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Jonathan Monsinjon

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Développement embryonnaire,
détermination du sexe sensible à la température
et phénologie des pontes sous contrainte du
changement climatique : le cas de la tortue
Caouanne (*Caretta caretta*)

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préparée à l'Université Paris Sud

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Thèse présentée et soutenue à Orsay, le 20 décembre 2017, par

Jonathan Monsinjon

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- Préambule -

Pendant ces trois années de thèse, j'ai travaillé sur le développement embryonnaire et la détermination du sexe sensible à la température sous contrainte du changement du climat chez trois espèces de tortue : la cistude d'Europe (*Emys orbicularis*), la tortue olivâtre (*Lepidochelys olivacea*) et la tortue Caouanne (*Caretta caretta*). L'objectif principal de mon travail était d'améliorer notre capacité à prédire le sex ratio des nouveaux nés en milieu naturel en modélisant le développement des embryons à partir de données de température enregistrée au sein des nids. J'ai concentré mes efforts sur la tortue Caouanne car c'est une espèce pour laquelle j'ai disposé d'un vaste jeu de données obtenu grâce à des collaborations internationales (Turquie, Libye, Afrique du Sud, Floride, Géorgie et Brésil). J'ai aussi travaillé sur la phénologie des pontes afin de mieux comprendre dans quelles mesures cette plasticité phénotypique peut s'avérer être une stratégie efficace pour contrecarrer l'effet du changement du climat. Le corps de ce manuscrit traite donc de l'effet de la température sur le développement embryonnaire, la détermination du sexe et la phénologie des pontes chez cette espèce de tortue marine. J'ai placé en annexe une étude détaillant la méthodologie pour prédire la température au sein d'un nid chez la cistude d'Europe (ANNEXE 1) ainsi que deux autres études auxquelles j'ai participé : l'une portant sur la norme de réaction thermique du taux de croissance embryonnaire chez la tortue olivâtre (ANNEXE 2) et l'autre portant sur la période d'incubation pendant laquelle la détermination du sexe est sensible à la température chez la tortue Caouanne (ANNEXE 3). Pendant ma deuxième année de licence de biologie j'ai réalisé un stage de terrain en République du Congo afin d'évaluer l'incidence des engins et techniques de pêche artisanale sur les captures accidentelles incluant notamment les tortues marines. Cette étude a abouti à une publication que j'ai ajoutée en annexe (ANNEXE 4).

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Introduction

1. Changement du climat et biodiversité

Sur une échelle de temps suffisamment longue, chaque espèce est vouée à disparaître. Une espèce peut évoluer sous la pression de la sélection naturelle et de la dérive génétique et ainsi donner naissance à de nouvelles espèces ou s'éteindre.

1.1. Evolution de la biodiversité au cours des temps géologiques

Depuis l'apparition des principaux embranchements des métazoaires au début du Cambrien (-542 Ma), l'évolution de la biodiversité a été ponctuée par des événements d'extinction et de diversification. Le registre fossile a permis de mettre en évidence 5 événements majeurs marqués par l'extinction de plus de 75% des espèces vivantes à ces époques sur un intervalle de temps géologique extrêmement court (1000 à 10000 ans) (Erwin 2014). On estime que 86% des espèces ont disparu pendant la crise de l'Ordovicien (-443 Ma), 75% pendant la crise du Dévonien (-359 Ma), 96% pendant la crise du Permien (-251 Ma), 80% pendant la crise du Trias (-200 Ma) et 76% pendant la crise du Crétacé (-65 Ma) (Barnosky et al. 2011). Ces grandes extinctions de masses ont été causées par un bouleversement rapide des conditions abiotiques déclenché, pour la plupart, par des événements de glaciation, de volcanisme intense et d'impact de météorites. Il est aussi probable que la disparition de certains groupes taxonomiques ait bouleversé le fonctionnement des écosystèmes et des relations trophiques entraînant, dans une seconde phase, d'autres espèces vers l'extinction (Hull and Darroch 2013, Hull et al. 2015).

Les taux d'extinctions actuelles suggèrent que l'Anthropocène (fin du 18ème siècle) marque le début d'une 6ème extinction de masse (Ceballos et al. 2015) causée par l'impact de l'Homme sur l'utilisation des ressources, la structure de l'habitat, l'établissement d'espèces envahissantes, la dispersion de pathogènes et le changement du climat (Myers 1990, Dirzo and Raven 2003, Wake and Vredenburg 2008, Hoffmann et al. 2010, Estes et al. 2011, Dirzo et al. 2014, Pimm et al. 2014). Cependant la question reste délicate. Premièrement, le registre fossile est un échantillon de la biodiversité biaisé vers les organismes vivants dans les zones favorables à la fossilisation (zones à forte sédimentation : côtes, lacs, rivières, marécages) et vers les organismes à parties dures. Le taux d'extinction actuel étant principalement basé sur des espèces de plantes et de vertébrés terrestres (mammifères, oiseaux, reptiles, amphibiens) il n'est pas aisément comparable avec les taux d'extinction basés sur le registre fossile qui est quant à lui biaisé vers les invertébrés marins à parties dures. Deuxièmement, nous pouvons identifier

les organismes actuels au genre et à l'espèce tandis que le registre fossile ne permet pas toujours une identification allant jusqu'à l'espèce. Troisièmement, nous pouvons actuellement définir l'extinction d'une espèce avec une fine résolution temporelle tandis que l'extinction d'une espèce basée sur le registre fossile n'est datable que sur un intervalle de temps allant de 1000 ans à plus d'un million d'années (Voir synthèse dans Barnosky et al. 2011). Une autre difficulté est liée au fait que l'espèce dans sa dimension temporelle (ressemblance morphologique) n'est pas la même chose que l'espèce biologique (interfécondité). Une affirmation de notre entrée dans la sixième extinction de masse est donc probablement prématurée et nécessite une amélioration des méthodes de comparaison. Une perspective d'amélioration serait de pouvoir identifier la raréfaction des groupes taxonomiques autrefois abondant ce qui offrirait une meilleure mesure de l'effondrement des réseaux écologiques et de la dynamique d'une extinction de masse (Hull and Darroch 2013, Erwin 2014, Hull et al. 2015). Dans le cas où la sixième extinction de masse serait avérée, elle ne pourra être enrailée qu'au moyen d'actions de conservation visant à préserver les trajectoires écologiques et évolutives (Sarrazin and Lecomte 2016).

1.2. Evolution actuelle de la biodiversité face aux changements de température

Entre 1850 et aujourd'hui, la température moyenne à la surface du globe a augmenté de près 0.8°C (IPCC 2014). Ce changement du climat induit par l'Homme a d'ores et déjà profondément affecté la biodiversité. Une méta-analyse réalisée sur 677 espèces (plantes, insectes, amphibiens, poissons et oiseaux) sur des intervalles de temps allant de 16 à 132 ans a révélé que 71% des espèces ont subi des modifications dans leur phénologie se traduisant principalement par un avancement des événements de reproduction en réponse à une augmentation de la température de l'environnement (Parmesan and Yohe 2003). Parmi 434 espèces (plantes, oiseaux, mammifères, reptiles, amphibiens, poissons, et invertébrés), 80% ont montré un changement d'aire de répartition vers de plus hautes latitudes et altitudes en réponse à une augmentation de la température de l'environnement sur des intervalles de temps allant de 17 à 1000 ans (Parmesan and Yohe 2003). Cette réponse des espèces au changement du climat a été très largement observée au sein de divers écosystèmes terrestres et marins (McCarty 2001, Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Perry et al. 2005, Parmesan 2006, Rosenzweig et al. 2007, Bellard et al. 2012, Poloczanska et al. 2013, Garcia et al. 2014). Il est important de noter que de telles modifications sur des échelles spatiales et temporelles sont hétérogènes à l'échelle des espèces et des populations ayant pour conséquence une altération des interactions trophiques dont les effets peuvent se ressentir à l'échelle des communautés et des écosystèmes (Gilman et al. 2010, Hoegh-Guldberg and Bruno 2010,

Walther 2010, Angert et al. 2013). Chez certains oiseaux, un changement de phénologie pour une population donnée peut procurer un avantage adaptatif lorsqu'il permet de suivre l'abondance de la ressource principale de l'espèce considérée (Charmantier et al. 2008) ou un désavantage lorsque la saison de reproduction est désynchronisée avec le pic d'abondance de la ressource en question (Both 2010, Visser et al. 2012). Même si de nombreuses espèces sont capables de modifier leur phénologie et leur aire de distribution en réponse au changement du climat, il est possible que leurs réponses ne soit pas suffisamment rapide pour suivre leur enveloppe climatique dans le contexte actuel de l'évolution du climat (Devictor et al. 2012).

Selon les scénarios du groupe d'expert sur l'évolution du climat, la température moyenne à la surface du globe pourrait augmenter de près de 4°C d'ici 2100 si les émissions de gaz à effet de serre ne sont pas diminuées (IPCC 2014). Dans cette situation, plusieurs questions se posent. Dans quelles mesures la biodiversité va-t-elle être affectée ? Quelles espèces sont susceptibles d'être les plus vulnérables ? Comment prédire la viabilité des populations ? Les organismes ectothermes, incluant principalement les espèces tropicales qui vivent actuellement aux limites de leurs tolérances thermiques, sont susceptibles d'être fortement affectés par des modifications dans leur environnement thermique car presque tous leurs traits d'histoire de vie dépendent de la température (Deutsch et al. 2008, Tewksbury et al. 2008, Huey et al. 2009). Plusieurs études prédisent un déclin de la diversité des espèces marine dans les zones tropicales, une augmentation aux moyennes latitudes et peu de changement aux hautes latitudes (Pereira et al. 2010, Jones and Cheung 2015, Molinos et al. 2015). Ces études utilisent des modèles de niche écologique qui visent à établir la niche écologique d'une espèce basée sur des corrélations entre la présence des individus et plusieurs paramètres abiotiques (température, humidité etc.) afin de prédire l'étendue de l'aire de distribution potentiellement favorable. Les hypothèses implicites de ces modèles sont que les populations sont démographiquement stables et que les individus occupent actuellement l'ensemble de leur niche fondamentale, c'est-à-dire l'aire de distribution bornée uniquement par des contraintes abiotiques. Ces hypothèses constituent une mauvaise approximation car une espèce est souvent organisée en métapopulations (populations sources et puits) et la distribution actuelle d'une espèce (niche réalisée) dépend des contraintes biotiques (compétition entre et au sein des espèces pour l'accès aux ressources, prédation, dispersion etc.) et des barrières écologiques. Les modèles de niche écologique ont donc tendance à sous-estimer les aires de distribution potentiellement favorables pour une espèce et, par conséquent, surestimer les risques d'extinction (Chevin et al. 2010). Une augmentation de la diversité des espèces aux latitudes moyennes (résultant de l'accumulation d'espèces

colonisatrices et résidentes) est probablement surestimée. Si une espèce étend son aire de répartition où d'autres espèces sont résidentes on peut s'attendre à une modification des interactions trophiques dont les conséquences à l'échelle des communautés et des écosystèmes sont difficiles à prévoir. L'utilisation de modèles mécanistes basés sur le potentiel adaptatif en termes de réponse évolutive et de plasticité phénotypique permettra de mieux appréhender la réponse des espèces au changement du climat (Chevin et al. 2010, Huey et al. 2012).

2. Potentiel d'adaptation des espèces

La vulnérabilité d'une espèce dépend de son exposition et de sa sensibilité aux perturbations ainsi que de son potentiel adaptatif (Butt et al. 2016). La capacité de réponse à la sélection et le degré de plasticité phénotypique déterminent le potentiel adaptatif.

2.1. Réponse évolutive

Une espèce à temps de génération court et disposant d'une importante variabilité génétique (maintenue par une taille de population suffisamment grande) est susceptible de répondre rapidement à une pression de sélection (Bradshaw and Holzapfel 2006, 2008, Visser 2008, Somero 2010). C'est notamment le cas de certains insectes, oiseaux et mammifères ayant d'ores et déjà expérimenté des modifications de fréquences alléliques au cours des générations dans la direction de la pression de sélection (Bradshaw and Holzapfel 2006). En revanche, ce type de réponse n'est pas évident chez des reptiles et amphibiens (Urban et al. 2014). En effet, le taux d'adaptation génétique peut s'avérer insuffisant si la vitesse du changement environnemental dépasse celle de la réponse à la sélection (Thomas et al. 2004, Nussey et al. 2005). La réponse à la sélection peut se calculer au moyen de l'équation des reproducteurs (Lush 1947, Kelly 2011) :

$$\Delta Z = h^2 \times S$$

Où ΔZ est le changement de la moyenne du caractère considéré en une génération, h^2 correspond à l'héritabilité (au sens étroit) du caractère et S étant l'intensité de la sélection (voir Hoffmann and Sgrò 2011 pour la prise en compte de plusieurs caractères en vue d'estimer la persistance d'une population).

