
Weight (173 MHz version):	800 g	800 g
Frequencies:	138 - 230 MHz	138-230 MHz
Bandwidth:	± 2 MHz	± 2 MHz
Gain:	6 dB	6 dB over dipole
Beamwidth (3dB):	80 ^{0}	80 ^{0}
Back-to-front ratio:	18 dB	18 dB
Impedance (nominal):	50 ohms	50 ohms
Receiver connector:	BNC	BNC
Replaceable Cable length:	1.5 m	1.5 m
Price (ex-VAT, from Jan-07):	£124	£105
Spare leads (ex-VAT, from Jan-07):	£15	

Polar diagram (both antennas)





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Articles sur la fixation des émetteurs

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Wilson, K.A.; Cavanagh, P.M. & Villepique, J. 2003. Radiotransmitter attachment technique for box turtles (*Terrapene* spp.). *Chelonian Conservation and Biology* 4:688-691.

Radio Transmitter Attachment for Turtle Telemetry

Variations in chelonian size, differences in behavior and habitat preference, and diverse research goals pose problems for fastening radio transmitters to turtles. Transmitters glued to turtles with aquarium sealant are quickly dislodged in the field; the bond from 5-minute clear epoxy deteriorates within one season (D. C. Holland, pers. comm.). Some fast-setting epoxies create a strong bond between transmitter and scute, but can emit enough heat to pose a burn hazard to underlying tissue (Renaud et al. 1993).

PC-7 (Protective Coating Co., Allentown, Pennsylvania, USA) is a slow-setting epoxy that emits no significant heat and secures material to scutes with a bond that endures for years. PC-7's viscosity will also hold a transmitter in place as it dries, and this simplifies application. Attachment with PC-7 (best started at night, so the epoxy can cure when the turtle is less active) proceeds as follows:

1) Thoroughly clean the attachment site with ethanol and a tooth brush (phenolic impurities are highly toxic to turtles [Hillyer 1993]).

2) Use thin strips of masking tape to cover all scute junctions near any area to be epoxied. Growth occurs at these boundaries and rigid adhesives (like PC-7) must not span them.

3) Lay a ring of caulking down along the perimeter of the scute to receive the transmitter. Reusable winter window caulking "rope" (FROSTKING FINGERTIP CAULK by Thermwell Products Inc.) is ideal for constructing this rim. The rim helps prevent epoxy from extruding onto neighboring scutes when the transmitter is pressed into place.

Aquatic Turtles.—For aquatic species, on which attached instruments can serve to snag and drown the turtle, the procedure continues as follows:

4) Apply epoxy within the caulk rim on the target scute and gently press the transmitter (AVM Instruments, Livermore, California, USA) into the well of epoxy. Do not apply pressure to the transmitter coils (red coiled wires) as this can detune the unit.

5) Epoxy the antenna in as wide an arc as possible just inside the marginal scutes. Trim the antenna to ensure that its arc will not constitute more than 75% closure of a circle when in its final position. A transmitter's signal is muted if the antenna's tip nears its base. To preclude snagging on underwater twigs, the unepoxied antenna segments at all scute junctions must be covered; a pliable adhesive like 5-minute epoxy should be used on these narrow interstices.

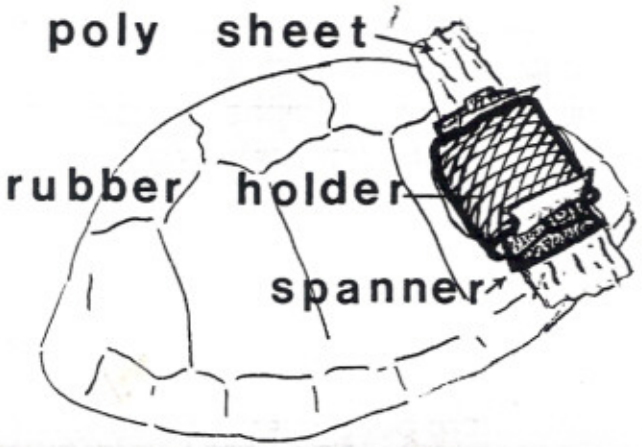

FIG. 1. Rubber holder with openings oriented laterally; plastic spanner in position.

While PC-7 provides a reliable bond that endures aquatic stress, it does make replacing transmitters difficult. Prying an old transmitter off can tear scutes bonded to the epoxy. Begin the removal process with a Dremel drill (with an emery cutting wheel), and follow the drill with a jeweler's saw to safely separate transmitter from scute. This is faster (one hour per transmitter) than using only a jeweler's saw to slice through the epoxy. An exacto knife will gently tease any epoxy residue off the scute.

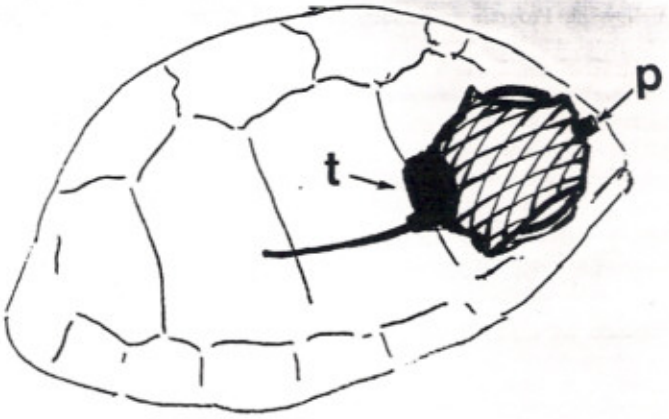

FIG. 2. Battery positioned within rubber holder; transmitter (t) projecting from posterior hole (at left) and anchoring pin (p) protruding through anterior puncture (at right).

Terrestrial Turtles.—For terrestrial turtles, our method can be adapted, after step 3, as follows:

4) Apply a thin patch of PC-7 epoxy within the putty rim on the target scute (on male box turtles, vertebral scute #2 is used; on females, pleural scute #1 is used instead, so the attached transmitter will not impede mounting by courting males). Work the epoxy into the scute's surface irregularities.

5) Cut a 3 cm length from a 1.25 inch bicycle inner tube. Make 3 to 5 holes of approximately 4 mm each in what will serve as the bottom half of this 3 cm rubber ring. The holes will allow epoxy, extruded from inside the ring in step #8, to bond the rubber holder to the expoxy on the scute.

6) Line the interior of the rubber ring's top half with tape and polyethylene to preclude its getting glued to the bottom.

7) Set a wad of epoxy on the center of a 3.5 cm x 8 cm polyethylene sheet. With its epoxy side down, slip one of the long ends of poly sheet into, and through, the rubber ring and draw the sheet into the ring until the epoxy is directly over the holes.

8) Coat a hard 3 cm x 4 cm plastic rectangle with vegetable oil so it can not adhere to epoxy. Slip this oiled plastic spanner into the narrow space above the polyethylene sheet's upper, clean surface. The space that the spanner creates between the sheet and the ring's roof further reduces the risk of gluing the ring's rubber walls together (Fig. 1). Insertion of the spanner extrudes epoxy through the holes in the bottom of the rubber ring, and out of its two ends.

9) Press the ring down, with its holes facing toward the carapace, onto the prepared scute. To ensure a strong bond, gently rotate the assembly to blend ring and scute epoxies. Orient the ring's blind ends sagittally, and its open sides laterally (Fig.1), so the antenna will emerge posteriorly in Step #11 (Fig.2).

10) After 12 h of epoxy curing, remove the plastic spanner and as much of the polyethylene and tape shielding as possible from inside the rubber ring. Cut a 1 cm hole in the posterior end of the rubber holder, and make a small puncture in its anterior end.

11) Thread the antenna, and then push the transmitter through the posterior 1 cm hole. The posterior hole's perimeter grips the

transmitter tightly. The short metal pin (built into the unit, upon request, by AVM Instruments) projecting from the end of the battery opposite the transmitter will anchor the anterior end when slipped into the puncture at the front of the rubber ring (Fig.2).

12) This secured rubber holder enables simple, fast (2 min) field replacement of transmitters. Covering the assembly with duct tape will protect the rubber from twig snags and abrasion.

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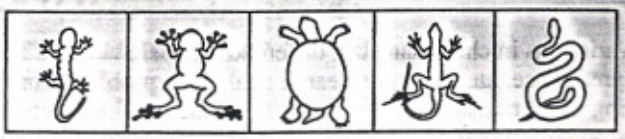

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Review of Radio Transmitter Attachment Techniques for Turtle Research and Recommendations for Improvement

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Abstract-How a radio, sonic, or satellite transmitter is attached to a turtle or tortoise may affect the transmitter's transmission range and the animal's behavior, survival, and reproductive success. We reviewed 113 scientific papers, reports, and semi-technical articles reporting on radio-tracking projects with turtles and conclude that little information is avail- able in the literature to evaluate the effects of transmitters on the study animals. We also provide step-by-step directions on a successful method we used to attach transmitters to desert tortoises *(Gopherus agassizii)* that minirnizes potential of affecting the animal's behavior, physiology, reproduction, or survival while maximizing distance of transmission. We believe this method can be used on many other species of turtles and tortoises.

Biotelemetry has become indispensable for studying turtle migration, dispersal, home range, habitat use, physiology, and the effectiveness of relocation efforts. The most common types of telemeters used on turtles are radio, sonic, and satellite transmitters, which each have advantages depending on the specific applications. An important consideration for using radio transn-dtters and D marking techniques is assuring they do not affect significantly the behavior, physiology, reproductive success, and survival of the animals (Anonymous 1987; Brander and Cochran 1969; Ireland and Kanwisher 1978; Kaufmann 1992a; Renaud et al. 1993b; Schubauer 1981; Schwartzman and Ohmart 1977). Therefore, non-invasive methods of transmitter attachment must be developed and tested (Anonymous 1987). Furthermore, as there are tradeoffs between transmitter weight, transmitter longevity, and transmission range (Brander and Cochran 1969; MacDonald and Amlaner 1980), transmitter attachment methods should be developed to optimize performance to meet study objectives.

We reviewed 113 published and unpublished accounts of the use of radio, sonic, and satellite tracking of turtles to determine the attachment methods used and to identify problems for the study animals caused by the transmitters. We also outline the method we have used for five years to attach transmitters to desert tor- toises *(Gopherus agassizii)* without causing physical harm to the study animals, while maxin-dzing transn-dtter longevity and range. This method can be used for multi-year applications with other species of turtles.