2.2. Plasticité phénotypique

La plasticité phénotypique, c'est-à-dire la capacité d'un génotype à exprimer plusieurs phénotypes selon des signaux environnementaux (Pigliucci 2001), peut être adaptative lorsqu'elle augmente la valeur sélective d'un individu, mal-adaptative lorsqu'elle diminue la valeur sélective d'un individu ou neutre si elle n'induit aucun changement de valeur sélective

(Ghalambor et al. 2007). Elle peut être réversible au cours de la vie d'un organisme (Gomulkiewicz and Kirkpatrick 1992) ou irréversible si elle s'exprime qu'une fois comme la métamorphose chez certains amphibiens (Newman 1994). La plasticité phénotypique est donc un élément central pour étudier le potentiel adaptatif des espèces (Nicoitra et al. 2010) mais n'est actuellement pas prise en compte dans les modèles de distribution des espèces (Chevin et al. 2010). Lorsque l'intensité de la sélection sur un caractère donné dépasse la vitesse de la réponse génétique, notamment chez les organismes à temps de génération long, la plasticité phénotypique peut fournir une alternative efficace à court terme pour atténuer ou neutraliser la pression de sélection (Nussey et al. 2005, Chevin et al. 2010, Hoffmann and Sgrò 2011, Huey et al. 2012, Refsnider and Janzen 2012). Cependant, la plasticité phénotypique a un coût lié à des contraintes développementales et des trade-offs évolutifs (DeWitt et al. 1998, Angilletta et al. 2003).

L'outil permettant de quantifier la plasticité phénotypique est la norme de réaction, c'est-à-dire la fonction qui décrit un changement de phénotype selon un gradient environnemental pour un génotype donné (Via et al. 1995). On parle donc de norme de réaction thermique lorsqu'il s'agit d'étudier un caractère dépendant de la température. Chez les organismes ectothermes, la majorité des traits d'histoire de vie dépendent de la température et sont donc définis par des normes de réaction thermique. Notre capacité à prédire le potentiel adaptatif des organismes ectothermes nécessite donc d'identifier les normes de réaction thermique des caractères étroitement liés à la valeur sélective des individus tels que le comportement et la physiologie (Huey et al. 2012) et de savoir dans quelles mesures ces normes de réactions sont héréditaires en milieu naturel (Nussey et al. 2007).

3. Développement embryonnaire chez les reptiles ovipares

Le stade embryonnaire est une étape critique dans le cycle de vie des reptiles ovipares puisque les œufs, en l'absence de soins parentaux, sont directement exposés aux variations environnementales.

3.1. Effet de la température sur le phénotype

La température d'incubation des œufs affecte la probabilité de survie des embryons (Van Damme et al. 1992), la durée de l'embryogénèse (Miller 1985), la morphologie, la couleur, la taille et les performances des nouveau-nés (Van Damme et al. 1992, Du and Ji 2003, Booth 2006) ainsi que le développement du système immunitaire (Freedberg et al. 2008) et le sexe chez les espèces à détermination du sexe sensible à la température (TSD) (Charnier 1966, Bull 1980, Janzen and Paukstis 1988, Pieau et al. 1995, Pieau 1996). Les effets de la température sur

les embryons en développement peuvent se ressentir sur le long terme affectant la physiologie, les performances, et le comportement (Burger 1989, Sibly and Atkinson 1994, Freedberg et al. 2004). Ces études ont majoritairement été conduites en laboratoire selon plusieurs températures maintenues constantes au cours de l'incubation des œufs. Cependant, la température d'incubation des œufs en milieu naturel n'est jamais constante chez les ectothermes ovipares car elle varie en fonction des fluctuations nyctémérales et saisonnières et selon les conditions météorologiques (précipitations, couverture nuageuse, vitesse du vent). Cette remarque est importante à prendre en compte puisque la magnitude des fluctuations thermiques pendant l'incubation des œufs a un effet sur le développement des embryons chez les organismes ectothermes (Les et al. 2009, Niehaus et al. 2012, Paaijmans et al. 2013, Bowden et al. 2014, Horne et al. 2014) et plus précisément sur le sex ratio chez les espèces à détermination du sexe sensible à la température (Valenzuela 2001, Georges et al. 2004, Georges et al. 2005, Schwanz and Proulx 2008, Du et al. 2009, Warner and Shine 2010, Neuwald and Valenzuela 2011, Georges 2013). Par conséquent, la valeur sélective des individus dépend étroitement de l'environnement thermique d'incubation des œufs.

3.2. Comportement de nidification

Une femelle peut influencer le phénotype, les performances et la probabilité de survie des nouveau-nés en sélectionnant un site de ponte particulier parmi divers micro-habitats thermiques disponibles sur des échelles spatiales et temporelles (Refsnider and Janzen 2010). Sa valeur sélective est donc étroitement liée à son comportement de nidification. La date de ponte affecte la survie des embryons et le sex ratio des nouveau-nés chez deux tortues aquatiques (Doody et al. 2004, Schwanz and Janzen 2008) mais il semblerait que cette plasticité dans la phénologie des pontes ne soit pas suffisante pour contrecarrer un changement rapide de l'environnement thermique d'incubation (Schwanz and Janzen 2008). La profondeur du nid influence le régime thermique d'incubation des œufs en diminuant l'amplitude des fluctuations thermiques à mesure que le nid est déposé plus en profondeur. Cependant, cette stratégie semble être inefficace pour pallier à des modifications dans l'environnement thermique d'incubation (Doody et al. 2006, Refsnider et al. 2013a) et certaines espèces, comme les tortues marines, sont contraintes par leur morphologie (Miller 1997). La stratégie qui semble être la plus efficace pour atténuer d'éventuelles variations thermiques défavorables pour le devenir des embryons est la sélection des sites de ponte en fonction de la surface ombragée (Doody and Moore 2010). Dès lors que l'habitat est suffisamment hétérogène (principalement médié par la couverture végétale), certains reptiles terrestres parviennent à éviter les variations thermiques potentiellement défavorables pour le devenir des embryons en choisissant des sites de ponte

plus ou moins exposés aux radiations solaires (Doody et al. 2006, Doody and Moore 2010, Refsnider et al. 2013b). Il est donc nécessaire de caractériser l'hétérogénéité thermique au sein d'un habitat afin de pouvoir évaluer dans quelles mesures les reptiles ovipares sont exposés aux perturbations thermiques liées au changement actuel du climat.

4. Détermination du sexe sensible à la température

La détermination du sexe sensible à la température a été découverte il y a 51 ans chez le lézard *Agama agama* (Charnier 1966). Ce mécanisme a ensuite été identifié comme étant la règle chez les crocodiliens (Lang and Andrews 1994, Deeming 2004) et les sphénodons (Cree et al. 1995, Nelson et al. 2004), majoritaire chez les chéloniens (Ewert et al. 1994, Ewert et al. 2004) et assez répandu chez les lézards (Viets et al. 1994, Harlow 2004) et les téléostéens (Ospina-Álvarez and Piferrer 2008, Baroiller et al. 2009).

4.1. Base moléculaire

Chez la majorité des reptiles à détermination du sexe sensible à la température, le sexe est irréversiblement déterminé par la température pendant une période de l'incubation correspondant au 2ème tiers de l'incubation à température constante (Pieau and Dorizzi 1981, Bull 1987, Mrosovsky and Pieau 1991). Après cette période thermosensible (appelée la TSP pour ThermoSensitive Period), les gonades poursuivent leur différenciation soit en testicules soit en ovaires en fonction du niveau endogène en hormones stéroïdiennes (androgènes et œstrogènes, respectivement) (Pieau et al. 1982, Raynaud and Pieau 1985). La régulation du niveau d'hormones stéroïdiennes est réalisée par l'enzyme Cytochrome P-450 Aromatase qui convertit la testostérone en œstradiol 17 β et l'androstènedione en œstrone. Chez les chéloniens, l'activité de cette enzyme au niveau des gonades est positivement corrélée avec la température d'incubation pendant la TSP (Desvages and Pieau 1991, 1992, Desvages et al. 1993). Cette enzyme est aussi transcrite et active dans le cerveau des embryons pendant la TSP ce qui a conduit certains chercheurs à penser que le cerveau est la source principale d'œstrogène impliquée dans la détermination du sexe (Jeyasuria and Place 1998, Salame-Mendez et al. 1998, Willingham et al. 2000). Cependant, une étude a récemment démontré que des gonades, préalablement isolées du reste du corps, se développent normalement en testicules ou en ovaire sous l'action de la température (Shoemaker-Daly et al. 2010). La transcription de l'enzyme Cytochrome P-450 Aromatase ainsi que d'autres facteurs impliqués dans la détermination du sexe est sous le contrôle d'un réseau de gènes régulateurs. L'expression d'un gène (ou de plusieurs gènes) en amont de cette voie de régulation est aussi sensible à la température (Rhen and Schroeder 2010, Czerwinski et al. 2016). Plusieurs gènes candidats ont été récemment

proposés (Rhen et al. 2015, Yatsu et al. 2015, Schroeder et al. 2016, Yatsu et al. 2016). Cependant, la tâche reste délicate car la structure du réseau de gènes régulateurs et le gène (ou les gènes) sensible à la température peuvent avoir emprunté différents chemins évolutifs et par conséquent être différents parmi les espèces (Bachtrog et al. 2014).

4.2. Evolution

La détermination du sexe est un trait de caractère labile au sein de nombreux taxons. En effet, deux espèces évolutivement proches peuvent présenter soit un déterminisme du sexe uniquement génétique soit un déterminisme du sexe sensible à la température (Valenzuela et al. 2013, Bachtrog et al. 2014). Par exemple, la tortue serpentine (*Chelydra serpentina*) présente un patron de détermination du sexe avec des femelles produites à basse et haute température et des mâles produit en proportions variables à des températures intermédiaires tandis que les espèces d'un genre évolutivement proche (*Sternotherus*) présente un patron de détermination du sexe avec des mâles produits à basse température et des femelles produites à haute température (Valenzuela et al. 2013). Deux espèces évolutivement proches de la tortue serpentine (*Chelydra serpentina*) ont quant à elles un déterminisme du sexe génétique avec des chromosomes sexuels homomorphes (*Claudius angustatus*) ou hétéromorphes (*Staurotypus salvinii*) (Valenzuela et al. 2013). Certaines études suggèrent que les transitions évolutives entre ces différents mécanismes de détermination du sexe peuvent se réaliser rapidement (Sarre et al. 2004, Quinn et al. 2011, Schwanz et al. 2013). En effet, une population de lézard (*Niveoscincus ocellatus*) vivant en haute altitude présente un déterminisme du sexe génétique tandis qu'une autre population de la même espèce vivant à basse altitude présente un déterminisme du sexe sensible à la température (Pen et al. 2010). Au sein d'une population d'un autre lézard (*Pogona vitticeps*), des mâles déterminés génétiquement ont subi une réversion de sexe en réponse à de fortes hausses des températures en milieu naturel (Holleley et al. 2015). Le fait que les femelles nouvellement formées soient fertiles laisse à penser que la réversion du sexe est le mécanisme proximal qui déclenche les transitions évolutives entre les différents modes de détermination du sexe (Holleley et al. 2016).

4.3. Signification adaptative

Sous sélection fréquence-dépendante du sexe, les individus qui produisent le sexe rare augmentent leur valeur sélective favorisant, in fine, les individus qui allouent autant d'énergie dans la production des deux sexes (Fisher 1930). Les espèces à déterminisme du sexe sensible à la température peuvent quant à elles produire l'un des deux sexes en plus grande proportion selon les changements de température ce qui a longtemps questionné la signification adaptative

de ce mécanisme de détermination du sexe. L'hypothèse qui prévaut propose que ce mécanisme de détermination du sexe soit favorisé par la sélection naturelle si la température affecte la valeur sélective des individus différemment chez les mâles et les femelles (Charnov and Bull 1977). En d'autres termes, la détermination du sexe serait une stratégie condition-dépendante de l'allocation au sexe. Cette hypothèse n'a été supportée que chez deux espèces de lézard (Warner and Shine 2008, Pen et al. 2010) probablement car mesurer la valeur sélective d'un individu reste un défi, notamment chez les espèces à temps de génération long. Chez les organismes à temps de génération long, il est probable que le chevauchement des générations atténue fortement la pression de sélection liée à l'allocation au sexe (Schwanz et al. 2010). Néanmoins, une étude prometteuse a récemment proposé que des variations dans l'âge à la maturité entre les deux sexes suffisent à sélectionner ce mécanisme de détermination du sexe (Schwanz et al. 2016).