Review of Transmitter Attachment Methods and Their Problems.-In the 113 publications, articles, and reports we reviewed, radio transmitters, which consist of three major components (body

of transmitter, battery, and antenna), were attached externally to the carapace of turtles by several means: cemented on with epoxy, silicone sealant, dental acrylic, or some other adhesive; strapped on with harnesses; or attached via bolts, wire, cable or nylon ties, or monofilament line passed through holes drilled in the carapace, usually through the posterior carapace or marginal scutes (Table 1). These methods were used to attach either the transmitter and battery directly to the carapace or to allow the transmitter to trail loosely behind the animal.

Some less conventional modes of attachment were used. In one instance, transmitters were sewn onto the carapace of soft-shelled turtles (Plummer and Shirer 1975). Transmitters also were attached with some success using black plastic electrical tape (Eckler et al. 1990; Moll and Legler 1971). Whereas implantation is the norm in snakes (Fitch and Shirer 197 1; but see Ikeda et al. 1979), it has been rarely employed in turtles (Table 1). Many authors (23%) did not mention how or where transmitters were attached, mak- ing it difficult to evaluate the potential effect of the transmitter on the animals, and hence the possible limitations on interpreting study results.

Problems caused by transmitters are well documented for birds and mammals (Kenward 1987; White and Garrott 1990), but are poorly known for turtles. We know of only three limited studies designed in part to test the effects of different transmitters or attachment methods on turtles. Tirnko and Kolz (1982) estimated that a satellite transmitter caused a captive loggerhead turtle to spend twice as much time on the water surface, but concluded the transmitter caused no "radical" change in behavior. However, their sample size was one, and no control was reported. Kenunerer et al. (1983) found that after equipping 20 loggerhead turtles with transmitters, the turtles spent more time on the surface during the first 3 days than the following 17 days of study. Beavers et al. (1992) found three different adhesive attachment methods had no effect on loggerhead turtle behavior, but their sample size was one per method and they made no mention of methods or criteria.

We located six papers reporting problems observed during the course of field studies with turtles. Keinath and Musick (1993) reported the transmitter and harness cemented to a leatherback *turtle (Dermochelys coriacea)* were bitten by a tiger shark *(Galeocerdo cuvieri),* the resultant damage causing the harness to chafe the turtle's skin. Equipment poorly attached to harnesses slapped against and severely damaged the carapaces of leatherback turtles (Eckert and Eckert 1986). Implanting transmitters into the oviducts of northern long-necked turtles *(Chelodina rugosa)* caused oviducal adhesion in at least two turtles, reducing reproductive output in the year studied, and the surgical procedure resulted in the death of one turtle (Kennett et al. 1993). The act of attaching transmitters may have caused up to 55% of fernale yellow mud turtles *(Kinosternonflavescens)* to move to new nesting locations (Iverson 1990); the transmitter attachment method was not noted, however. Brill et al. (1995) found submergence behavior of green turtles *(Chelonia mydas)* was affected for up to three hours after they attached transmitters to the rear marginals of the carapace by inserting nylon straps (tie-wraps) through drilled holes. Some shell deformation occurred in hatchling gopher tortoises *(Gopherus Polyphemus)* because epoxy holding on the transmitters encroached growth areas between scutes (Butler et al. 1995). On the other hand, Hopkins and Murphy (I 98 1) reported no damage to carapace or flippers from transmit- ters on 37 loggerhead turtles.

Although not published, other problems have occurred. For instance, J. Congdon (pers. comm.) found transmitters placed on the carapaces of painted turtles *(Chrysemys picta)* became entangled in filamentous algae preventing the turtles from diving. C. K. Dodd, Jr. (pers. comm.), has made similar observations on common mud turtles *(Kinosternon subrubrum).* H. Avery (pers. comm.) observed female desert tortoises impeded by transmit- ters, which were mounted on the anterior carapace, that got hooked by stems of desert shrubs. We found one desert tortoise shell that became deformed because normal shell growth was inhibited by a transmitter antenna that was attached improperly for one year. Similar results from desert tortoises were reported by K. Berry (pers. comm.) and A. Karl (pers. comm.). Such deformation is most likely to occur in animals that experience relatively rapid growth during the course of study (e.g., juveniles or animals equipped for several years). Although unreported, drilling holes into the shell and underlying bone may lead to potentially harin- ful infection, and this effect may not be observable until some- time after the transmitters have been removed (B. Homer, pers. comm.). Bertram (1979) did comment on the absence of any wounds after removing a transmitter that had been bolted onto the carapace of a hingeback tortoise *(Kinixys belliana)* two years earlier.

Transmitters may attract the attention of predators (Keinath and Musick 1993; cf. Renaud et al. 1993b) or people (Stoneburner 1982). To reduce the potential for such effects, transmitters should be camouflaged in some way. For instance, Dizon and Balazs (1982) covered their transmitters with roofing tar and sand be- fore attaching to Hawaiian green turtles *(Chelonia mydas).* Schwartzman and Ohmart (I 977) mixed neutral color compounds to the epoxy or painted the dried epoxy after attachment to desert tortoises. Satellite transmitters placed on sea turtles are routinely painted black (C. K. Dodd, Jr., pers. comm.).

Authors occasionally mention transmitter failures, problems, or malfunctions (Table 1), but rarely are the causes known, mentioned, or hypothesized. We found several accounts in the lit6rature of the loss of transmitters. Stonebumer (1982) laments the theft of seven out of eight buoy transmitters attached to logger- head turtles *(Caretta caretta).* Timko and DeBlanc (I 98 1) lost 4 of 22 transmitters and Tiniko and Kolz (I 982) lost their only trans- mitter when the linen lanyard used to attach floating transmitters to Kemp's ridley turtles and a loggerhead turtle became abrade
and parted (see also Renaud et al. 1992; Renaud et al. 1993b; Renaud and Carpenter 1994; Schubauer 198 1). After being in place for five months, the verticahy-protruding antenna broke off a transmitter attached to a hingeback tortoise *(Kinixys belliana,* Bertram 1979). In one study of the desert tortoise, 9% of transmitters (10 of I I 1) fell off the animals over four years (EG&G 1993).

Attaching Transmitters to Desert Tortoises.-For nearly two decades, researchers have been attaching transmitters to the carapaces of desert and gopher tortoises with epoxy cement (for example, see Schwartzman and Ohmart 1977). We modified the methods used by Schwartzman and Ohmart (1977), Mike Cor- nish (pers. comm.), Charles Peterson (pers. comm.), and others to attach radio transmitters securely to desert tortoises apparently without causing shell deformation, predator attraction, mating disruption, or transmitter loss, while also yielding greater trans- mitter range. We present the following step-by-step description of the protocol we used so that the method can be adapted to other species of turtles and tortoises.

We used three different types of transmitters depending on the size of the tortoise. Two-stage battery-powered transmitters (AVM Instruments SB-2*), weighing 35 g, were attached 108 times to 43 tortoises (171-296 mm midline carapace length [MCL], 1075- 5200 g). One-stage battery-powered transmitters (AVM Instru- ments SM- I H), which are smaller (26 g) and weaker, were attached 24 times to 14 tortoises between 146 and 239 mm MCL (800-3150 g).

One-stage solar-assisted transmitters (AVM Instruments SM-1H-solar), weighing 4.2 g, were attached 41 times to 21 immature and subadult tortoises between 97 and 207 nun MCL (220-1800 g). Whip antennas on the larger two transn-dtters ranged from 280 to 320 mm in length and were made of 20 gauge, insulated, stranded wire. The whip antennas for the solar transmitters were 150 mm long and made of single 24 gauge, insulated, stranded wire.

We used the following step-wise procedure to attach the non-solar assisted transmitters to 57 desert tortoises (Fig. la):

1. We tested the transmitter to confirm that it worked.
2. All dirt was brushed off of the carapace.
3. We pre-positioned the transmitters to the first left or first right costal scute of the tortoise's carapace, as flush to the carapace as possible.
4. To position the antenna, we cut short sections of flexible 3 mm plastic tubing, and epoxied each section to the first four vertebral scutes (see also Butler et al. 1995). Each section was cut slightly shorter than its associated scute. Super glue was used to hold each section of tubing in place while we applied a quick drying, pli- able putty epoxy (Power Poxy Adhesives, Inc., Power Poxy® #40001 *) over each section of tubing in a continuous layer from the scute surface on one side of the tube to the scute surface on the opposite side of the tube. We were cautious not to get any epoxy on the scute sutures or on neighboring scutes.
5. We ran the antenna through the tube sections leaving approximately 50-120 mm of antenna hanging loose beyond the posterior of the animal.
6. The transmitter was then attached with putty epoxy, using care not to bridge the scute margins. Spaces between the transmitter and carapace were filled in with epoxy to prevent the transmitter from getting caught in vegetation.
7. Both the transmitter and the putty epoxy were painted with a flat colored, lead-free paint to reduce reflectivity and contrast.
8. Finally, the transmitter was checked again for proper operation and the tortoise was released immediately.

The entire procedure takes approximately 15 min. The transmitters were removed about every two years for battery replacement by cutting through the epoxy with a pocket knife, a simple process that took less than 10 min.

Using similar procedures, solar-assisted transmitters were attached to the fifth vertebral scutes of 21 tortoises using putty ep- oxy, but the antenna was left loose. We did not use any tubing to attach the antenna to the tortoise. Some transmitters were attached with the antenna oriented vertically and others horizontally.

To simplify and expedite transmitter removal during future scheduled battery replacement, we initially attached a brass base plate with Devcon® Five-Minute Epoxy®* to the carapace, then attached the transmitter to a metal post on the plate. Transmitters attached in this manner became detached 22 times between day 1 and 26 months later. No additional losses were experienced after eliminating use of the brass plate (i.e., using the methods described above).