4.4. Conséquences génétiques et démographiques

Un sex ratio fortement biaisé à l'échelle d'une population peut avoir des conséquences néfastes sur la dynamique démographique de cette dernière. La recherche de partenaire peut s'avérer coûteuse si l'un des deux sexes est majoritaire car la probabilité de rencontre de deux individus prêts à se reproduire diminue à mesure que le biais est important, conduisant à un effet Allee démographique (Courchamp et al. 1999) médié par un sex ratio biaisé (Soldaat et al. 1997). Cet effet Allee peut être renforcé lorsque le sex ratio est biaisé vers les mâles si ces derniers ont un comportement agressif lors de l'accouplement (Le Galliard et al. 2005). D'autre part, l'effet Allee peut être atténué lorsque le sex ratio est biaisé vers les femelles si au moins l'un des deux sexes cherche activement et efficacement un partenaire (Berec et al. 2001). La dynamique d'une population dépend aussi du sex ratio opérationnel, c'est-à-dire le sex ratio des adultes reproducteurs corrigé par un dimorphisme sexuel physiologique ou comportemental. En effet, le sex ratio opérationnel dépend du nombre de femelles fécondables et du nombre de mâles sexuellement actifs (Emlen and Oring 1977). Dans un cas où le sex ratio serait biaisé vers les femelles, le sex ratio opérationnel serait quant à lui moins biaisé (voir rééquilibré) si les femelles sont capables de stocker le sperme sur plusieurs saisons de reproduction et si les mâles sont capables de féconder plusieurs femelles sur une saison de reproduction. Ce type de situation est généralement associé à un fort taux de croissance de la population (Dyson et al. 2004, Rankin and Kokko 2007).

Un sex ratio biaisé à l'échelle d'une population peut avoir des conséquences néfastes sur la diversité génétique de cette dernière dès lors que sa taille efficace diminue à mesure que le biais

augmente. La taille efficace d'une population (N_e) correspond à la taille d'une population idéale qui aurait une fluctuation du polymorphisme similaire à celle d'une population naturelle (Crow and Kimura 1970). Elle se calcule comme suit :

$$N_e = \frac{4 \times Nm \times Nf}{Nm + Nf}$$

Où Nm est le nombre de mâles dans une population et Nf le nombre de femelle. $N_e = 2Nm$ si le sex ratio est égale à 0.5 mais diminue à mesure que le sex ratio tend vers 0 ou 1. Par conséquent, une population dont le sex ratio est biaisé est plus susceptible d'être exposée à la dérive génétique et donc d'accumuler des mutations délétères (Charlesworth and Charlesworth 1998), conduisant à un effet Allee génétique (Luque et al. 2016). Jusqu'à présent aucune étude n'a considéré l'effet Allee génétique chez les espèces à détermination du sexe sensible à la température.

4.5. Prédiction du sex ratio en milieu naturel

Prédire le sex ratio d'une ponte en milieu naturel chez les reptiles ovipares à détermination du sexe sensible à la température reste un défi majeur à l'heure actuelle. C'est pourtant une étape nécessaire pour tester les hypothèses évolutives quant à la signification adaptative de ce mécanisme de détermination du sexe et pour évaluer la vulnérabilité des espèces présentant ce trait de caractère dans le contexte actuel de l'évolution du climat. La méthode la plus précise serait de compter le nombre de mâle et le nombre de femelle au sein d'une population. Il va de soi que ce n'est pas une tâche aisée lorsque les individus ne sont pas facilement observables et d'autant moins s'ils ne présentent pas de caractères sexuels secondaires facilement interprétables. Dans cette situation il faut examiner les gonades, soit histologiquement ou en utilisant la méthode de la laparoscopie. La première méthode nécessite de sacrifier l'individu, ce qui est difficilement justifiable si l'espèce est protégée ou menacée. La seconde méthode requiert de la technique et beaucoup de temps et d'argent notamment lorsqu'il est nécessaire d'élever l'organisme en captivité afin qu'il atteigne une taille suffisante pour mener l'intervention (Wyneken et al. 2007). Par ailleurs le maintien en captivité d'animaux pose des problèmes éthiques et sanitaires. Des méthodes indirectes pour estimer le sex ratio ont été proposées mais leur précision n'a pas été évaluée correctement.

La méthode la plus simple utilise la durée de l'incubation comme mesure indirecte de l'environnement thermique dans lequel les embryons évoluent pendant l'incubation. En effet, certaines études réalisées en laboratoire à température constante pendant l'incubation ont relié le sex ratio et la durée de l'incubation (Webster and Gouveia 1988, Mrosovsky et al. 1999).

Cette méthode a été très largement utilisée pour prédire le sex ratio de pontes de tortue marine en milieu naturel car la durée d'incubation est une mesure facile à réaliser sur le terrain (Marcovaldi et al. 1997, Godley et al. 2001, Jribi et al. 2013, dei Marcovaldi et al. 2014, dei Marcovaldi et al. 2016). Pourtant, il a été démontré très tôt que cette méthode est inefficace lorsque la température d'incubation varie pouvant introduire jusqu'à 100% d'erreur dans l'estimation du sex ratio (Georges et al. 1994). Une autre erreur commune dans la littérature est l'utilisation de la température moyenne pendant le 2ème tiers de l'incubation basé sur l'observation que la période thermosensible pour la détermination du sexe correspond au 2ème tiers du développement (en termes de stades embryonnaires). Cependant, cette période correspond au 2ème tiers de l'incubation uniquement si la température d'incubation est maintenue constante (Georges et al. 2004, Georges et al. 2005, The Chu et al. 2008, Woolgar et al. 2013, Stubbs et al. 2014), ce qui n'est jamais le cas en milieu naturel. De plus, la simple moyenne arithmétique de la température est un mauvais indicateur de l'effet de la température sur le développement embryonnaire, et a fortiori sur la détermination du sexe, car les normes de réaction thermique des taux biologiques, comme le taux de croissance embryonnaire, sont non linéaires et souvent non monotones dans la gamme de température d'incubation en milieu naturel (Georges et al. 2004, Georges et al. 2005, Warner and Shine 2010, Neuwald and Valenzuela 2011). Il est donc nécessaire de modéliser la croissance des embryons afin de déterminer les bornes de la période thermosensible pour la détermination du sexe au cours de l'incubation et d'intégrer l'effet différentiel de la température sur le développement embryonnaire.

5. Objectifs de thèse

Ce manuscrit vise à prédire le sex ratio primaire chez l'une des 7 espèces de tortue marine, la tortue Caouanne (*Caretta caretta*), dans un environnement thermique changeant. Le premier chapitre explore d'éventuelles différences dans les normes de réaction thermique du taux de croissance embryonnaire entre deux populations qui nidifient en Méditerranée (Turquie et Libye). Le second chapitre a pour objectif de déterminer si les estimateurs du sex ratio disponible à l'heure actuelle produisent des résultats similaires avec l'exemple d'une population nidifiant au Brésil. Le troisième chapitre propose de quantifier le décalage requis dans la phénologie des pontes pour pallier aux conséquences néfastes liés à un réchauffement du climat au sein de plusieurs populations répartis en Méditerranée, dans l'océan Atlantique et dans l'océan Indien. Le quatrième chapitre évalue l'effet combiné de la température et de la disponibilité en ressource sur la dynamique de la saison de ponte avec l'exemple d'une

population nidifiant au Brésil. Le dernier chapitre propose une nouvelle méthode pour prédire le sex ratio d'une ponte chez les organismes à détermination du sexe sensible à la température en utilisant des données de sex ratio provenant des plusieurs sites de ponte localisés en Floride. Je discute ensuite l'implication de ces résultats dans notre capacité à estimer la vulnérabilité des tortues marines dans le contexte actuel de l'évolution du climat.

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Chapter 1: Embryonic growth rate thermal reaction norm of Mediterranean *Caretta caretta* embryos from two different thermal habitats, Turkey and Libya

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Abstract: Ectothermic species are strongly affected by thermal changes. To assess the viability of these species under climate change constraints, we need to quantify the sensitivity of their life-history traits to temperature. The loggerhead marine turtle (*Caretta caretta*) nests regularly in the Oriental basin of the Mediterranean Sea. The different populations are separated because of time (<12,000 yrs) and very different thermal habitats; it is hotter in the southern coast (Libya) than in the northern ones (Cyprus, Greece, Turkey). Patterns of embryo growth response to incubation temperatures have been searched for these 2 populations. We found that both populations have similar thermal reaction norms for embryonic growth rate. This

highlights that 12,000 yrs is not enough time for this species to adapt to specific thermal habitats and raises the question of the persistence of these populations in the context of rapid climate change.

Keywords: Climate change, phenotypic plasticity, embryonic development, temperature, sea turtles, loggerhead

1. Introduction

Green and loggerhead marine turtles (*Chelonia mydas*, and *Caretta caretta*, respectively) are the 2 marine turtles nesting commonly along Mediterranean coasts (Casale and Margaritoulis 2010). Whereas green turtle nesting sites are restricted to the East of the oriental basin of the Mediterranean, the distribution of nesting sites of loggerheads encompasses most of the oriental basin. The main aggregations occur along the Cypriot, Turkish, and Greek coasts at the East and in Libya at the West of the oriental basin. Although the Cypriot, Turkish, and Greek populations have been well studied (Casale and Margaritoulis 2010), the Libyan population has not and it may be one of the most important populations in the Mediterranean region (Saied et al. 2012).

Loggerhead nesting populations in the Mediterranean are genetically separated into 3 groups (Garofalo et al. 2009): One at the West of the oriental basin nests in Calabria (Italy), one at the East of Turkey, and a central one nesting in Greece, West-Turkey, Lebanon, Cyprus, and Libya (Fig. 1). The population nesting in Libya emerged as the oldest population in the Mediterranean, dating from the Pleistocene ca. 65,000 yrs ago (20,000–200,000) (Clusa et al. 2013). This suggests that the Libyan population might have settled in the Mediterranean basin before the end of the last glacial period. The remaining populations from the central part (Greece, West-Turkey, Lebanon, Cyprus) originated as a result of a more recent Holocenic expansion after the last glacial era some 12,000 yrs ago (Clusa et al. 2013). The Libyan population belongs to the central genetic group but nests in a thermal environment that is very different from the other populations (Figs. 2 and 3), because it is the only major nesting along the southern part of the Mediterranean. The differences between Libyan and Turkish populations gives the opportunity to test whether this species has been able to adapt rapidly (<12,000 yrs) in different thermal environments (Figs. 2 and 3).

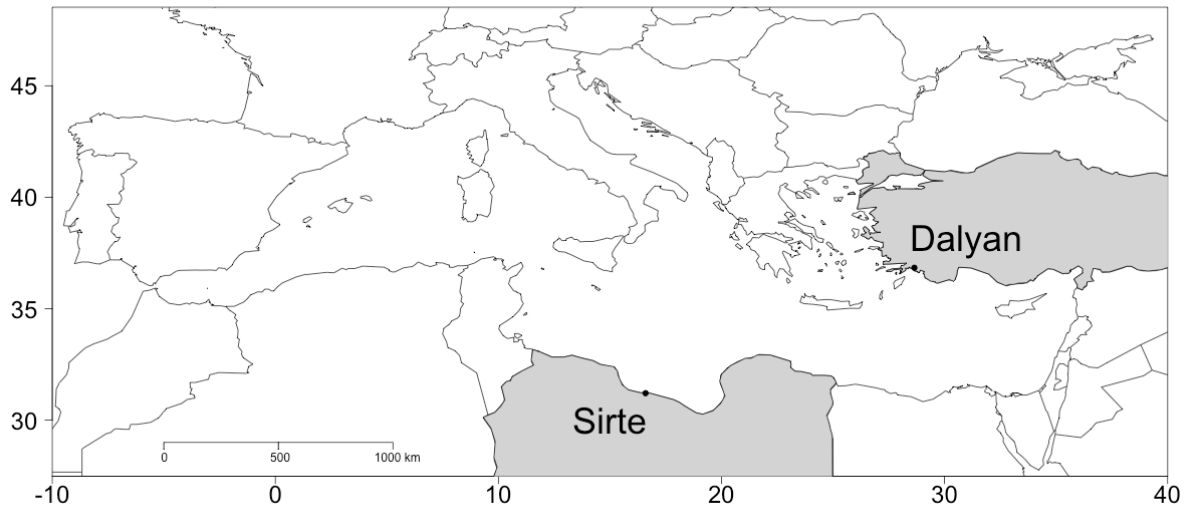


Figure 1: Map of the Mediterranean Sea with Libya and Turkey indicated by shading. Position of Sirte and Dalyan cities are shown. Nests are located 20 km west of the city of Sirte and 5 km south of the city of Dalyan.