For the first two years, we attached the antenna to the marginals, partially encircling the animal. Later, we began attaching the trans- mitter to the first right or left costal, as described above, which facilitated placement of the antenna over the vertebral scutes and letting the distal 50-120 mm of antenna trail behind the tortoise. This improvement increased transmission range by approximately 2O% (pers. obs.). Diverting the antenna down to the last one or two costal scutes on females would keep it from possibly inter- fering with copulation, although we have observed unimpeded copulations with the antenna attached to all vertebral scutes. Leaving antennas loose on solar-assisted transmitters caused antennas to break 19 times, but was necessary to maximize the range of these weaker transmitters. Vertical orientation of antennas also resulted in greater range compared to horizontal orientation, but made the antenna more vulnerable to breakage. To reduce the breakage problem, a smaller, more resilient gauge antenna was used and the base of each antenna was enclosed in a small spring.

Placed on the vertebrals, the tubing allowed the antenna to be pulled through the tubes as the tortoise grew, thus preventing shell deformation. We have attached antennas in this manner to 57 tor-

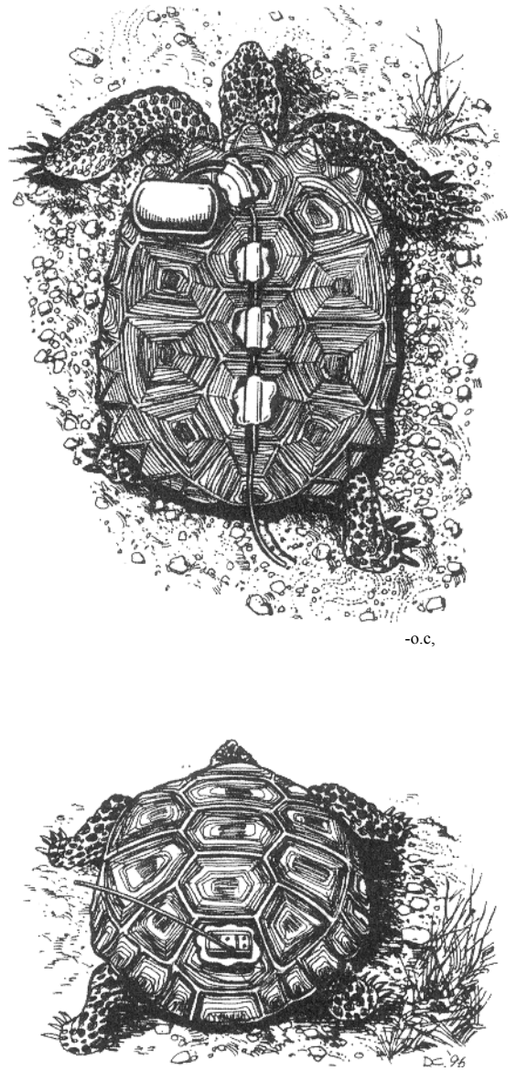

FIG. 1. Drawing showing how we attached radio transmitters to the carapaces of desert tortoises: (a) larger battery-powered transmitters were attached to tortoises larger than 146 mm (midline carapace length; 800 g) and (b) smaller solar-assisted transmitters were attached to immature and subadult tortoises between 97 and 207 *m*m (220-1 800 g).

TABLE 1. Methods of transmitter attachments in chelonians. Methods are categorized as one of the following classifications: "adhesive" (transmit- ter was attached to the shell of the turtle with glue, epoxy, dental acrylic, or fiberglass), "harness" (transmitter was strapped around the shell without otherwise disturbing the shell), "hole in shell" (holes were drilled, screwed, or punched through the shell, and bolts, string, wire or other filament was strung through the holefs] to attach the transmitter), "implantation" (transmitters were surgically implanted within the body), "tape" (transmitter was attached with electrical tape), "sewn" (the transmitter was sewn into the shell of a soft shell turtle), or "not mentioned" (method was not evident). Papers that reported on problems are indicated by superscripts. An "*" notes data on effect of the transmitter on the health, development, behavior, or ecology of the turtle. A "+" denotes problems with transmitters failing off or otherwise being lost from the turtle. A "t" notes a non-specified problem with transmitter use on a turtle.

Method	Species	Source
adhesive	*Caretta caretta*	Beavers et al. 1992; Hays et al. 1991; Renaud et al. 1992+; Renaud and Car- penter 1994
	Chelonia mydas	Renaud et al. 1992+; Renaud et al. 1993b+
	Chelydra serpentine	Ireland and Kanwisher 1978
	Clemmys guttata	Lovich 1990, pers. comm.
	Clemmys mar?norata	Rathbun et al. 1992
	Clemmys muhlenbergii	F-ckler et al. 1990t; Larson 1984; Lovich et al. 1992, pers. comm.
	Dermochelys coriacea	Standora et al. 1984t
	Gopherus agassizii	Barrett 1990; Bulova 1994; Burge 1977b-, Esque 1994; Goldsmith and Shaw 1994; Martin 1995; O'Connor et al. 1994a, b; Peterson 1993; Schwartzman and Ohmart 1977; Stewart 1993; Turner et al. 1984; Zimmerman et al. 1994 Tom 1994
	Gopherus flavomarginatus	
	GopheruspPolyphemus	Butler et al. 1995*; Diemer and Moler 1982; Diemer 1992t; Smith 1995; Wilson et al. 1994
	Lepidochelys kempii	Renaud et al. 1993a
	L.epidochelys olivacea	Beavers and Cassano 1996; Plotkin et al. 1995, 1996
	Sternotherus depressus	Dodd et al. 1988
	Terrapene carolina	Madden 1975+
	Terrapene omata	Nieuwolt 1993
	Trachemys scripta	Moll 1994, pers. comm.
	Testudo kleinmanni	Geffen and Mendelsson 1988t, 1989
	generic	Belzer and Reese 1995
hamess	*Caretta caretta*	Stonebumer 1982+
	Chelonia mydas	Ireland 1980; Standora et al. 1982
	Dermochelys coriacea	Duron-Dufrenne 1987; Eckert and Eckert 1986*; Eckert et al. 1986; Keinath and Musick 1993*
hole in shell	*Lepidochelys kempii*	Byles 1989*+
	Caretta caretta	Byles and Dodd 1989+; Hopkins and Murphy 198 1 *; Keinath et al. 1989*+, Kemmerer et al. 1983; Standora et al. 1982; Renaud and Carpenter 1994; Wibbels et al. 1990t; Yano and Tanaka 199 It
	Chelonia mydas	Baldwin 1973; Brill et al. 1995*; Dizon and Balazs 1982; Mendonqa 1983; Ogden et al. 1983f
	Chelydra serpentine	Froese 1974; Galbraith et al. 1987t; Ireland and Kanwisher 1978; Brown and Brooks 1991; Brown et al. 1990; Obbard and Brooks 1981
	Chrysemys picta	
	Clemmys insculpta	Taylor and Nol 1989; Christens and Bider 1987t
	Emydoidea blandingii	Kaufmann 1995, 1992a, b
	Gopherus pokvphernus	Ross and Anderson 1990; Rowe and Moll 1991 t
	Kinixys belliana	Diemer 1992t
	Lepidochelys kempt .i.	Bertram 1979+
	Macroclemys temminckii	Byles 1989*+; Tiniko and DeBlanc 198 1 +
	Pseudemys concinna	Harrel et al. 1996; Sloan and Taylor 1987
	Terrapene ornata	Buhimann and Vaughan 1991
	Trachemys scripta	Doroff and Keith 1990; Eliner and Karasov 1993; Legier 1971
	generic	Florence 1975t; Moll and Legier 197 It; Schubauer et al. 1990
implantation	*Chelonia rugosa*	Schubauer 1981
	Geochelone gigantea	Kennett et al. 1993*t
	Gopherusflavomarginatus	Swingland and Frazier 1980
sewn tape	*Apalone mutica*	Aguirre et al. 1984
	Clemmvs muhlenbergii	Plummer and Shirer 1975
	Trachemvs scri.pta	Eckler et al. 1990
not mentioned	*Batagur baska*	Moll and Legier 197 I t
	Caretta came	Moll 1980
	Clielonia mvdas	Soma and Ichihara 1977; Soma 1985; Timko and Kolz 1982+
	Chelydra serpentine	Ireland 1979; Carr 1967
		Ultsch and Lee 1983

TABLE 1. cont'd

Method	Species	Source
not mentioned	*Gopherus agassizii*	Berry 1974; Burge 1977a; Christopher et al. 1993; DeFalco 1995; EG&G 1993*+; Henen 1994; Jennings 1993; Turner et. al. 1987, 1986; Wallis et al. 1992
	Gopherus potyphei-nus	McRae et al. 1981
	Kinosternonflavescens	Iverson 1990*
	Kinosternon subrubum	Burke et al. 1994
	Mauremysjaponica	Yabe 1992
	Pseudemydura umbrina	Fullagarl967
	Terrapene carolina triunguis	Kiester et al. 1982; Schwartz et al. 1984
	Testudo hermanni	Swingland et al. 1986

toises for up to five years, and have observed only ond shell that became slightly deformed when the widened distal end of the antenna failed to slide through the tubing. We now use antennas with continuous surfaces rather than ones with additional insula- tion at their ends.

Attachment to the first right or left costal prevented the transmitter from interfering with mating when males mounted females. We did not measure the effect of transmitters on tortoise behav- ior, but did observe several instances of males mounting females unobstructed by the transmitter and two transmittered animals successfully righting themselves after falling on their carapace.

Attaching the transmitter to the first right or left costal scute generally resulted in a fairly flush alignment with the top of the carapace, thus minimizing problems that could occur when tortoises with transmitters turn around inside their burrows. Three of our transmittered animals were found stuck in collapsed burrows following an unusually rainy winter, but we were unable to determine if the transmitters contributed to burrow collapse or tortoise entombment. None of three known mortalities of our transmittered animals were attributed to the presence of the trans- mitter (one was a road kill, one probably died from a respiratory disease, and one died of unknown causes).

Discussion.-Based on five years of observation, the method described herein successfully reduced loss of transmitters, increased transmission range, and prevented deformation of the shells, while minimally altering the animals'behavior. However, experiments designed explicitly to measure transmitter effect were not conducted.

Transmitter design is a three-way compromise between battery size, longevity, and transmission range (Brander and Cochran 1969; Macdonald and Amlaner 1980; Mech 1983). We found an- tenna orientation to affect transmission range. We found that trans- mission range was increased by allowing the antenna to lie across the top of the carapace. This orientation likely reduced nulls in the transmission signal caused by an open loop and reduced slightly ground attenuation (Mech 1983).

Allowing the transmitter and/or antenna to bind together two or more scutes may cause deformation of the shell as the animal grows. If the antennas were attached directly to the shell with epoxy, they would connect several scutes together for as long as the transmitter was attached; which may be the life of the animal if the animal becomes lost with the transmitter still attached. This would be particularly critical in rapidly growing turtles (e.g., hatchlings and juveniles). Although undocumented, shell defor- mations could be hazardous if they impede normal behavior or damage underlying tissues (B. Homer, pers. comm.).

We found that our transmittered tortoises were still able to mate apparently unimpeded by the transmitter and were able to successfully right themselves if tipped over during mating or aggression. Eckler et al. (1990) also observed the behavioral effect of attaching transmitters to 45 bog turtles *(Clemmys muhlenbergii),* and reported seeing successful foraging, mating, and nesting. They epoxied the transmitters to the fourth costal scute and attached the antennae directly to the carapace.

The method chosen for attaching radio transn-titters depends on the size, behavior, potential future growth, and catchability of the species, as well as characteristics of the environment and the principal study objectives (e.g., length of study, type of data desired). It is essential that the transmitter not affect significantly the behavior, survival, or reproductive success of the study animals. Therefore, for relatively long-term applications (the length of time depends on the animal's growth rate, which depends in part on the animal's age), attachment should avoid causing shelf deformation. Studies should be conducted to evaluate the effect that transmitters and their attachment methods have on turtles and tortoises with the results reported in the literature. Furthermore, stud- ies usinc, radio transmitters should provide sufficient detail on attachment methods to allow readers to evaluate the potential ef- fect the transmitters may have on the animals and the study's re- sults.

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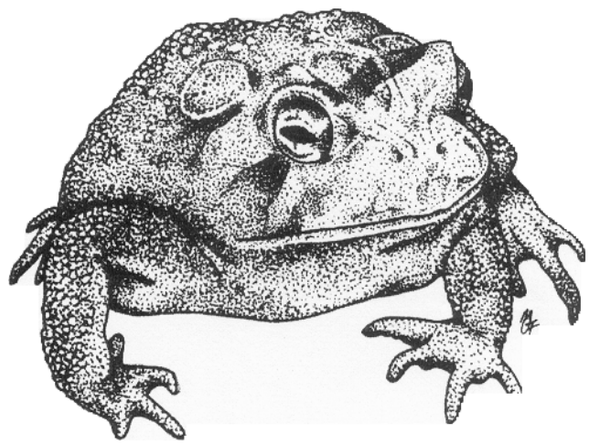

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Radiotransmitter Attachment Technique for Box Turtles (*Terrapene* spp.)

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Terrestrial chelonians are often difficult to locate and monitor under natural conditions because many species are cryptic in coloration and behavior. For example, the eastern box turtle (*Terrapene carolina carolina*) has a carapace often spotted with yellow or orange, and brown or black, a pattern that easily blends into substrate of leaf litter. In addition, eastern box turtles bury themselves in leaf litter (i.e., in forms) and spend long periods underground in burrows. In the northeastern United States, eastern box turtles may be easily overlooked during visual surveys and marked individuals often cannot be relocated with any regularity (pers. obs.). Radiotelemetry, which allows observers to relocate an individual on demand, has become an essential tool for studying the movements, home range, habitat use, and other characteristics of box turtles and other chelonians that are difficult to locate and observe.

Application of radiotelemetry to the study of small animals, such as terrestrial turtles, can be challenging. Biologists must consider not only the objectives and constraints of the study, but also the welfare of the animals; many universities and research institutions now require that methods used in live-animal studies be reviewed and approved by an institutional animal care and use committee before implementation. Most studies require that a radiotransmitter remain attached to an individual for an extended period of time; however, the radiotransmitter and attachment must not affect the behavior, physiology, reproductive success, or survival of the study animal if accurate observations are to be obtained. In the case of box turtles, the radiotransmitter and attachment must not excessively burden the animal, become entangled in vegetation, increase rate of detection by predators, pose an obstruction to burrowing, or disrupt copulation or other behaviors. Methods of attachment, such as adhesives or hardware, must be nontoxic and should not increase chances of infection or disease, cause significant injury or developmental abnormalities, or produce potentially damaging amounts of heat. Ideally, the method of attachment should allow the radiotransmitter to

be replaced or removed as necessary without causing injury or stress to the study animal.

We attached radiotransmitters to adult eastern box turtles (*T. c. carolina*) as part of a study of their habitat use, home range, and movements in southeastern Massachusetts. To attach the radiotransmitters, we modified a method used by Boarman et al. (1998) to attach radiotransmitters to larger adult desert tortoises (*Gopherus agassizii*). Our goals in selecting a radiotransmitter and attachment method were to minimize: 1) percent of the turtle's body mass of the radiotransmitter and its attachment; 2) the profile of the radiotransmitter and attachment against the carapace, 3) the likelihood the radiotransmitter would interfere with copulation; and 4) handling time and duration of captivity required during radiotransmitter attachment and replacement. We also wanted to maximize the range of signal detection and radiotransmitter life. Here we describe our adaptation of Boarman et al.'s (1998) method and report on the results.

Methods. — We used Advanced Telemetry Systems (Isanti, MN) Model 7PN radiotransmitter (164 MHz range with 1/4 wave antenna) modified for use on box turtles. We selected this model, in part, because its dimensions allowed us to attach it to a single pleural scute with little or no overlap of scute sutures. Radiotransmitter length (mean = 40.0 mm, $n = 11$) was similar to the length of a pleural scute of an adult turtle, while width (mean = 18.8 mm, $n = 11$) was much smaller than the width of the scute. The profile of the radiotransmitter was relatively flat (mean height = 9.6 mm, $n = 11$) and would not greatly increase the profile of the animal when attached. Finally, radiotransmitter mass was low (mean = 12.5 g, $n = 30$), and radiotransmitters were guaranteed to emit a signal for 130–260 days.

After locating an adult study turtle, we attached a radiotransmitter to it using one of two modifications made to the method described by Boarman et al. (1998). Our first modification ("epoxy base") was to attach the radiotransmitter by bonding the entire bottom surface of the radiotransmitter to the third pleural scute of the carapace with waterproof epoxy putty (as described by Boarman et al., 1998), then threading the antenna through pieces of aquarium tubing bonded to pleural scutes (Figs. 1 and 2). Our second adaptation ("silicon base") was to attach the radiotransmitter to the carapace by creating two "brackets" of putty on the edges of the radiotransmitter, with a cushion of nontoxic aquarium silicon sealant between the radiotransmitter and the carapace (Figs. 1 and 2). Both attachment methods are described as follows.

A radiotransmitter attachment kit was prepared in anticipation of capturing turtles. This kit contained 2–4 radiotransmitters, nitrile gloves, cotton balls, isopropyl alcohol, non-toxic silicon aquarium sealant, non-toxic adhesive epoxy putty (Power Poxy, Plug n' Patch Epoxy, Plumbers Formula, #40102, Power Poxy Adhesives, Inc., New Berlin, WI), pre-cut 5 and 10 mm pieces of 3 mm internal diameter aquarium tubing, masking tape, a pocket knife, non-toxic markers (Sharpie, Sanford Corp., Bellwood, IL) and paint pens (Speedball Painters, Hunt MFG Co., Statesville, NC), metric ruler, calipers, and a portable electronic balance (Ohaus Model LS 2000). All radiotransmitters were tested before being placed into the kit, and kit materials were replenished as needed.

Upon locating a turtle we put on nitrile gloves. These gloves remained on during the attachment process. Before attaching the radiotransmitter we measured and recorded carapace length, width, turtle mass, and the time of capture.

We used a cotton ball moistened with isopropyl alcohol to remove dirt from the pleural scutes. We then placed the turtle in a cardboard box and allowed the scutes to dry. Once the alcohol dried we covered the sutures of the third pleural scute, typically the left side, with masking tape. The third pleural scute, posterior to the widest part of the carapace, was selected as the attachment location so that vegetation and other materials would pass over the carapace without getting caught on the radiotransmitter.

Our initial attachment method ("epoxy base") was to cover the bottom of the radiotransmitter with a layer of adhesive epoxy putty (Fig. 2). Our second approach ("silicon base") substituted a thick layer of silicon sealant for the adhesive putty (Fig. 2). In both methods the radiotransmitter, with the antenna extending forward, was then positioned on the bottom of the third pleural scute. The radiotransmitter was pressed against the carapace and held in place. When using the silicon base method, we were careful to leave a layer of silicon approximately 4 mm thick between the radiotransmitter and the carapace and not to squeeze the silicon out from under the sides of the radiotransmitter.

We then prepared approximately 1/5 package of nontoxic epoxy putty according to the directions on the packag-

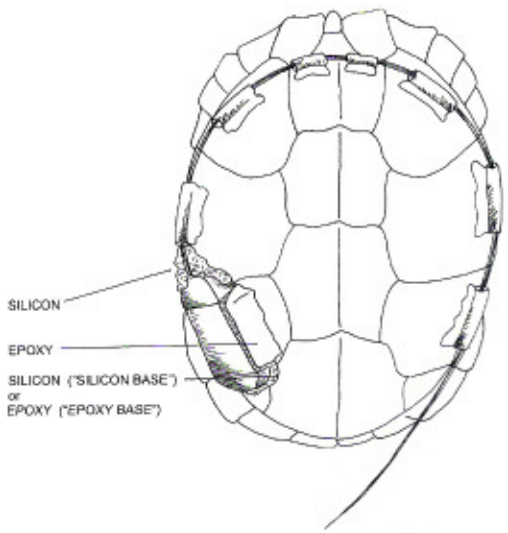

Figure 1. Dorsal view of a box turtle carapace showing position of radiotransmitter, antenna, and attachment materials.

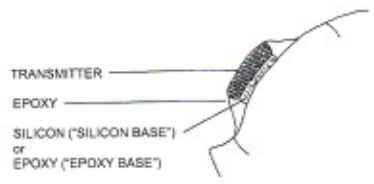

Figure 2. Cross sectional view of a box turtle carapace showing position of radiotransmitter and attachment materials.

ing. We prepared two strips of putty, each approximately 40 mm long by 10 mm wide, and placed a strip lengthwise along each side of the radiotransmitter. This epoxy putty was either added to the putty under the radiotransmitter (epoxy base), or formed brackets that held the radiotransmitter to the carapace (silicon base). We pressed the putty onto the radiotransmitter and scute and removed excess putty with a pocketknife. We removed the masking tape and any putty from the sutures.