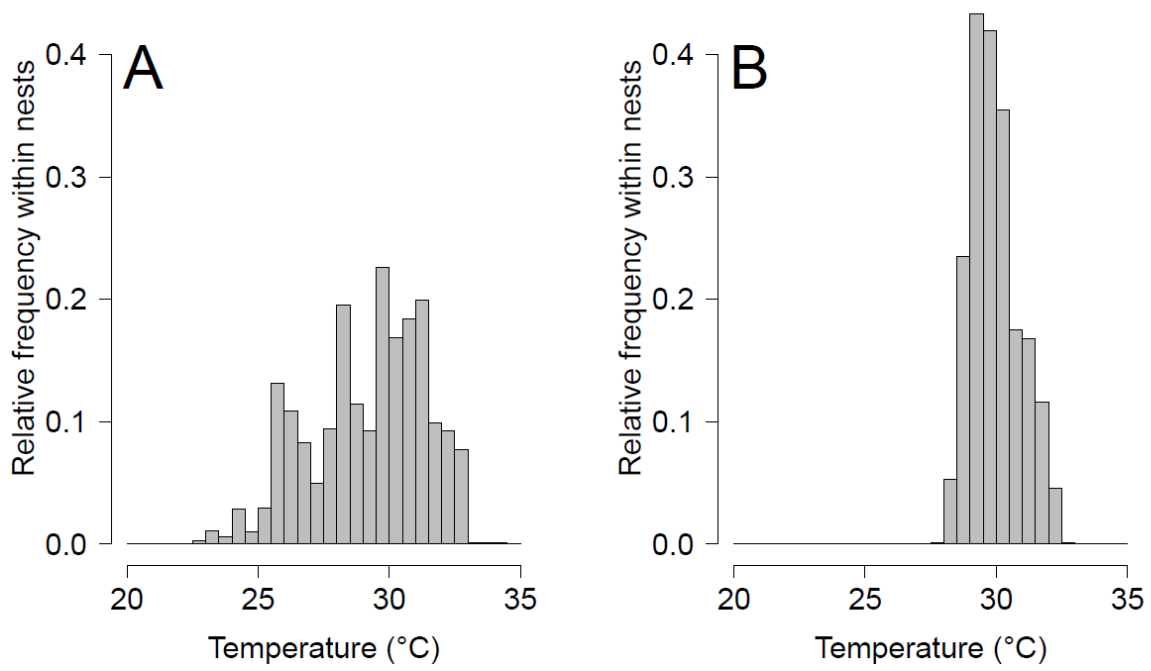


Figure 2: Temperatures recorded in Turkish (A) and Libyan (B) nests.

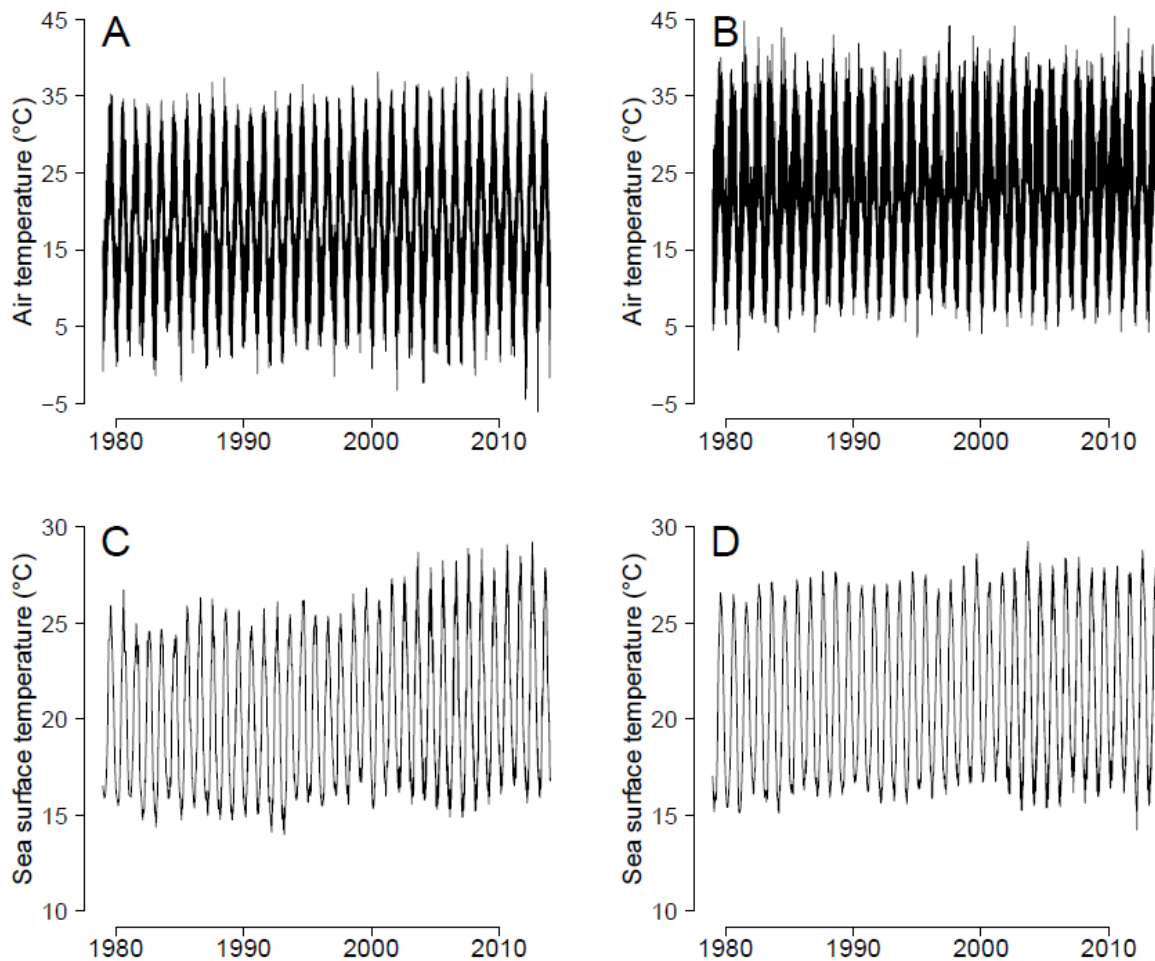


Figure 3: Air temperature (AT) and sea surface temperature (SST) close to Dalyan, Turkey (lat 36.791, long 28.619) (A and C, respectively), and AT and SST close to Sirte, Libya (lat 31.212, long 16.566) (B and D, respectively).

Virtually all biological rates of ectothermic animals are affected by temperature including development times (Gillooly et al. 2002) and growth rates (Gillooly et al. 2001). Egg incubation temperatures affect the duration of embryogenesis (Miller 1985), the probability of embryo survival (Van Damme et al. 1992), the sex for species with temperature-dependent sex determination such as *Caretta caretta* (Pieau 1996), and the morphology and body size at hatching (Du and Ji 2003). In addition, long-term effects of incubation temperature on the physiology and behaviour of hatchlings has been observed (Burger 1989, Sibly and Atkinson 1994). Thus, the fitness of developing embryos and hatchlings is strongly dependent on the temperature within the nest during incubation.

Recently, a general way to model sigmoidal embryo growth with variable incubation temperature obtained from field data was proposed and tested with data from *Caretta caretta* from Dalyan beach, Turkey (Girondot and Kaska 2014a). We apply this model to new data of nest temperatures gathered in 2009 in Libya to test if embryos of both populations react

differently to incubation temperature. Finally, we discuss how the different temperature-dependent life-history traits could interact with each other to mitigate or enhance the effect of climate change.

2. Materials and methods

2.1. Field data

In Turkey, temperatures of 21 loggerhead turtle nests were examined via electronic temperature recorders during the 2010 nesting season on Dalyan Beach. Tiny talk temperature recorders (resolution $\pm 0.37^\circ\text{C}$, Orion Components Ltd., UK) were used to measure temperature. They were placed into the middle of the nests (ca. 45 cm depth) during oviposition or on the same night or following morning in the case of relocated nests. For each of the 21 monitored nests of *Caretta caretta*, temperatures were recorded every 90 min from the time of egg deposition to the emergence of hatchlings.

In Libya, temperatures were recorded with 32 electronic temperature recorders LogTag HAXO-8 Humidity and Temperature Data Logger (LSTechnology, UK) during the 2009 nesting season (July to September) in 14 loggerhead nests on several beaches (Al Ghbeba, Althalateen, Shash, Al-Arbaeen and west of Althalateen) located at the East of Sirte city, Libya, in the Gulf of Sirte (Fig. 1). One to four dataloggers were placed in nests found in the morning during beach monitoring. All loggers were programmed to record temperature every 15 min. The dates of emergences of hatchlings were also recorded. Some nests were equipped several days after nesting occurred for logistic reasons and missing temperatures were reconstructed as described in next section. Dataloggers measures have been compared to temperatures recorded by mercury thermometer and no systematic deviation was observed.

2.2. Filling the temperature gaps

Based on the observation that nest temperature is correlated with air temperature (AT) and with the sea surface temperature (SST) in front of the beach (Girondot and Kaska 2014b), we collected such data for years 1979 to 2014 from the European Centre for Medium-Range Weather Forecasts (ECMWF) data sets, which provide temperature every 6 hrs (UTC) for several decades at 0.1° resolution (Dee et al. 2011). SST and AT at a height of 2 m and the closest available to the nesting sites were extracted for the entire nesting period (Fig. 3).

We modelled the average daily temperature using a generalized linear mixed model with Gaussian distribution and identity link function:

$$NT_{j,i} = \mu + \alpha \cdot SST_{i-dSST} + \beta \cdot AT_{i-dAT} + \gamma \cdot d_i + \epsilon_j$$

Where $NT_{j,i}$ is the daily average temperature of the nest j on day i ($i = 0$ for nest deposition), μ is a constant term, α and β are the coefficients associated with the linear relationship between NTx and SST and AT lagged by $dSST$ and dAT days, respectively. The analysis was performed for $dSST$ and dAT from 0 to 10 days and the best combination was discriminated using AIC. AIC is a measure of the quality of fit, which penalizes the quality of the fit for too many parameters in the model (Burnham and Anderson, 2002). When several combinations had similar AIC support ($\Delta AIC < 2$), the solution with the lowest $dSST + dAT$ was retained. Nest temperature is also affected by metabolic heating, which is the increase of temperature within the nest as compared to adjacent sand temperature resulting from metabolic reactions within the nest. The parameter γ is the maximum difference of temperature between sand and nest at the end of incubation and d_i is the proportion of incubation time for the nest (0 is for day 0 and 1 is for the end of incubation). Random effect was the nest identity, which includes its position on the beach and a temporal autocorrelation structure of order 1 was used (AR1 model). Average daily amplitudes of temperatures (AmpT) have been calculated for all days and all nests using daily maximum [from noon to noon] – daily minimum [from twelve to twelve] (Eccel 2010a).

Using the fitted μ , α , β and γ values and the daily amplitude of temperatures, it was possible to reconstruct the missing temperatures following a similar method used by Eccel (2010b) available in the R packages HelpersMG (Girondot 2016). In short, time series of temperatures are modelled using series of sinus functions in which a maximum at day i is observed for ($x = T_{max}, y = NT_{j,i} + \text{AmpT}/2$) and the minimum for ($x = T_{min}, y = NT_{j,i} - \text{AmpT}/2$). Only time sequences with less than 5 days missing were retained to limit the influence of the reconstructed time series of temperatures on the final results. As a consequence, data from three nests from Libya were discarded.

2.3. Growth rate thermal reaction norm

The model of embryo growth integrates in a single framework both the growth rate dependency on temperature and the embryo growth based on growth rate (Girondot and Kaska 2014a). The growth rate dependency on temperature and the embryo growth model were fitted using maximum likelihood to best describe the observed hatchling size according to time series of temperature within nests (Girondot and Kaska 2014a). This method is summarized briefly and some changes to the original method are emphasized.

Biological temperature-dependent rate models based on Arrhenius' and Eyring's equations were formulated by Sharpe and DeMichele (1977). The original formulation of Sharpe and DeMichele was modified by Schoolfield et al. (1981) to remove the very high correlations of parameter estimators (eq. 1).

$$r(T) = \frac{\rho_{(298\text{ K})} \frac{T}{298} \exp\left[\frac{\Delta H_A^\ddagger}{R} \left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R} \left(\frac{1}{T_{1/2L}} - \frac{1}{T}\right)\right] + \exp\left[\frac{\Delta H_H}{R} \left(\frac{1}{T_{1/2H}} - \frac{1}{T}\right)\right]} \quad [\text{Equation 1}]$$

$r(T)$ is the mean development rate at temperature T (time^{-1}), T is temperature in K (298 K = 24.85°C), and R is the universal gas constant ($\text{J K}^{-1} \text{mol}^{-1}$). The original model defined R in $\text{cal K}^{-1} \text{mol}^{-1}$; however, this has been converted to SI units.