We attached sections of aquarium mini-tubing to the first and second pleural scutes on the side with the radiotransmitter, first vertebral scute, and the first through third pleural scutes on the side opposite the radiotransmitter. A single 10 mm long piece of tubing encased in a small amount of putty was positioned on the bottom, center of each pleural scute and pressed against the carapace. Two 5 mm sections of tubing, one to either side of the keel, were attached to the vertebral scute. We threaded the antenna through the tubing as we attached each piece, and removed any putty that blocked the openings in the tubing or covered a suture. We then checked the antenna to ensure that it moved freely and would pull out of the tubing if it became entangled in vegetation. We removed excess putty from the sides of the tubing by cutting it away.

To prevent entanglement in vegetation or debris we filled and contoured the anterior and posterior ends of the radiotransmitter with silicon sealant. We filled the space between the anterior end of the radiotransmitter, antenna, and first piece of tubing (Fig. 1), and also filled the space between the posterior end of the radiotransmitter and the carapace with silicon.

Once the putty had set (ca. 10 min) we used non-toxic paint pens and permanent markers to camouflage the radiotransmitter and putty with colors and patterns similar to those of the turtle's carapace. We then measured the combined mass of the turtle and radiotransmitter. We wrapped masking tape over the radiotransmitter to cover the silicon until it was dry, and used non-toxic paint pens and markers to camouflage the tape.

We released the turtle and checked the signal. We relocated the turtle within two days of radiotransmitter attachment and removed any remaining masking tape. We located each turtle 1-3 times per week with a Telonics Model TR-2000 receiver and an "H" antenna from the day of capture through mid-November 1998 and sporadically thereafter through July 1999. We attempted to obtain visual confirmation of each turtle's exact location and to inspect radiotransmitter condition whenever we monitored our study animals.

Results. — We attached radiotransmitters to 19 adult eastern box turtles using the putty base, and to two turtles with the silicon base method. Handling time for both methods, including radiotransmitter attachment and data collection, was 38–70 min (mean = 49 min). Handling time generally decreased as personnel became more proficient in radiotransmitter attachment. Radiotransmitters and attachments (i.e., tubing and adhesives) added 2.5–5.0% (mean = 3.9%, $n = 19$, excluding two individuals that urinated during handling) or 14–26 g (mean = 19.71 g) to an animal's mass.

Radiotransmitters functioned properly and remained firmly attached to our study animals for as long as 407 days; in some cases, however, radiotransmitters apparently failed after only a few weeks. Despite extensive searches, we were only able to locate three individuals through days 16, 71, and 75 after radiotransmitter attachment; we believe that these radiotransmitters failed. One other individual left the study area and we were unable to track its exact location, although we were able to confirm that the radiotransmitter was working 165 days after it was attached. Two individuals died during the study; one of unknown causes 23–32 days after radiotransmitter attachment, and the second was crushed by land-clearing equipment off our study area 5 days after radiotransmitter attachment. Between May and July 1999, nine radiotransmitters were still functioning 347–407 days after attachment. Seven were removed and replaced and two were left "as is" on the turtles (M. Ciaranca, Camp Edwards Environmental Protection Office, *pers. comm.*).

Our ability to detect a signal varied with the location and behavior of the turtle. When turtles were underground in burrows (generally ≤ 0.3 m below the surface), the distance at which we could detect the signal was highly variable. In most cases, signals from burrows could be detected several hundred meters from the burrow. However, in a few instances antennas appeared to have grounded out against the walls of the burrow and signal strength was greatly reduced. In one such case we were able to detect a signal from a turtle in a burrow only from approximately 10 m away. This turtle's signal was later discernable 100–200 m from the burrow (although the turtle remained inside). When turtles were active or in forms above ground, however, we could monitor their signals from as far as 963 m away through flat woodland habitat. Signal range also increased when part of the antenna pulled out of the plastic tubing and trailed freely. In all cases, we were able to identify an individual's exact location whenever a signal was detected and the turtle accessible.

Field observations suggest that our radiotransmitter attachment design did not impede reproduction. On two separate occasions we observed females with radiotransmitters copulating with unmarked males.

Discussion. — We attached radiotransmitters to the first 19 adult turtles that we captured during our study by bonding the entire bottom surface of the radiotransmitter to the carapace (epoxy base) with the same brand of putty adhesive used by Boarman et al. (1998). Although the manufacturer's stock number of the epoxy putty that we used was different from that used by Boarman et al. (1998), the manufacturer assured us that the two products were identical with the exception of package size. We therefore expected similar results with radiotransmitter removal. When one of the turtles in our study died 15–21 days after radiotransmitter attachment, however, we tried to remove the radiotransmitter from the frozen specimen with a pocketknife (as recommended by Boarman et al., 1998) and found that we were unable to do so. Our attempts to cut away the putty failed and

eventually a large section of the scute broke away from the carapace. Had the turtle been alive, it may have been seriously injured. We had similar results when trying to remove a radiotransmitter from the remains of a second individual that was killed by land-clearing equipment. These observations reinforce the caution made by Belzer and Reese (1995), who noted that prying off radiotransmitters bonded to turtles with epoxy can tear scutes. However, in May 1999, approximately 12 months after the start of our study, radiotransmitters that had been attached to turtles for 347–366 days were easily removed without damaging scutes (M. Ciaranca, *pers. comm.*). Whether the putty degraded over time or whether natural sloughing of scute material, or both, caused the radiotransmitters to loosen over time is unknown. Regardless of cause, it appears the putty base method of attachment is acceptable if radiotransmitters are expected to remain attached to turtles for extended periods of time, such as the 11–12 months that elapsed between attachment and removal in this study. However, we are hesitant to recommend this method in cases where radiotransmitter life is shorter, incidence of radiotransmitter failure is high, or recovery of radiotransmitters after only brief periods of attachment is otherwise anticipated.

Because attempts to remove radiotransmitters from two dead turtles resulted in damage to scutes, we further modified the method used by Boarman et al. (1998) in order to minimize the amount of putty in contact with the carapace. Our silicon base method created a space between the radiotransmitter and carapace that provided working room for tools that would later be used to remove the radiotransmitter. We anticipated that a carefully used cordless rotary hobby tool (e.g., Dremel) with a cutting disk could readily cut through the strips of epoxy putty along the sides of the radiotransmitter. Because the radiotransmitter was not in direct contact with the carapace (due to the silicon pad) the cutting disk would not contact the scute and therefore would not damage the turtle. After the old radiotransmitter was removed, a new radiotransmitter could be attached to the putty that remained bonded to the carapace.

We believe the silicon base method appropriate for both long and short-term attachment of radiotransmitters. One turtle with a silicon base mounted radiotransmitter was relocated 357 days after attachment, indicating that the longevity of this attachment method is comparable to that of the putty base method. The main benefit of the silicon base method is that it permits the safe removal of radiotransmitters at any time without requiring either the epoxy or scute surface to degrade. The ability to remove radiotransmitters shortly after attachment may be especially important in the event of premature radiotransmitter failure. We recommend this approach for all applications because it minimizes the likelihood of turtles being injured during the radiotransmitter removal process.

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Rediscovery of the Critically Endangered River Terrapin, *Batagur baska*, in Cambodia, with Notes on Occurrence, Reproduction, and Conservation Status

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The river terrapin, *Batagur baska* (Gray, 1831), inhabits coastal rivers, estuaries, and mangrove swamps from eastern India and Bangladesh, to Myanmar, southern Thailand, Cambodia, Cochinchine (southern Vietnam), peninsu-

Annexe 4 :

exemple de fiche de suivi par radiopistage

Annexe 4 : exemple de fiche de suivi par radiopistage

Numéro identification[1]	Fréquence[2]	Date[3]	HHMM[4]	R[5]	Site fixe[6]	X-récep[7]	Y-récep[8]	Azimut[9]	Observations[10]

1) Numéro identification (selon le système de marquage) : numéro d'après la lecture des encoches.
2) Fréquence : fréquence de l'émetteur.
3) Date : jour du suivi.
4) HHMM : heure et minute de la localisation (moment où l'on détermine l'azimut).
5) R : récepteur (dans le cas d'utiliser des récepteurs différents, indiquer toujours celui qui a été utilisé).
6) Site fixe : il y aura normalement des sites « fixes » de localisation (les sites les plus favorables) ; il faut prendre leurs coordonnées avec précision, ce qui nous épargnera le travail de déterminer chaque fois la position à l'aide du GPS (p.ex. site nº1 : bout de la digue sud, sur le promontoire de terre).
7) X-récep : coordonnées X avec le GPS de la position du récepteur (si on n'est pas dans un « site fixe »).
8) Y-récep : coordonnées Y avec le GPS de la position du récepteur (si on n'est pas dans un « site fixe »).
9) Azimut : angle par rapport au nord indiqué par la boussole.
10) Observations : si la cistude est observée, indiquer l'habitat et le comportement. D'autre remarques / problèmes avec la localisation.

Chaque localisation d'une cistude doit être faite avec deux contacts (2 lignes de la fiche), car il faudra trianguler pour connaître la position de la cistude dans la mare. Avec deux récepteurs et deux personnes, les localisations peuvent être simultanées. Sinon il faudra faire les deux localisations sans délai (± 5 – 10 minutes entre localisations).

Annexe 5 :

article d'évaluations par étapes

Bertolero, A. 2003. Assessment of reintroduction projects: the case of the Hermann's tortoise. Proceedings of the IUCN Turtle Survival Alliance 2003 Conference. Orlando, Florida.