$\rho_{(298\text{ K})}$ is the development rate at 24.85°C assuming no enzyme inactivation (time^{-1}), ΔH_A^\ddagger is the enthalpy of activation of the reaction that is catalyzed by the enzymes (J mol^{-1}), $T_{1/2L}$ is the temperature in K at which the enzymes are ½ active and ½ low temperature inactive, ΔH_L is the change in enthalpy associated with low temperature inactivation of the enzymes (J mol^{-1}), $T_{1/2H}$ is the temperature in K at which the enzymes are ½ active and ½ high temperature inactive, ΔH_H is the change in enthalpy associated with high temperature inactivation of the enzymes (J mol^{-1}). To ensure that $T_{1/2H} \geq T_{1/2L}$, a new variable ΔT has been setup with $T_{1/2H} = T_{1/2L} + |\Delta T|$. Thus the fitted variables were $T_{1/2L}$, ΔT , ΔH_H , ΔH_L , ΔH_A^\ddagger , and $\rho_{(298\text{ K})}$.

This model can be simplified taking into account only 4 parameters (Schoolfield et al. 1981):

$$r(T) = \frac{\rho_{(298\text{ K})} \frac{T}{298} \exp\left[\frac{\Delta H_A^\ddagger}{R} \left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_H}{R} \left(\frac{1}{T_{1/2H}} - \frac{1}{T}\right)\right]} \quad [\text{Equation 2}]$$

For equation 2, the fitted variables were $T_{1/2H}$, ΔH_H , ΔH_A^\ddagger , and $\rho_{(298\text{ K})}$.

2.4. Change of embryo size with time

The growth of embryos was modeled using a modification of the Gompertz model proposed by Laird (1964):

$$X(t) = K \exp\left(\ln\left(\frac{X(0)}{K}\right) \exp(-r(T) t)\right) \quad [\text{Equation 3}]$$

Where $X(0)$ is the size or mass at nesting time ($\text{time} = 0$), $r(T)$ is the growth rate at the beginning of the curve, and K is the carrying capacity with $\lim_{t \rightarrow +\infty} X(t) = K$. Note that hatching occurs

generally before the time when the embryo reaches a size or mass K . The K parameter can be viewed here simply as a way to slow down the growth at the end of incubation (Girondot and Kaska 2014a).

The dynamic of $X(t)$ is governed by the Gompertz differential equation (eq. 4):

$$X'(t) = r(T) \ln\left(\frac{K}{X(t)}\right) X(t) \text{ [Equation 4]}$$

At oviposition the gastrula is approximately a disk 1.7 mm in diameter and this size was used as $X(0)$ (Kaska and Downie 1999).

2.5. Parameter fitting

$r(T)$ can be calculated with the 4 or 6 parameters of the Schoolfield et al. (1981) model and an incubation temperature T . Knowing $X(0)$, K , and a time-series of $r(T)$, the pattern of change of size for this nest was evaluated using Runge-Kutta method of order 4 for the approximation of solutions of ordinary differential equations. The mean straight carapace length (SCL) at hatching for the turtles in Mediterranean loggerhead nests is 39.33 mm (SD = 1.92 mm) (Girondot and Kaska 2014b).

Estimation of parameters was performed using maximum likelihood with a Gaussian distribution of SCL and an identity link. Matrix of variances-covariances for fitted parameters was estimated using the inverse of the Hessian matrix (matrix of second order derivatives for all couples of parameters) and confidence interval of $r(T)$ was estimated using delta-method (Bates and Watts 1988).

2.6. Comparison between Libyan and Turkish populations

First, $r(T)$ was fitted separately to data from nests from the Libyan and Turkish populations and, second, $r(T)$ was fitted to the data grouped in a single dataset. We used AIC and Akaike weight to test whether a single model for both populations was sufficient or not to describe the data. Akaike weight gives the relative statistical support of several models tested on the same dataset (Burnham and Anderson 2002).

3. Results

3.1. Nest temperature reconstruction

To reconstruct missing parts of nest temperature from Libya, daily average temperatures were modelled using a generalized linear mixed model (Girondot and Kaska 2014b). The best AIC was for $dSST = 3$ days and $dAT = 0$ day (AIC = -634.93). SST, AT, and metabolic heating were all significant factors ($\alpha = 0.19$, $p = 2.26 \times 10^{-5}$; $\beta = -0.024$, $p = 2.53 \times 10^{-5}$; $\gamma = 2.05$, p

$= 5.31 \times 10^{-16}$, respectively) and were used to predict daily average temperature during the missing incubation period.

A sinusoidal function was applied to generate periodic hourly-based temperature time series. The amplitude of daily thermal fluctuations was low with little variability (mean, $0.34^{\circ}\text{C} \pm 0.17^{\circ}\text{C}$ SD), the lowest and highest temperatures being observed at $T_{\min} = 11 \text{ h } 28 \text{ min UTC}$ (SD = 2 h 7 min) and $T_{\max} = 23 \text{ h } 52 \text{ min UTC}$ (SD = 1 h 54 min), respectively (local time is UTC – 5 h). Time-series with missing temperatures were completed by the modelled ones.

3.2. Growth rate dependent on temperature

Temperatures recorded within nests were much lower in Turkey (mean, 29.18 ± 2.18 SD; range, 22.7–34.1) than in Libya (mean, 29.98 ± 0.93 SD; range, 27.7–32.6). The range of temperatures for each location is defined as the informative temperatures (Girondot and Kaska 2014a); it means that only the portion of $r(T)$ within this range should be discussed. The temperatures recorded in Sirte in 2009 were not exceptionally high and the ones recorded in Dalyan in 2010 were not exceptionally low based on the air data temperatures and sea surface data temperatures recorded for the last 35 yrs (Dee et al. 2011) (Fig. 3).

Two equations relating temperature and embryo growth rate (eq. 1 and 2) were compared using AIC for the Libyan data. The simplest form (eq. 2) of the Schoolfield et al. (1981) model was strongly supported (AIC = 67.48, Akaike weight = 0.81) compared with the 6-parameters equation (eq. 1; AIC = 70.49, Akaike weight = 0.18). Same result was obtained for the Turkish population (Girondot and Kaska 2014a). Only the 4-parameters equation (eq. 2) will be used further in this work.

To test whether a difference between the responses of embryos from Libya and Turkey to temperature (Figs. 4) could be detected, all the data were grouped and fitted with a common 4-parameters model (Figs. 5). Then this model with a single set of parameters for both locations (AIC = 151.3) was compared with the model with one set of parameters for each location (AIC = 157.8). The probability that a single set of parameters is sufficient to explain the combined data was highly supported (Akaike weight = 0.96) (Fig. 6). The growth rate reached its maximum at 31.5°C and the growth rate at 20°C was only one-fifth of the one observed at 31.5°C .

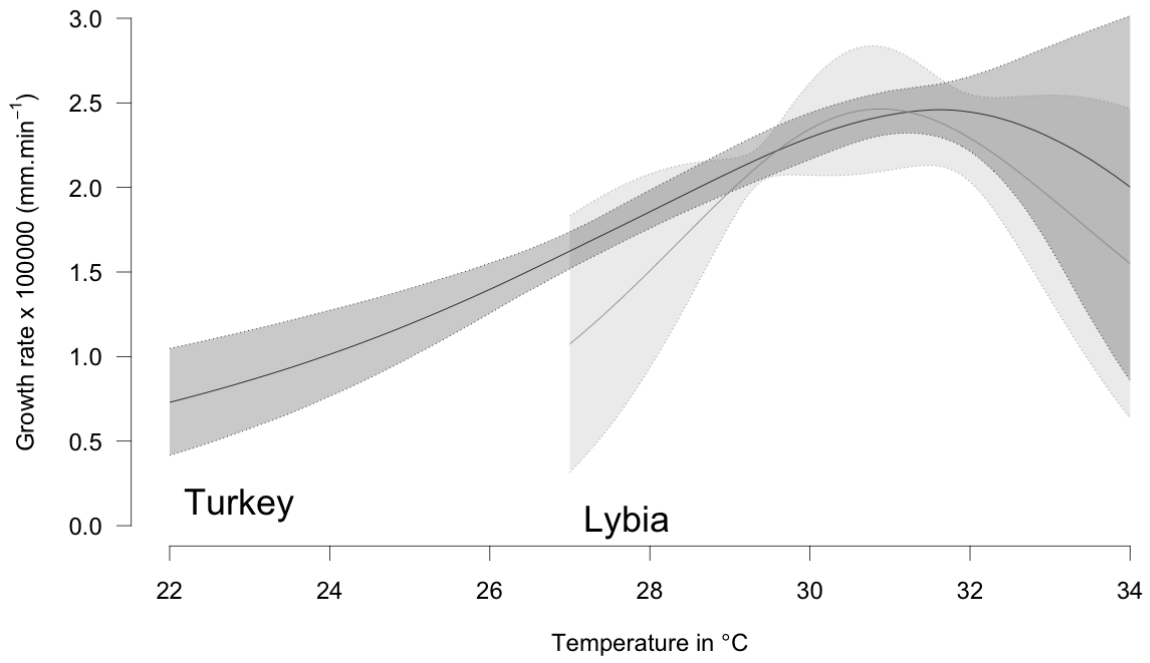


Figure 4: Fitted growth rate $r(T)$ of straight carapace length (SCL) of *Caretta caretta* depending on incubation temperature T in Libyan (in light grey) and in Turkish (in dark grey) nests. The area within the dashed lines indicate the 95% confidence interval.

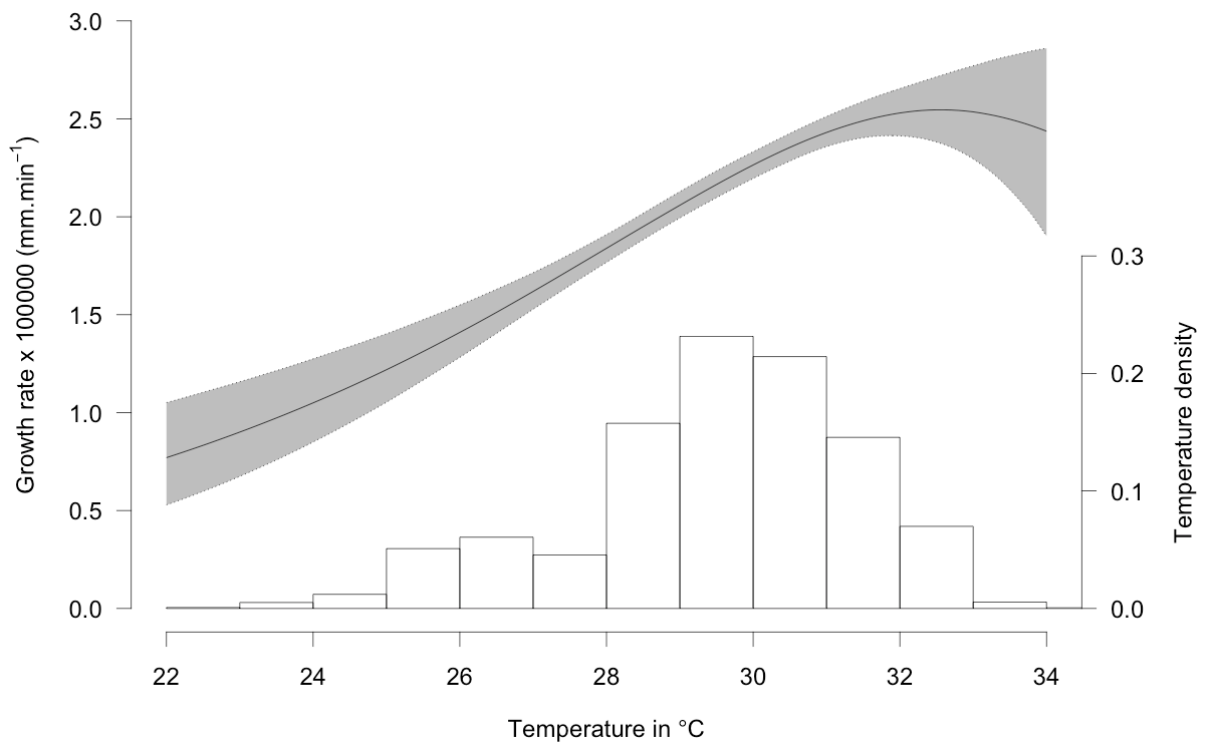


Figure 5: Common model for Libyan and Turkish nests for fitted growth rate $r(T)$ of straight carapace length (SCL) of *Caretta caretta* depending on incubation temperature T . The area within the dashed lines indicate the 95% confidence interval. The histogram shows the temperatures among all these nests.