Orlando, Florida August 18-19, 2003

TSA

turtle survival alliance

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IUCN Turtle Survival Alliance 2003 Conference

Sunday August 17

Evening ice breaker at Disney – Lonnie McCaskill

Monday August 18

8:00 Welcome – Lonnie McCaskill
8:10 James Barzyk – TSA overview
8:20 Kurt Buhlmann- Update on the Turtle Conservation Fund
8:50 Dwight Lawson- his role in TSA
9:00 Rick Hudson- - A Vision for the Future of TSA
9:20 Hans Dieter Phillipen- TSA Europe update
9:40 Gary Ades- Chelonian conservation at Kadoorie Farms Botanical Garden, Hong Kong

10:00 Break

10:20 Darryl Sennecke – TSA Website update
10:40 Gerald Kuchling: Recovery plan for Australasian turtles
11:00 John Behler- Conservation of Pyxis planicauda
11:20 Kurt Buhlmann and Gary Ades – Natural history and conservation of Platysternon
11:40 David Fabius – Natural history and conservation of Acanthochelys
12:00 Cris Hagen- Observation of Leucocephalon yuwonoi in the field
12:20 Questions

12:30 Lunch

1:30 Susan Donoghue – Captive chelonian nutrition
1:50 Kurt Buhlmann - Gopherus relocation project at Savannah River Ecology Laboratory
2:10 Gerald Kuchling - Pseudemydura captive breeding and conservation
2:30 Gerald Kuchling - Conservation of river turtles in Burma
2:50 Albert Bertolero- Assessment of reintroduction projects: the case of Hermann's tortoise in Spain
3:10 Jean Marc Ducotterd- Centre de protection et de recuperation des tortues (Switzerland)

3:30 BREAK

3:40 Ghislaine Guyot-Jackson- The centre d'elevage et de protection de la tortue, A cupulatta, Corsica
4:00 Dwight Lawson - Cheng Du Zoo Manouria impressa program and other activities of Zoo Atlanta
4:20 Richard Ogust and Susan Tiedeman- Tewksbury Institute of Herpetology: Operational Status
4:40 Hans Dieter Phillipen -Update on the Allwetter Zoo Asian Turtle Captive Breeding Project
5:00 Kamuran Tepedelen – Chelonian field observations and commercial trade in Sulawesi and Sumatra
5:20 Questions

5:30 Dinner

7:30 Studbook and Population Management workshop for TMG Coordinators– Sarah Long, AZA Population Management Center

Tuesday August 19

8:30 Open microphone: Questions for speakers and TSA Steering Committee
9:30 Chuck Schaffer- A survey of Manouria emys in Malaysia and Thailand
9:50 Vic Morgan – Update on Manuoria emys Taxon Management Group and captive breeding
10:10 Terry Norton –Medical management of Manouria emys and other endangered tortoises at Saint Catherine's Island

10:30 Break
10:40 Don Moll – Studies of Chitra chitra in Thailand
11:00 Scott Davis- Captive husbandry of Asian softshell turtles
11:20 Phil Spinks: Phylogenetic hypotheses for the turtle family Geoemydidae
11:40 Paul van der Schouw: Tips on establishing and coordinating a Taxon Management Group

12:00 Lunch

1:00 Working sessions: Taxon Management Groups - Sarah Long, AZA Population Management Center

2:20 Hugh Quinn- The species recovery plan for the river terrapin (Batagur baska): integrating components of a species recovery plan.
2:40 Mike Forstner- Heosemys grandis Taxon Management Group status; and the significance of Hemogregarine parasites in captive chelonia
2:50 Mike Forstner – Important genetic issues for small captive populations
3:00 Mike Forstner- Pond systems and filtration design
3:10 Gerald Kuchling- Erymnochelys captive breeding/conservation

3:30 Break

3:40 Elliott Jacobson- Implications of infectious disease in captive management and relocation of chelonians
4:00 Peter Pritchard- Galapagos tortoises: 100 years of management, good and bad
4: 20 Charles Innis – Update on the TSA Veterinary Group, Leucocephalon yuwonoi Taxon Management Group, and intranuclear coccidiosis in Indotestudo forsteni
4:40 Questions and Closing remarks

Poster Session
Ghislaine Guyot-Jackson: Manouria: how to reach the public for conservation causes

Chuck Schaffer: A comparison of in-situ and ex-situ studies of Manouria emys

Assessment of reintroduction projects: the case of the Hermann's tortoise

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Key words: Hermann's tortoise, reintroduction, *Testudo hermanni*.

Introduction

Due to the worrying situation in which many chelonians populations are, different reintroduction projects have been carried out in numerous countries both in marine species as in freshwater and terrestrial. In the future, as the situation of many chelonian populations will worsen, it is foregone that the number of conservation projects will increase; in which one of the performance phases will be the reintroduction of specimens in the environment.

A reintroduction project bears the realization of four main phases, which in summary are made up of:
1 - Planning. It is the starting point in which the viability of the project should be analyzed.
2 - Performance. If the evaluation has been positive this second phase, which is formed by the selection and liberation of the specimens in the environment, will follow.
3 - Evaluation of the project. It will allow to know if success has been reached or not and which are the reasons for the obtained result.
4 - Publication of the results. It will allow to approach new projects in a better way and not to repeat mistakes.

As logical as this procedure might seem, many re-introduction projects only pay attention to the second phase; therefore, several projects are undertaken without any planning or an assessment of their results (Scott and Carpenter, 1987). Unfortunately, when this happens, the following points are not usually valued:
1 - The survival of the liberated specimens is put in danger. This is inadmissible, especially when we are dealing with threatened species.
2 - Economic resources are wasted. All the phases of a reintroduction project have an economic expense; even getting and liberating the specimens can constitute an expensive process (Scott and Carpenter, 1987; Boyer and Brown, 1988; Nielsen, 1988; Fischer and Lindenmayer, 2000). That's why, wasting funds that can contribute in the conservation of nature is also inadmissible (Seigel and Dodd, 2000).
3 - If a reintroduction project is not evaluated it is not possible to determine its results or correct its errors, and therefore, it is impossible to determine if it has reached its objectives (Dodd and Seigel, 1991; Fischer and Lindenmayer, 2000).
4 - If the planning and/or the results are not available for the rest of the society, they don't serve as base for new projects. In this way, new reintroduction projects cannot benefit from the experience obtained in previous projects, wasting successful handling options or repeating erroneous handling options.

The purpose of this work is to determine how the obtained results can be evaluated in a reintroduction project from the accumulated experience during the twelve years of monitoring

of the conservation project of the Hermann's tortoise, *Testudo hermanni*, in Delta de l'Ebre Natural Park (NE Spain).

Brief antecedents of the reintroduction project

The population of Hermann's tortoise studied originated from 44 adults tortoises introduced in Delta de l'Ebre Natural Park between 1987 and 1988 with the objective of preserving this species in the county of Tarragona (Bertolero et al., 1995; Bertolero, 2003). A second group of 22 adults tortoises was liberated in the same area between 1997 and 1998. Between 1991 and 2002, an intensive pursuit of the population was carried out and 448 tortoises were marked. They all belonged to the first and second generation born in freedom (Bertolero, 2003). This population lives in dune "islets" with small slopes fixed by psammophile and halophile vegetation. The space around the islets lacks vegetation and sea cover depends on the season and weather.

Structure of the Evaluation Process

Once the realization of a reintroduction project has been valued positively and the specimens have been liberated in the environment, a new stage of the project, which consists on its evaluation, begins. Most species of tortoises are characterized by a great longevity, reaching the sexual maturity after several years and being iteroparity. Due to this, the only way of carrying out a real evaluation of the project is by means of planning a long term pursuit. According to Dodd and Seigel (1991), it should be superior to 20 years in chelonians. However, the necessity to carry out a long term pursuit does not imply that it is intensive along this time.

The evaluation of the project determines if a reintroduction has been successful or if it has failed. Moreover, it provides (or tries to determine) the causes that give this result. In a succinct way, one can say that success has been reached when a self-sufficient population is obtained from the liberated specimens (Griffith et al., 1989; Dodd and Seigel, 1991; Fisher and Lindenmayer, 2000). On the contrary, it will have failed if the population has extinguished or if it is not self-sufficient. In both cases, it is important that the causes that give these results are analyzed, since they will allow to improve the design of new reintroduction projects. On the other hand, in many works of reintroduction different definitions of success have been used according to the expectations in the given moment of the evaluation (eg, Burke, 1989; Short and Turner, 2000; Nelson et al., 2002), because long term pursuits are usually required in order to determine the viability of a population, which is not always possible to carry out. Determining the viability of a population can seem a too distant objective in many reintroduction projects, it is proposed in this assignment that it is better to define an evaluation in stages if the required objectives are reached in each one of them. In this way, objectives can be evaluated in short and medium-term, which will be useful for the identification of possible problems that arise in the development of the reintroduction project to finally evaluate the long-term objective that should always be getting a self-sufficient population. Nevertheless, because a great variation exists in the vital cycles of the different chelonians species, it is not possible to establish a general time rule which should last each evaluation term. Therefore, the objectives and the duration of the terms in the evaluation of a reintroduction project of the Hermann's tortoise will be indicated here. In addition, it is proposed that these objectives and terms can serve as reference to evaluate reintroduction projects in other chelonians species, provided the necessary adaptations for each species are carried out. When the species is present and what is intended to be done is a reinforcement or translocation, some of the considered recommendations should be set out differently.

To evaluate the reaches of a reintroduction it is very important to keep in mind the age of the liberated specimens. As Bertolero suggests (2003) for the Hermann's tortoise, the reintroduction of adults or youngsters from six years of age is more advantageous than to carry it out with younger specimens, in which this age group will be mainly considered here. Lastly, some of the

considered points require to carry out an intensive monitoring, while in others it needs to be made along several years, but without having to be intensive each year.

Evaluation of short-term objectives

It is considered that the ideal situation is to liberate most individuals equipped with radio transmitters (if it is not feasible, in a representative sample) and to carry out a radiotracking of at least a year of duration. Some of the objectives developed in this section are only reachable by means of this methodology, while in others it is only required to carry out appropriate samplings.

Short-term is defined by the time that lapses between the moment of liberation of the specimens and the first three years that they remain in freedom. During this time, it was considered important to evaluate the following aspects (summary in Table 2): 1) body condition of the liberated specimens; 2) growth in the event of being liberated young; 3) dispersion of the liberated specimens; 4) reproduction in case the specimens are liberated mature; 5) survival; and 6) permanency of the liberated specimens.

Body condition of the liberated specimens
The liberated tortoises should adapt themselves to the new habitat conditions they find. In this period of adaptation, they can experience a decrease in their physical condition, but once this process of adaptation is overcome their physical condition should recover. As an index of body condition, the one proposed by Hailey (2000) for the Hermann's tortoise can be used. One must keep in mind that if the tortoises come from captivity, they can show a body mass superior to the normal one. Hence, it is more appropriate, if possible, to compare their body mass with one in wild populations.