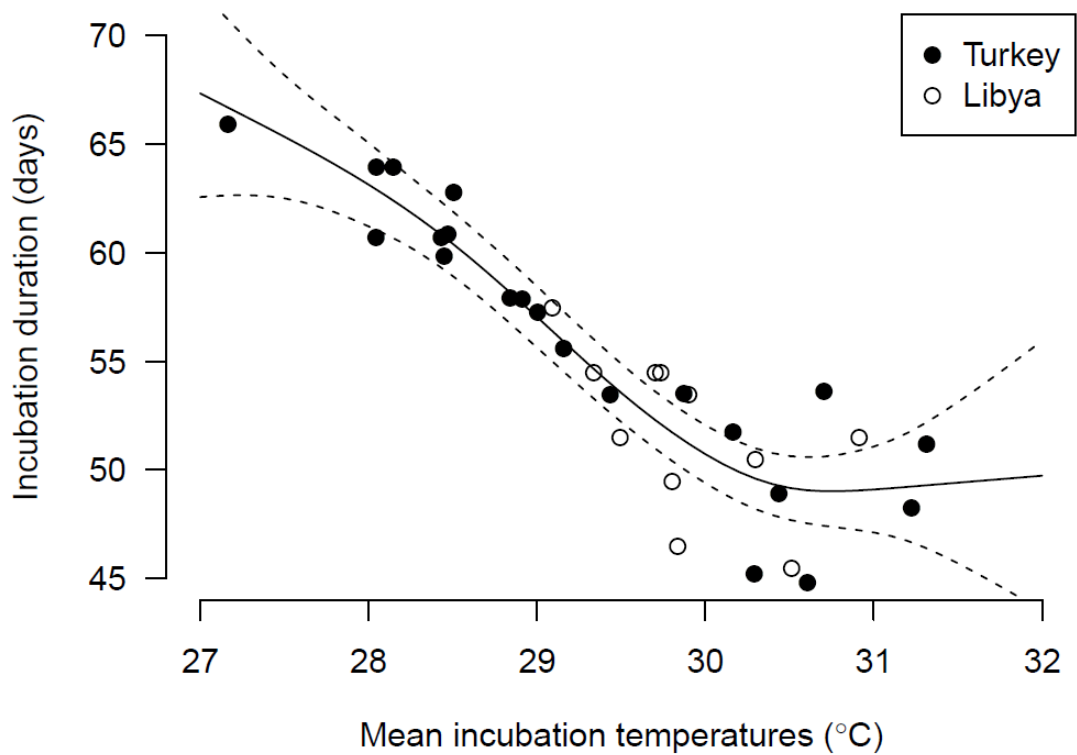


Figure 6: Relationship between mean incubation temperatures and incubation duration for the *Caretta caretta* nests monitored in Libyan and Turkish coast. Best GAM fit curve and ± 1.96 SE is shown.

4. Discussion

Nest temperatures for nests incubated in Libya were modelled to fill missing temperatures at the beginning of incubation period. As previously demonstrated for nests incubated in Turkey (Girondot and Kaska 2014b), the relative contribution of AT and SST to explain nest temperature differs both quantitatively and temporally. Whereas the effect of SST lagged by 3 days, the effect of AT was not delayed in Libya. These results differ from those obtained in Turkey, where the effect of SST lagged by 1 day and the effect of AT lagged by 2 days (Girondot and Kaska 2014b). However, because solar irradiation is moderated by reflectance, albedo, thermal capacitance, and thermal conductivity, this difference could be to the result of beach-specific physical characteristics. In addition, we found that the relative contribution of SST in front of the beach as compared to AT was higher in explaining nest temperature. This finding is logical because water is a better heat conductor than air (Girondot and Kaska 2014b). Also, metabolic heating within the nest was significant: nests were 2.05°C warmer than the surrounding sand at the end of incubation. This result is consistent with previous studies on metabolic heating for sea turtles which range from 0.07°C to 5°C (Booth and Astill 2001, Broderick et al. 2001, Godley et al. 2001, Zbinden et al. 2006). Daily patterns of thermal fluctuations were reconstructed to represent the best as possible the time series of nest temperatures. Because nests of loggerhead turtles are incubated at a 45-cm depth (depth of the center of nest), it was not surprising that amplitude of thermal fluctuation was low. Then, we found that extreme daily temperatures are observed on the late morning and near midnight (for minimum and maximum, respectively). The times when extreme daily temperatures occur during a day vary depending of latitude and season according to earth orbital parameters (Eccel 2010b, Eccel 2010a). However, setting extreme temperatures at the same moment during a day is not a strong hypothesis for nests incubated at low latitudes and when analysis is performed for few months (incubation period of monitored nests ranged from the beginning of July to the end of September).

Temperature during incubation of ectothermic animals can have profound consequences on the fitness of individuals and selection acts to adapt response of embryos to temperature (Angilletta et al. 2003). Here we study 2 genetically close populations, separated by at most only 12,000 yrs, but that experience very different thermal nesting habitats. We show that we cannot detect difference in the response of growth of embryos to temperature for these both populations, whereas temperatures within nests were different. In both populations, the growth rate reached its maximum at 32°C (Figs. 4 and 5) or 31.5°C (Fig. 6) and decreased below and above but this

effect is masked by large confidence interval. Particularly, we show that nests incubated at very high temperatures have longer incubation duration than those incubated at 31.5°–32°C (the optimum temperature). Our results raise several questions about the adaptability of this population to change in temperature in the context of global change. We did not detect a difference between the 2 populations of Mediterranean Sea separated for less than 12,000 yrs but nesting in very different thermal habitats. These populations have not had enough time to adapt to the different thermal contexts. It should be noted also that the high longevity of marine turtles hampers a rapid adaptation (Tucek et al. 2013).

Negative effects of climate change could be mitigated by shift of nesting phenology to fit when a suitable thermal environment for the development of embryos is available. Such a phenotypic plasticity in the timing of nesting has been reported to be another temperature-dependent life-history trait for sea turtles: Several studies have shown that warmer sea surface temperatures at the foraging and breeding sites result in earlier nesting events for the loggerhead sea turtle (Weishampel et al. 2004, Mazaris et al. 2009, Weishampel et al. 2010, Ben Hassine et al. 2011, Lamont and Fujisaki 2014) and the green sea turtle (Weishampel et al. 2010). On the other hand, a delay in the first nesting events related to warmer sea surface temperatures at the foraging and breeding sites has been reported for the leatherback sea turtle (Neeman et al. 2015) and the green sea turtle (Dalleau et al. 2012). The effect of temperature on the temporal parameters of nesting phenology could be indirectly mediated by the availability of resources at the foraging sites. If warmer conditions are associated with fewer resources being available, females will spend more time accumulating reserves before starting migration to breeding and nesting sites. Therefore, first nesting events will be delayed. Temperature also affects the rate of biological processes such as oogenesis, vitellogenesis, and metabolism. Consequently, higher temperatures will promote a departure of females from the foraging sites earlier in the season and then earlier first nesting event. In such situation, there are no rules and each population will react in different ways depending on the thermal conditions at the foraging, breeding and nesting sites and the distance between them.

Marine turtles exhibit all temperature-dependent sex determination with eggs incubated at lower temperatures producing more males and eggs incubated at higher temperature producing more females (Hulin et al. 2009). It has been proposed that the larger the range of temperatures producing both sexes (TRT), the more the population can adapt sex ratio to a climate change (Hulin et al. 2009). The TRT has been studied in 8 populations of *Caretta caretta* and the population of Greece exhibits the second lower TRT after South-African population (Hulin et

al. 2009), perhaps as a result of the founder effect when this population colonized the Mediterranean Sea (Clusa et al. 2013). The nearby population nesting in Kuriat Islands (Tunisia) has a low protein diversity (Chaieb et al. 2010). It should be noted that individuals from Atlantic origin still enter the Mediterranean Sea but they seem not to participate to reproduction (Carreras et al. 2011). Thus, Mediterranean populations of *Caretta caretta* may have difficulties adapting rapidly to climate change and its effect on sex ratio. It would be very interesting to predict primary sex ratio for both the Turkish and the Libyan populations studied here but sex ratio thermal reaction norm for Libyan population is unknown. Thus, we are forced to hypothesize that Turkish and Libyan populations have the same sex ratio thermal reaction norm and the test will not be conclusive because of the circularity in hypothesis testing.

This temperature-dependent temporal adjustment of nesting leads to three scenarios: embryos will be exposed to (i) warmer conditions, (ii) colder conditions, or (iii) the same thermal environment during incubation. In the first situation, negative effects of climate change on the development of embryos are exacerbated leading to a low survival and a female biased hatchling sex ratio. In the second situation, negative effects of climate change are mitigated because embryos are not exposed to lethal temperatures and a more balanced sex ratio is probably produced. In the last scenario, negative effects of climate change are neutralized. Consequently, this system with 4 temperature-dependent life history traits (embryonic survival, sex ratio, incubation duration, and nesting phenology) should be correctly parametrized to quantify the effect of climate change on sea turtle populations but further work is needed to obtain reliable parameters.

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Chapter 2: Sex ratio estimates for species with temperature-dependent sex determination differ according to the proxy used

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Abstract: Knowledge of the sex ratio of a population is crucial to understand their structure and dynamics. For species, such as marine turtles, with temperature-dependent sex determination, this knowledge provides a baseline in advance of climate change. Determining the primary sex ratio for marine turtle populations is challenging since offspring lack sexually dimorphic external characteristics. Therefore several proxies have been used to estimate the primary sex ratio of marine turtle populations. However, no study to date has compared estimations of sex ratio when using different proxies to determine the most accurate and to detect potential bias. To address this, we estimated the sex ratio of natural loggerhead, *Caretta caretta*, nests using 8 different proxies: two based on constant temperature equivalent (average of temperature or average temperature weighted by the growth of embryos during each time step) both for three developmental periods (the whole incubation, the middle third of incubation and the middle third of development) as well as two proxies based on incubation duration (duration of the whole incubation and of the middle third of development). Sex ratio estimates differed greatly depending on the proxy being used. Here we discuss the differences among proxies based on the biological relevance of underlying hypotheses and highlight the need for

studies to accurately determine the thermosensitive period and to obtain appropriate estimates of embryo growth rate to estimate marine turtle sex ratio.

Keywords: Marine turtles, climate change, temperature-dependent sex determination, loggerhead turtle, reptile

1. Introduction

Knowledge of the sex ratio of a population is crucial to understand their structure, dynamics and their evolutionary potential; this informs population viability analysis and effective population size calculations, are relevant to the management and conservation of species (Brook et al. 2000, Ewen et al. 2001). An understanding of primary sex ratio is also important in the context of a warming climate, where populations of species with temperature-dependent sex determination (TSD), may have their sex ratios altered with changes in temperatures (Stubbs et al. 2014, dei Marcovaldi et al. 2016).

Determining the primary sex ratio of populations can be challenging, especially for species that lack heteromorphic sex chromosomes and when offspring lack sexually dimorphic external characteristics. This is the case for marine turtles which exhibit TSD, where incubation temperature triggers the development of either testes or ovaries in embryos (Yntema and Mrosovsky 1980, Mrosovsky 1982, Janzen and Paukstis 1991), and they are not externally dimorphic until puberty (Miller 1997, Wyneken 2001, Ceriani and Wyneken 2008). Thus, reliable sexing of marine turtle hatchlings requires the sacrifice of animals and histological examination of the gonad, which is difficult to justify for threatened species

Since empirical estimates of *in situ* primary sex ratios are difficult to obtain, several proxies (e.g., incubation temperature and duration) have been used to estimate the primary sex ratio of marine turtle populations (Girondot et al. 2010, Wyneken and Lolavar 2015). Wyneken and Lolavar (2015) recently categorized them based on how many steps are necessary to determine sex from the actual embryonic sex ratio, with an increase in proxy “distance” decreasing the reliability of the sex ratio estimate. First level proxies includes samples of hatchlings from nests, second level proxies includes the use of incubation temperature during the Thermosensitive Period (TSP) – developmental period for which temperature influences gonadal differentiation (Mrosovsky and Pieau 1991), and third level proxies includes, among others, incubation duration to infer TSP, the use of Constant Temperature Equivalent (CTE) to transform field incubation temperatures to a constant temperature that would result in the same developmental rate to determine sex ratio based on laboratory curves, and the use of climatic data to infer nest

temperatures. It has been widely highlighted that these proxies need to be validated with actual sex ratio expressed in nests and that caution is necessary when interpreting sex ratio estimates from proxies (Girondot et al. 2010, Wyneken and Lolavar 2015). Unfortunately, for ethical, logistical and economic reasons validation is challenging (Gross et al. 1995, Wyneken et al. 2007), and consequently several studies have used and continue to use these proxies. Even though these proxies have been reviewed separately, no study to date has compared estimations of sex ratio when using different proxies to determine the most pertinent and detect potential bias. To address this, we estimated the sex ratio of natural loggerhead, *Caretta caretta*, nests using 8 different proxies and discuss the differences among methods based on the biological relevance of underlying hypotheses.

2. Materials and methods

2.1. Incubation temperatures and durations from field

The temperature of 43 nests of loggerhead turtles at Praia de Forte, Bahia, Brazil was recorded during the 2014-2015 nesting season (21st October 2014 to the 11th of March 2015). Five of them were removed from analysis because they showed abnormal temperature patterns that were likely due to predation and consequent exposure events. This region is an important loggerhead nesting area for the Southwest Atlantic loggerhead Regional Management Unit (Wallace et al. 2010) and an intensive study area for TAMAR (for information on TAMAR, see Marcovaldi and de Marcovaldi 1999). Of the 38 nests used for this study, 16 nests were monitored using Minilog data loggers (VEMCO Minilog-II-T, accuracy $\pm 0.1^{\circ}\text{C}$, resolution= 0.01°C) and 22 others with Thermochron iButtons (DS1922L-F5, accuracy $\pm 0.5^{\circ}\text{C}$, resolution= 0.0625°C). Dataloggers were placed at the middle of the clutches while females laid their eggs and removed just after hatchlings emerged. Incubation durations, are defined here as the time between when the eggs were laid and when the first hatchlings emerged. Air temperature at 2 m above the ground level and sea surface temperature in front of the beach were retrieved from the European Centre for Medium-range Weather Forecasts (ECMWF) which provide a global reanalysis (of climate temperature for the last 37 years on earth at a 0.125° spatial resolution (ERA-Interim project), where models used historical records to provide smoothed temperature time hindcasts. We retrieved data every 6 hours from the 1st January 1979 to the 31th December 2015 (latitude 12.375°S and longitude 38°W).

Temperature time series recorded within natural nests can be viewed as an autocorrelative process by which temperature recorded at time i depends on temperature at time $i-1$. Statistical properties of such time series are analyzed by autoregressive moving-average (ARMA) models

which describe stationary stochastic process using two parameters, p and q (p is the lag for the coefficient of auto regression AR and q is the lag for the moving average MA). The ARMA model that best describe daily nest temperature was estimated for $p=0$ or 1 and $q=0$ or 1 (with 0 being time i and 1 being time $i-1$). We summed the Akaike Information Criterion (AIC) for each of the 38 temperature time series recorded in natural conditions for each of these p and q combinations. AICs are then compared to test which ARMA model best describes statistical properties of nest temperature time series.

2.2. Proxies for sex ratio estimations

The primary sex ratio, for each monitored nest, was predicted using 8 different proxies: 2 based on constant temperature equivalent (average of temperature or average temperature weighted by the growth of embryos (in term of embryo size measured by straight carapace length during each time step) both for 3 developmental periods (the whole incubation, the middle third of incubation and the middle third of development) as well as 2 proxies based on incubation duration (duration of the whole incubation and of the middle third of development) (Table 1).

Proxy ID	Proxy base	Temporal scale	Biological assumptions
1	Constant Temperature Equivalent averaged temperature (CTE _A) in °C	Whole incubation	Sex is determined only by temperature and during the whole incubation period.
2	Constant Temperature Equivalent averaged temperature (CTE _A) in °C	Middle third of incubation	Sex is determined only by temperature and only during the middle third of incubation period.
3	Constant Temperature Equivalent averaged temperature (CTE _A) in °C	Middle-third of development (TSP)	Sex is determined only by temperature and only during the thermosensitive period for sex determination (TSP).
4	Constant Temperature Equivalent averaged temperature weighted by the growth of embryos (CTE _w) in °C	Whole incubation	Sex is determined by the interaction between temperature and growth rate and during the whole incubation period.
5	Constant Temperature Equivalent averaged temperature weighted by the growth of embryos (CTE _w) in °C	Middle third of incubation	Sex is determined by the interaction between temperature and growth rate and only during the middle third of incubation period.
6	Constant Temperature Equivalent averaged temperature weighted by the growth of embryos (CTE _w) in °C	Middle-third of development (TSP)	Sex is determined by the interaction between temperature and growth rate and only during the thermosensitive period for sex determination (TSP).
7	Duration-based equivalent in days	Whole incubation (linearly proportional with the middle third of incubation)	Indirect measure of the effect of the thermal environment on both growth and gonadal differentiation throughout the whole incubation period.
8	Duration-based equivalent in days	Middle-third of development (TSP)	Indirect measure of the effect of the thermal environment on both growth and

			gonadal differentiation throughout the thermosensitive period for sex determination (TSP).
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Table 1: Description of each proxy. Proxies 1 to 3 are averaged temperature CTE_A , proxies 4 to 6 are averaged temperature weighted by the growth of embryos CTE_W and proxies 7 and 8 are based on the duration of two developmental periods, respectively the whole incubation period and the middle third of development which corresponds to the thermosensitive period for sex determination (named TSP in this study).

2.2.1. Temperature-based proxies (proxies 1 – 6)

The relationship between marine turtle sex ratio and temperature or incubation duration (reaction norm of sex ratio) is first obtained with incubation experiments at constant temperature in laboratory conditions. This reaction norm is then applied to measures obtained in the field for the same population (see Marcovaldi et al. 1997, The Chu et al. 2008, dei Marcovaldi et al. 2014, dei Marcovaldi et al. 2016). Indeed for the population studied here, this relationship was determined with two clutches collected in 1995 and incubated artificially at 6 constant temperatures ranging from 27.4 to 30.6 °C (Marcovaldi et al. 1997). However, in natural conditions, incubation temperature fluctuates according to circadian cycle or from day to day, and is not constant. Therefore, temperature time series recorded within nests cannot be used as such to predict sex ratio from the relationship established at constant temperatures. Thus, to increase the accuracy of sex ratio estimates from nest profiles, a Constant Temperature Equivalent (CTE) has been used for different incubation periods to determine sex ratios (see Georges et al. 1994, Georges et al. 2005, The Chu et al. 2008, Woolgar et al. 2013). It has been proposed that average incubation temperature during any developmental periods (named here CTE_A) is not able to reflect correctly the effect of temperature on gonadal differentiation (Valenzuela 2001). This is because the growth of embryos (or cell proliferation) is also dependent on temperature. In other terms, the higher the temperature (which is more likely feminizing) the faster the growth and the lower the temperature (which is more likely masculinizing) the slower the growth. Considering this, embryos that spend equal time at masculinizing and feminizing temperatures would develop into females because more cells have proliferated during the feminizing period rather than during the masculinizing one. CTE weighted by embryonic growth (named here CTE_W) is another type of proxy that accounts for the rate of gonadal differentiation according to the differential growth of embryos at different temperatures. Here we propose to calculate CTE_W by multiplying the incubation temperature by the fraction of development realized for each period from k to N:

$$CTE = \frac{\sum_{i=k}^{N-1} T_i \times W_i}{\sum_{i=k}^{N-1} W_i} \text{ [Equation 1]}$$

Where T_i is the temperature at the moment i during incubation with k being the first recorded temperature during the considered developmental period. W_i is the growth of embryos in term of straight carapace length (SCL) between two records (i.e. the difference between the embryonic size at time $i+1$ and i). N is the last recorded temperature during the considered developmental period. The following developmental periods were considered for temperature-based proxies (CTE_A and CTE_W – proxies 1-6, Table 1): (i) the total incubation duration, (ii) the middle third of incubation period and (iii) the middle third of development (which corresponds to the thermosensitive period for sex determination and named TSP in this study – as determined in section 2.3) for each of the 38 natural nests monitored.

2.2.2. Duration based proxies (proxies 7 and 8)

Temperature has a direct effect on marine turtle embryonic growth and thus on the timing to complete embryonic development (Miller 1985). Consequently, the duration of the total incubation period (from a freshly laid egg to a completely developed embryo) is an integrated measure of thermal incubation conditions. Given this, incubation durations recorded in the field (from nest laying date to hatching date) have been used as a proxy to predict sex ratio of clutches (Fuller et al. 2013, dei Marcovaldi et al. 2014, Jribi and Bradai 2014, dei Marcovaldi et al. 2016). However, given daily variability in thermal fluctuations in natural conditions, the total incubation duration does not necessarily reflect thermal conditions during the TSP. The TSP occurs during incubation when gonads form and until a critical threshold when gonads are sufficiently developed to differentiate along the male or female pathways. This period corresponds to the middle third of development (Mrosovsky and Pieau 1991). Thus, the progression of embryonic development during incubation needs to be modeled to access information about TSP boundaries during incubation and then its duration.

2.3. Modeling embryonic development

To determine sex ratio for proxies 3, 6, and 8, which are related to the middle third of development, the growth of embryos throughout incubation needed to be calculated to calculate the TSP boundaries. The change of embryonic size can be modeled using a growth function where the growth rate depends on temperature (see section 2.3.1). Therefore, the thermal reaction norm for growth rate (i.e. the pattern of growth rate across a range of temperature) needs to be estimated to model the growth of embryos accounting for such phenotypic plasticity. Here we modeled both the thermal reaction norm for growth rate and the progression of embryonic size in term of straight carapace length (SCL) for the 38 natural nests. Thereafter, we determined TSP boundaries and incubation duration for each nest. We used SCL metric

rather than mass to ensure that the first-order derivative of the chosen metric can never be negative as does the Gompertz equation (see section 2.3.1).

2.3.1. Progression of straight carapace length during incubation

Thermal reaction norm for growth rate can be estimated from traces of temperature recorded within natural nests (Girondot and Kaska 2014). Here we used the 4-parameters equation to describe the effect of temperature on the embryonic growth rate for loggerhead turtles as per Girondot and Kaska (2014). The equation is given below:

$$r(T) = \frac{\rho_{(298\text{ K})} \frac{T}{298} \exp\left[\frac{\Delta H_A^\ddagger}{R} \left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_H}{R} \left(\frac{1}{T_{1/2H}} - \frac{1}{T}\right)\right]} \quad [\text{Equation 2}]$$

Where $r(T)$ is the rate of embryonic growth at temperature T (time^{-1}), R is the universal gas constant ($\text{J.K}^{-1}.\text{mol}^{-1}$), $T_{1/2H}$ is the is the temperature in K at which the enzymes are 1/2 active and 1/2 inactive due to high-temperature effect, ΔH_H is the change in enthalpy associated with the high-temperature inactivation of the enzymes (J.mol^{-1}), ΔH_A^\ddagger is the enthalpy of activation of the reaction catalyzed by the enzymes (J.mol^{-1}) and $\rho_{(298\text{ K})}$ is the developmental rate at 24.85°C (298 K) assuming no enzyme inactivation (time^{-1}). Thus the fitted variables are $T_{1/2H}$, ΔH_H , ΔH_A^\ddagger and $\rho_{(298\text{ K})}$.

The change of size of embryos upon incubation time is described by the Gompertz equation which have been modified by (Laird 1964):

$$X(t) = K \exp\left(\ln\left(\frac{X(0)}{K}\right) \exp(-r(T) t)\right) \quad [\text{Equation 3}]$$

Where $X(0)$ is the size of a gastrula at nesting time ($\text{time} = 0$), $r(T)$ is the growth rate and K is the asymptotic value when $t \rightarrow \infty$. $X(t)$ is the size of embryos at any time during incubation and depends on the rate of growth which depends on temperature. To inform this, we measured a subset of hatchlings ($n = 70$) from one monitored clutch at Praia do Forte during the study period. The straight carapace length of hatchlings was measured, and an average size of 40.97 mm (SD 0.96 mm) was recorded. The confidence interval of the thermal reaction norm for growth rate has been obtained by simulating the posterior distribution of the four parameters using MCMC with Metropolis-Hastings algorithm (Chib and Greenberg 1995) during 100 000 iterations. An adaptive algorithm has been used to satisfy the acceptance rate close to 0.234 by adjusting the distributions of the new proposals every 100 iterations (Rosenthal 2011). Initial parameter values during the MCMC search corresponded to the set of values that maximized

likelihood (for more details see Girondot and Kaska 2014). The priors are U (500) for ΔH_A^\ddagger , U (100000) for ΔH_H , U (500) for $\rho_{(298\text{ K})}$ and N (308; 7) for $T_{1/2_H}$. The thermal reaction norm model was informed by the 38 temperature times series recorded in the field at Praia de Forte and the 12 constant temperature experiments from Marcovaldi et al. (1997).

2.3.2. Determining period of development (whole incubation, the middle-third of incubation duration, and the middle-third of development)

CTEs (proxies 1 to 6) were implemented for three incubating periods (i) the whole incubation period, (ii) the middle third of incubation period and (iii) the middle-third of development (TSP), and the duration-based proxies (proxies 7 and 8) were implemented for (i) the whole incubation duration and (ii) the middle third of development. We did not calculate the duration of the middle third of incubation as a third duration-based proxy because this measure is directly proportional to the whole incubation duration and would therefore provide redundant results.

The whole incubation period is defined from a freshly laid egg at gastrula stage to a completely developed at hatchling. The middle third of incubation starts at the 1/3 of the whole incubation duration and ends at 2/3 of the whole incubation duration. The TSP starts when embryos reach 1/3 of their final size before hatching and ends when embryos reach 2/3 of their final size.