After liberation, a detailed monitoring of the evolution of the body mass will determine if the liberated tortoises adapt themselves to their new habitat and/or if this has the conditions to guarantee their survival (partial success). On the other hand, if its weight diminishes continually, it is a symptom that the adaptation fails or that the habitat doesn't offer the necessary resources (failure). For example, pursuits of this type have been carried out with angonoka, *Geochelone yniphora*, in Madagascar (Pedrono and Sarovy, 2000) and Hermann's tortoise in Castelló, Spain (Marta Aguiló, com pers.).

Growth in the event of being liberated young
If the liberated young tortoises have adapted themselves appropriately to the conditions of their new habitat, it can be considered as partial success that their rates of growth are comparable to those that are shown in wild specimens of the same age. In general, if the specimens don't grow during the period of activity, it should be considered that the process of adaptation has failed or that the habitat conditions are not the appropriate ones to satisfy their necessities.

Dispersion of the liberated specimens
The reaction of the specimens in the moment they are liberated in a new environment can be as follows: 1) they stay near the area of liberation; 2) they scatter until they find a favourable area or one they like; 3) they don't settle down in any particular place and they acquire a nomadic behaviour; and 4) each specimen reacts in a particular way and because of this, the three previous situations could be present within one group. In the face of these situations, the dispersion can be evaluated from at least two perspectives: 1) home range size; and 2) density of specimens.

Adults Hermann's tortoises show stable home range and a high percentage of intraindividual overlap along the years (Bertolero, 2003). Hence, it would be interpreted as partial success that each individual's home range have a stable size and a high overlap percentage during the first three years. This would also indicate that the tortoises have developed a homing behaviour in

the area of liberation, as it has already been described in natural populations of the Hermann's tortoise in Italy (Chelazzi and Francisci, 1979, 1980).

If the introduced specimens are dispersed from the point of liberation, it is not possible to obtain a good density of specimens to be able to constitute a population. In the case of the Hermann's tortoise, there is abundant information on the densities in which different populations are found (revision in Cheylan, 2001), so a good approximation to use would be a minimum value of three individuals, which is the inferior range where several of them are located. Moreover, to reference title, the density of introduced specimens in Ebre Delta in 1988 was of at least 4.6 individuals (not including two males that died in 1988 and a female that died on an unknown date, but between 1987 and 1990). On the other hand, for the approximation of density to make sense, the initial area where the population should settle down should be established with precision (without including, if there were any, the possible dispersion areas, expansion and/or colonization).

Reproduction in the case of liberating mature specimens

As Dodd and Seigel (1991) suggest, the fact of finding hatchlings is not enough to demonstrate the success of a project, because the only thing that this demonstrates is that the species has been able to reproduce in its new atmosphere. However, according to the evaluation approach in stages it can be considered as partial success, since it provides information on the conditions where reproduction takes place. This way, if the reintroduced tortoises have adapted to the conditions of the environment and there they find the resources and appropriate setting places, it would be hopeful to find hatchlings starting from the first or second breeding season. If it doesn't turn out that way, it could be due to the lack of appropriate breeding places and, therefore, the habitat wouldn't show the required conditions to maintain a population. Another cause could be that the tortoises have not been capable enough to adapt to the new conditions in which they are, or that negative factors of type abiotic exist (eg, contamination) or biotic (eg, high rates of nest depredation) with a great incidence in the process of the population's formation. Lastly, it is important not confuse the non reproduction with the non detection of hatchlings, because during the monitoring samplings should be designed in order to check the reproduction (eg, intensification of the samplings at the time of nestings or hatches).

Survival

If radiotracking of the liberated specimens is made, their survival can be calculated through a variety of calculation methods (see revision in White and Garrott, 1990). The methods of capture-recapturing don't allow to obtain good enough estimates in the period of time considered in the short-term evaluation.

After the phase of adaptation, in which you can detect a liberation cost (Sarrazin and Legendre, 2000), estimates of survival similar to those of the wild populations should be obtained. The value of minimum survival that could be accepted as reference is 0.81, which is the inferior limit that is found in one of the wild populations of the Hermann's tortoise (revised in Cheylan, 2001). During the short term-evaluation, if very low estimates of survival are obtained in comparison to those in wild populations (<0.81), this could be inferred as a lack of adaptation of the liberated specimens or that the habitat doesn't gather the appropriate conditions for survival (lack of resources, refuges, areas with favourable microclimates, excess of predators and/or spoliation effects).

Permanency of the liberated specimens

From the moment that the tortoises are liberated, the realization of censuses or samplings allows to establish the population's size for a definite period of time (eg, at the end of the activity time). Considering that the population is closed, diverse capture-recapture methods exist to estimate its size (revisions in White et al., 1982; Pollock et al, 1990; Williams et al., 2002). The quotient among the estimated number of specimens that exists in the area at the end of a certain period and the number of exemplary reintroduced (which is known) can be used like a permanent

estimate of the exemplary reintroduced in the liberated area. In fact, this quotient is an estimate of minimum survival when the population is closed and it can be compared with the values of the wild populations' survival as in the previous section. However, in this case, low values (<0.81) can indicate an effect of dispersion of the exemplars reintroduced (that is equal to mortality at population's level) and not a real inferior survival.

Evaluation of medium-term objectives

It is defined by medium-term the time that lapses from the fourth year since the first tortoises were liberated and the first year in which the tortoises born in freedom reproduce (obtaining the first generation in freedom starting from specimens already born in freedom). During this period, the aspects to evaluate are (summary in Table 2): 1) survival; 2) reproduction of the specimens born in freedom; 3) structure of the population; and 4) colonization.

Survival

The annual rate of survival of the reintroduced specimens should be calculated by means of capture-recapture analysis (eg, Lebreton et al., 1992). Thus, it is important to carry out a minimum of samplings every year in order to obtain better estimates of survival and not to have to include years with a recapturing probability similar to zero. This way, from the moment when the reintroduction is carried out until the end of the first year of freedom, it is possible that the introduced specimens show a higher mortality than the expected one in a natural population of specimens of the same age. The causes of this mortality include lack of adaptation to the new habitat and unknown of the favourable places for their survival (eg, hibernation places). However, it is also expected that at the beginning of the second year, the survivors are already better adapted to their new habitat and, consequently, the rates of survival from the second year onwards should be similar to those of the natural populations. This type of hypothesis (liberation cost; Sarrazin and Legendre, 2000) can be tested by means of analysis of survival (Bertolero, 2003). Like in the case of short-term objectives, an indicator of success will be obtaining estimates of average survival similar to those that the natural populations show. In this case, it is considered that they must be superior to 0.90, the approximate value of the lowest average estimates found in stable natural populations of the Hermann's tortoise (revision in Cheylan, 2001).

Reproduction of the specimens born in freedom

It is important to determine if the individuals born in freedom reach the reproduction age and if they reproduce regularly, since they are the key for the formation of a viable population (Bertolero, 2003). Different approximations exist to determine if the tortoises can reproduce. In the males, you can determine if they are reproductive by observing their copulative behaviours (Hailey, 1990; Bertolero, 2003). In the females, this method is not considered so effective (Hailey, 1990), but other ways of determining their reproduction exist, such as: 1) the observations of nesting behaviours (although they require an important observation effort); 2) by means of inguinal palpation to detect the presence of calcified eggs (Ewert, 1989; Bertolero and Marín, 2000); 3) radiography (Gibbons and Greene, 1979); and, 4) by means of ultra-sound scanning, with the advantage that not only does it detect calcified eggs, but it also allows to evaluate the follicular activity (Kuchling, 1998). Lastly, partial success would have been reached if it had been detected that both sexes of the tortoises born in freedom were reproductive.

Structure of the population

From the number and the age of the existing juveniles every year, you can determine if the tortoises that are born are represented in all the possible age classes. In general, if all the age classes are represented, this indicates that the reintroduced tortoises reproduce regularly every year and that the reproduction is not an isolated or sporadic fact. On the other hand, this also indicates that the tortoises born in freedom show some rates of appropriate survival to be represented in all the age classes of the population. This situation was found in the population of

the Hermann's tortoise introduced in Delta de l'Ebre. In every year, between 1991 and 2002, tortoises were registered in all the age classes that the population could show (Bertolero, 2003). To determine the number of breeding of each age class, capture-recapture estimates can be used (Pollock et al., 1990; Williams et al., 2002; however, see Koper and Brooks, 1998) or total recounts (in small areas and with intense sampling), as it was used in Delta de l'Ebre. High rates of average survival for each age class, evaluated by means of capture-recapture analysis can also be interpreted as if the tortoises are in an appropriate habitat, thus allowing their survival. The fact that the breeding of all the possible ages that can be present in a certain year and that the survival estimates of the tortoises born in freedom are high, can be interpreted as partial success.

Colonization

If the liberated individuals are able to establish a population and it continues growing, then it is foregone that, as the density of specimens in the original area increases, an emigration and colonization toward new favourable areas is carried out. Starting from the place of reintroduction, the expansion toward new areas is considered partial success as well as the regular detection of reproduction in these areas. Contrary to the dispersion, the colonization implies the emigration of more than a specimen toward the new areas and that they settle and reproduce.

In the population of studied Hermann's tortoises, an expansion of the population toward the two nearest "islands" with appropriate habitat was detected from the initial nucleus in 1996. However, only one was colonized. At present, the population has successfully colonized two "islands", extinguished one which was occupied for four years and used up to six more "islands" during dispersive movements (Table 1). This has been interpreted as if the population is in phase of growth and expansion.

Year	Occupied regularly	Occupied temporaly
1987-1988	1	
1991-1995	1	
1996	2	1
1997	2	3
1998	4	1
1999	4*	0
2000	4*	2
2001	4*	0
2002	3	1

Table 1. Number of occupied "islands" according to the years of monitoring. Occupied regularly, number of "islands" occupied regularly, here they register hatchlings regularly; Occupied temporaly, number of "islands" occupied temporarily, they are considered as dispersion areas. * in one of these "islands" the tortoises have not reproduced again, but the breeding born in 1998 stay there until 2001.

Evaluation of long-term objectives

The long-term evaluation is the only one that allows us to determine if the success has come from the reintroduction project, which is the formation of a self-sufficient population. Subsequently, the evaluation can only be carried out from the moment in which there is evidence that the tortoises born in freedom are already reproducing. It is considered that the aspects to evaluate are (summary in Table 2): 1) growth rate population; and 2) population viability analysis.

Growth rate population

To calculate the growth rate population it is necessary to determine a series of demographic parameters (the size of the clutches, the clutch frequency, age of sexual maturation, the population's size according to ages or categories, sex-ratio and survival according to ages or categories), the results can imply an important and long field work. It is believed that a self-sufficient population is obtained if the rate of the population's growth is the same or superior to one. In the different models that were considered in the population of the Hermann's tortoise in Delta de l'Ebre, estimates were >1.0 in all of them (Bertolero, 2003). Therefore, it is confirmed that this population is in phase of growth and that, for its viability, it only depends on the specimens born in freedom.

Population viability analysis (PVA)

The use of models of population viability analysis is a tool with which the results of a reintroduction project can be evaluated, as well as to determine the administration strategy to continue in reduced populations (Reed et al., 1998). To be able to apply the PVA it is necessary to determine a series of demographic parameters (see previous chart) and their results should be analyzed with caution (revisions in Beissinger and Westphal, 1998; Reed et al., 1998; Coulson et al., 2001), because they are predictive models that depend on the reliability of the included parameters and of the considered assumptions (Reed et al., 1998; Coulson et al., 2001). Keeping these considerations in mind, their results are useful to determine the elasticity (or sensibility) of the used parameters and to be able to carry out more effective administration strategies (Reed et al., 1998), or to compare the results of models that include different scenarios or administration strategies (Beissinger and Westphal, 1998). Consequently, if it is possible to carry out the analysis of the population's viability, an approach to define the success can be based on obtaining models with extinction probabilities below 5% in a 50 year-old horizon (Beissinger and Westphal, 1998). The models carried out for the population of the Ebre Delta fulfilled the previous expectation, since their predictions of extinction are inferior to 5%, if the current conditions stay this way for the next 50 years (Bertolero, 2003).

Evaluation of short-term objectives		
Objectives	Successful	Failed
Body condition	stable or increasing	decreasing
Growth of young	yes	no
Dispersion (home range)	stable and big intraindividual overlapping	no stable and small intraindividual overlapping
Dispersion (density)	>3 individuals/ha	<3 individuals/ha
Reproduction of adults	yes	no
Radiotracking survival	>0.81	<0.81
Permanency of the liberated specimens	>0.81	<0.81
Evaluation of medium-term objectives		
Objectives	Successful	Failed
Capture-recapture survival	>0.90	<0.90
Reproduction of the specimens born in freedom	yes	no
Structure of the population	yes	no
Colonization	yes	no
Evaluation of long-term objectives		
Objectives	Successful	Failed
Growth rate population	$\lambda \geq 1$	$\lambda < 1$
Population viability analysis	survival >50 years with an extinction probability <0.05	can survive or not 50 years and with an extinction probability >0.05

Table 2. Summary of the evaluation process according to the objectives considered. For details see text.

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Annexe 6 :

exemple de fiche de contrôle du piégeage, utile également pour prendre des données des conditions hydrologiques ou autres

Annexe 6 : exemple de fiche de contrôle du piégeage, utile également pour prendre des données des conditions hydrologiques ou autres

Site[1] :										
Date[2]	Piège[3]	Type[4]	X[5]	Y[6]	HHMM[7]	Cistudes[8]	Conductivité[9]	Tº eau[10]	Profondeur[11]	Observations[12]
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exemples										
3/04/07	1	V	318036	4506868	1045	-				
	2	V	318442	4506846	1100	-				
	3	N	318219	4507056	1115	-				
5/04/07	1				1200	3				
	2				1245	0				
	3				1300	1				

1) Site : Estagnol / Bagans.
2) Date : jour du piégeage.
3) Piège : chaque piège a un numéro d'identification à une place fixe (coordonnées X-Y).
4) Type de piège (verveux / nasses / etc).
5) et 6) X et Y : coordonnées de la localisation de chaque piège (il faudra les prendre seulement la première fois).
7) HHMM : heure et minute de la mise en place du piège, de la surveillance surveille ou de l'enlèvement.
8) Cistudes : nombre de cistudes capturées chaque fois qu'on surveille le piège.
9), 10) et 11) exemples des variables que l'on peut prendre à chaque surveillance.
12) Observations : si un piège reste à sec par le changement du niveau de l'eau ; si un piège a été volé ; etc.

Commentaires de l'exemple :
La séance de piégeage va du 3 au 5 avril, mais les pièges ne sont pas surveillés chaque jour, mais toutes les 48 heures (±). Le premier jour on a mis 3 pièges (2 verveux et 1 nasse), on a enregistré leurs coordonnées (cela est seulement nécessaire la première fois dans l'année) et l'heure. Le 5 avril on retire les pièges ; le premier a travaillé pendant 49 heures et 15 minutes, et les deux autres pendant 49 heures et 45 minutes. Dans le premier piège on a capturé 3 cistudes ; pas de captures dans les deux autres.

Annexe 7 :

exemple de matrice d'histoires de vie des cistudes capturées

Annexe 7 : exemple de matrice d'histoires de vie des cistudes capturées

			2008					2009					2010			
Individu	Sexe	Age	mars	avril	mai	juin	juillet	mars	avril	mai	juin	juillet	mars	avril	mai	juin
Ind 1	1	Ad	0	1	1	0	0	0	0	0	0	0	1	0	0	0
Ind 2	1	Ad	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Ind 3	2	Ad	0	0	0	0	0	0	1	1	1	0	1	1	1	0
Ind 4	3	J	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Ind 5	2	Ad	1	0	1	0	1	1	1	0	1	0	1	0	0	1
Ind 6	1	Ad	0	1	0	1	0	1	1	1	0	0	0	0	0	0
Ind 7	1	Ad	1	1	1	0	1	0	0	0	1	1	1	1	1	0
Ind 8	2	Ad	1	1	0	0	1	1	0	1	1	0	1	1	1	1
Ind 9	2	Ad	0	1	1	1	0	0	0	0	0	1	1	1	1	0
Ind 10	2	Ad	0	1	0	1	0	1	0	1	1	0	0	0	1	0

Les trois premières colonnes montrent les informations d'identification, sexe et âge de chaque cistude (sexe : 1=mâle, 2=femelle, 3=indéterminé ; âge : Ad=adulte, J=juvénile). Avec cette information on peut faire les analyses de façon stratifiée (par sexe, par âge, par sexe et âge, ou tout ensemble) s'il y a suffisamment de données pour faire les groupes.
Dans les colonnes suivantes, il y a un exemple des résultats des captures pendant les trois premières années (chaque année, piégeage de mars à juillet) : un « 0 » si la cistude n'a pas été capturée pendant la session de piégeage (chaque session est de cinq jours) et un « 1 » si elle a été capturée au moins une fois dans la session de piégeage.

Sous-matrice pour calculer la taille de la population

A partir de la sous-matrice de 2008 on calcule la taille de la population pour l'année 2008 en choisissant l'une des méthodes de calcul (Schnabel, CAPTURE ou Jolly-Seber). La même méthode devra être utilisée pour les années 2009 et 2010.

			2008				
Individu	Sexe	Age	mars	avril	mai	juin	juillet
Ind 1	1	Ad	0	1	1	0	0
Ind 2	1	Ad	1	1	1	0	0
Ind 3	2	Ad	0	0	0	0	0
Ind 4	3	J	0	0	0	0	0
Ind 5	2	Ad	1	0	1	0	1
Ind 6	1	Ad	0	1	0	1	0
Ind 7	1	Ad	1	1	1	0	1
Ind 8	2	Ad	1	1	0	0	1
Ind 9	2	Ad	0	1	1	1	0
Ind 10	2	Ad	0	1	0	1	0

0	1	1	0	0
1	1	1	0	0
1	0	1	0	1
0	1	0	1	0
1	1	1	0	1
1	1	0	0	1
0	1	1	1	0
0	1	0	1	0

Sous-matrice pour calculer les taux de survie apparente

A partir des captures annuelles on calcule les taux annuels de survie apparente.

			2008					2009					2010			
Individu	Sexe	Age	mars	avril	mai	juin	juillet	mars	avril	mai	juin	juillet	mars	avril	mai	juin
Ind 1	1	Ad	0	1	1	0	0	0	0	0	0	0	1	0	0	0
Ind 2	1	Ad	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Ind 3	2	Ad	0	0	0	0	0	0	1	1	1	0	1	1	1	0
Ind 4	3	J	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Ind 5	2	Ad	1	0	1	0	1	1	1	0	1	0	1	0	0	1
Ind 6	1	Ad	0	1	0	1	0	1	1	1	0	0	0	0	0	0
Ind 7	1	Ad	1	1	1	0	1	0	0	0	1	1	1	1	1	0
Ind 8	2	Ad	1	1	0	0	1	1	0	1	1	0	1	1	1	1
Ind 9	2	Ad	0	1	1	1	0	0	0	0	0	1	1	1	1	0
Ind 10	2	Ad	0	1	0	1	0	1	0	1	1	0	0	0	1	0

			2008	2009	2010
Individu	Sexe	Age			
Ind 1	1	Ad	1	0	1
Ind 2	1	Ad	1	0	0
Ind 3	2	Ad	0	1	1
Ind 4	3	J	0	0	1
Ind 5	2	Ad	1	1	1
Ind 6	1	Ad	1	1	0
Ind 7	1	Ad	1	1	1
Ind 8	2	Ad	1	1	1
Ind 9	2	Ad	1	1	1
Ind 10	2	Ad	1	1	1

Les différentes captures annuelles sont résumées à une capture annuelle. Dans cet exemple, les cinq sessions mensuelles de 2008 (en jaune) sont condensées dans une seule session annuelle. Par exemple, la cistude 1 (Ind 1) a été capturée 2 fois pendant l'année 2008 (avril et mai), d'où le « 1 » de la colonne 2008 ; elle n'a pas été capturée en 2009, d'où le « 0 » ; et, finalement, elle a été capturée une fois en 2010, ce qui est représenté par un « 1 ». Pour chaque cistude on fait de la même façon et on obtient une colonne pour chaque année de suivi.