2.4. Reaction norm of sex ratio at constant temperature

Loggerhead turtles species exhibits a male/female pattern of TSD which corresponds to the production of males at low temperatures and females at high temperatures (see Wibbels 2003 for a review). For the population in focus here, their sex ratio determined histologically, the constant incubation temperature and the total incubation duration have been previously established for 185 hatchlings from two nests (Marcovaldi et al. 1997). We modeled the embryonic development (as described in section 2.3.1) for these 6 constant incubation temperatures and calculate the duration of the TSP.

The sex ratio thermal reaction norm can be modeled using several equations whereas the global pattern is summarized by the pivotal temperature at which sex ratio is equal to 0.5 (P) and the range of temperature within which both sexes are produced is called the Transitional Range of Temperatures (TRT). The relationship between sex ratio and incubation duration can be modeled using similar equations and it permits to define the pivotal duration (PD) at which sex ratio is equal to 0.5 and the range of duration within which both sexes are produced is called the Transitional Range of Durations (TRD). It should be noted that the pattern describing sex ratio versus temperature is reversed as compared to the pattern of sex ratio versus incubation duration. Indeed, embryos that incubate at high temperature develop faster and are more

feminized than those incubated at cooler temperature. However, this is true only for monotonic relationship between temperature and rate of development. This relationship cannot be used if incubation duration slowdown at very high temperature.

Genotypic Sex Determination (GSD) is modeled as a constant 0.5 sex ratio for all temperatures or durations. Four other equations can be fitted if sex is determined by temperature (for more detailed see Hulin et al. 2009). The simplest equation is the logistic model:

$$sr(proxy) = \frac{1}{1+e^{\left(\frac{(P-proxy)}{S}\right)}} \text{ [Equation 4]}$$

Where, $sr(proxy)$, is the sex ratio (here the proportion of males) in terms of the considered proxy. This equation can be modified to account for a logarithmic scaling in the shape of the transition between the production of 100% males and 100% females. This model is the Hill equation:

$$sr(proxy) = \frac{1}{1+e^{\left(\frac{(\log P - \log proxy)}{S}\right)}} \text{ [Equation 5]}$$

To account for a potential asymmetrical transition in the sex ratio produced around the pivotal value of the considered proxy, a third parameter K can be introduced. This model is the Richards equation:

$$sr(proxy) = \left[\frac{1}{1+(2e^{K-1}) e^{\left(\frac{(P-proxy)}{S}\right)}} \right] e^{\frac{1}{K}} \text{ [Equation 6]}$$

In this equation, the parameter K does not depend on the value of the proxy. Given this, a sharp transition before the inflection point of the sigmoid curve implies a smooth transition after and vice versa. To account for an asymmetrical pattern with a potential similar shape above and below the pivotal value of the proxy, two parameters K_1 and K_2 replace the previous one. This equation is named Hulin model is:

$$sr(proxy) = \left[\frac{1}{1+(2e^{K_1 \cdot proxy + K_2 - 1}) e^{\left(\frac{(P-proxy)}{S}\right)}} \right] e^{\frac{1}{K_1 \cdot proxy + K_2}} \text{ [Equation 7]}$$

To select the most appropriate equation/model that describes the relationship between sex ratio and the considered proxy, we compared their Akaike information criteria (AIC) with a correction for finite sample sizes (AICc) using the Akaike weight which gives relative support for each model (Burnham and Anderson 2002).

This model selection was conducted independently for sex ratio against (i) temperature to be used with proxies 1 to 6, (ii) incubation duration for proxy 7, and (iii) TSP duration for proxy 8. The 3 resulting equations are used further to predict the sex ratio of natural nests using each proxy.

2.5. Quantifying distances among sex ratio estimates using different proxies

Once sex ratio estimates have been obtained using each proxy (proxy 1 to 8), we calculated the Euclidean distance for each pairwise comparison of proxy-specific sex ratio estimates in order to quantify differences among proxies. Those distances are then clustered using unweighted pair group method (UPGMA). Thereafter, results are represented using a dendrogram to visualize distances among proxy-specific sex ratio estimates.

3. Results

3.1. Thermal incubating environment

Temperature recorded within nests ranged from 23.6 °C to 35.5 °C (mean=31.34 °C, sd =1.62 °C) with an average daily amplitude of 0.61 °C (Fig. 1). Temperature recorded within nests was higher than air temperature and sea surface temperature.

Although a temporal trend of sea surface temperature and nest temperature shows a slight increase along the nesting period (October to March), air temperature did not show a clear seasonal trend within this temporal window (Fig. 1).

Four ARMA models have been fitted to nest temperature time series (with $p=0$ or 1 and $q=0$ or 1). The lowest AIC is observed for the ARMA model with $p=1$ and $q=0$ (AIC=36355.33) as compared to the following models: $p=0$ and $q=0$ (AIC=65615.86), $p=0$ and $q=1$ (AIC=56357.71) and $p=1$ and $q=1$ (AIC=36396.15). This suggest that nest temperature time series can be viewed as an autoregressive process of first order. In other words, temperature at time t is correlated with temperature at time $t-1$ and no moving average component is needed.

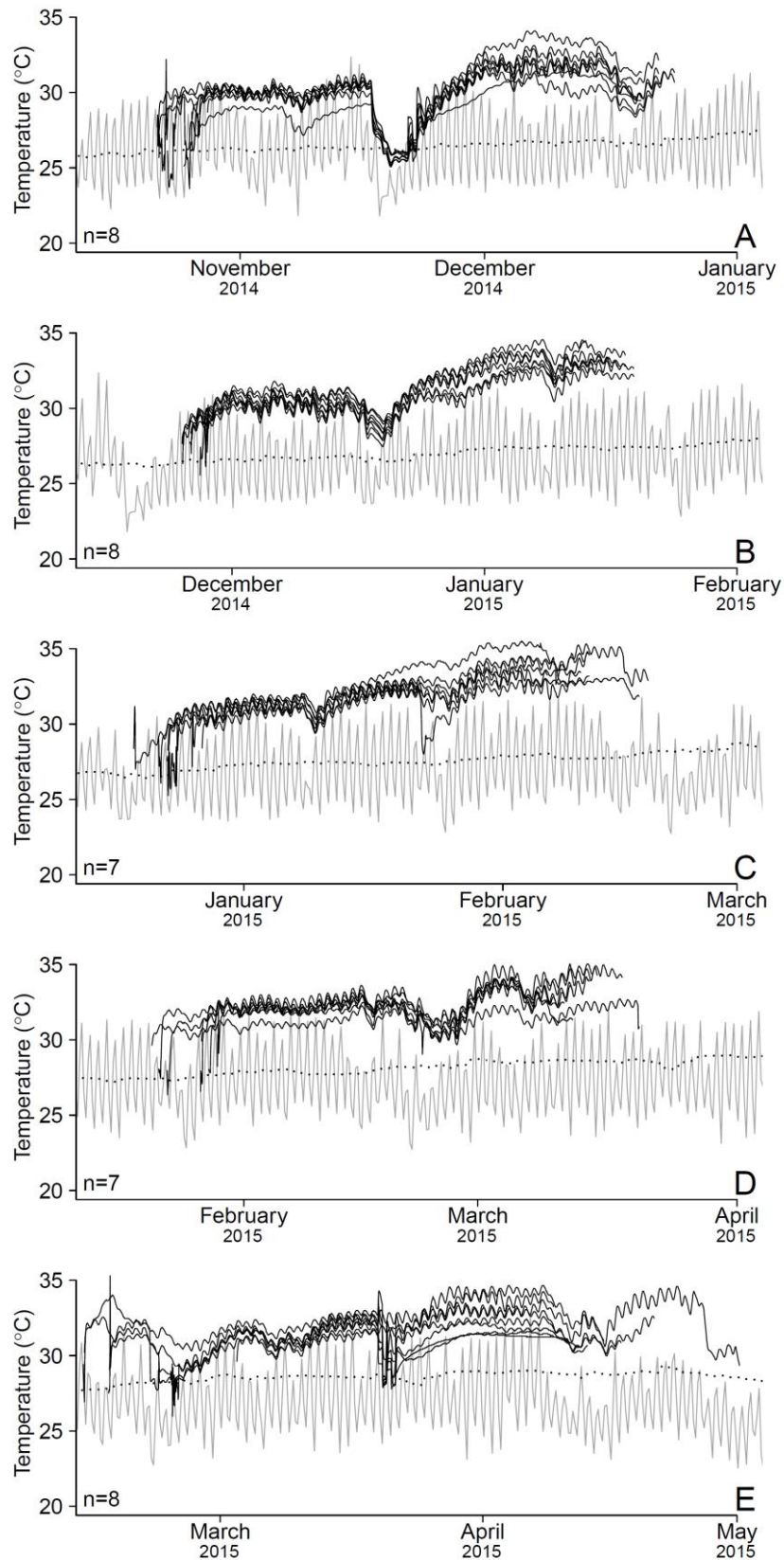


Figure 1: Variation in incubation temperature for 38 natural nests (black lines), along with: air temperature at 2 m above the surface (grey lines) and sea surface temperature in front of the nesting beach (dotted lines). Nests were laid in A) October 2014, B) November 2014, C) December 2014, D) January 2015, and E) February 2015. Note that time frames for each plot are shifted by one month depending when the nest was laid.

3.2. Embryonic development model

The thermal reaction norm for embryonic growth rate shows a non-monotonic pattern where the growth rate increases with temperature to reach a maximum at 34.5°C. The embryonic growth rate at 34.5°C is 2.7 times higher than at 20°C and decreases sharply when nest temperature exceeds 34.5°C (Fig. 2).

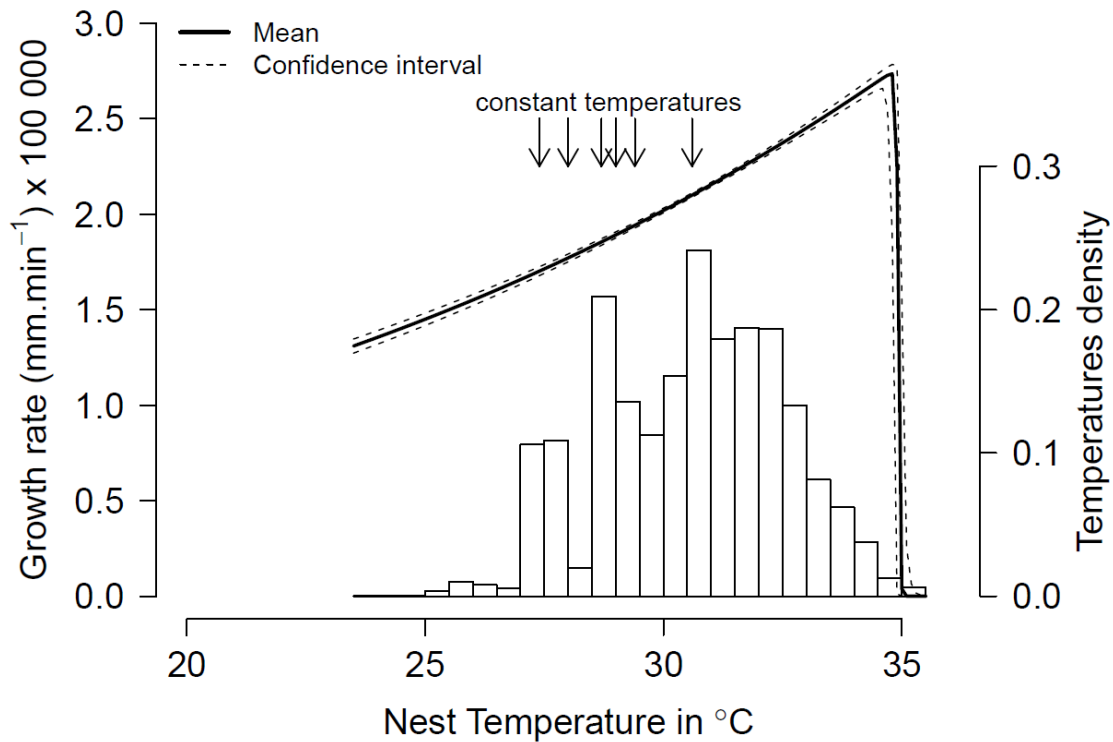


Figure 2: Thermal reaction norm for embryonic growth rate for Brazilian loggerhead population nesting at Praia de Forte. The confidence interval (dotted lines) is based on quantiles 2.5% and 97.5%.

TSP durations varied from 20.94 days to 22.57 days for nests which have been laid during October 2014 and from 17.17 days to 20.02 days for those after mid-November 2014 (Appendix A). The TSP does not always fit with the middle third of incubation in natural conditions (Fig. 3). For instance, the TSP is delayed from up to 2 days (Fig. 3, A and B) or it starts earlier (Fig. 3, C and D) as compared to the middle third of incubation period for a set of 4 nests which have incubated after mid-December 2014.

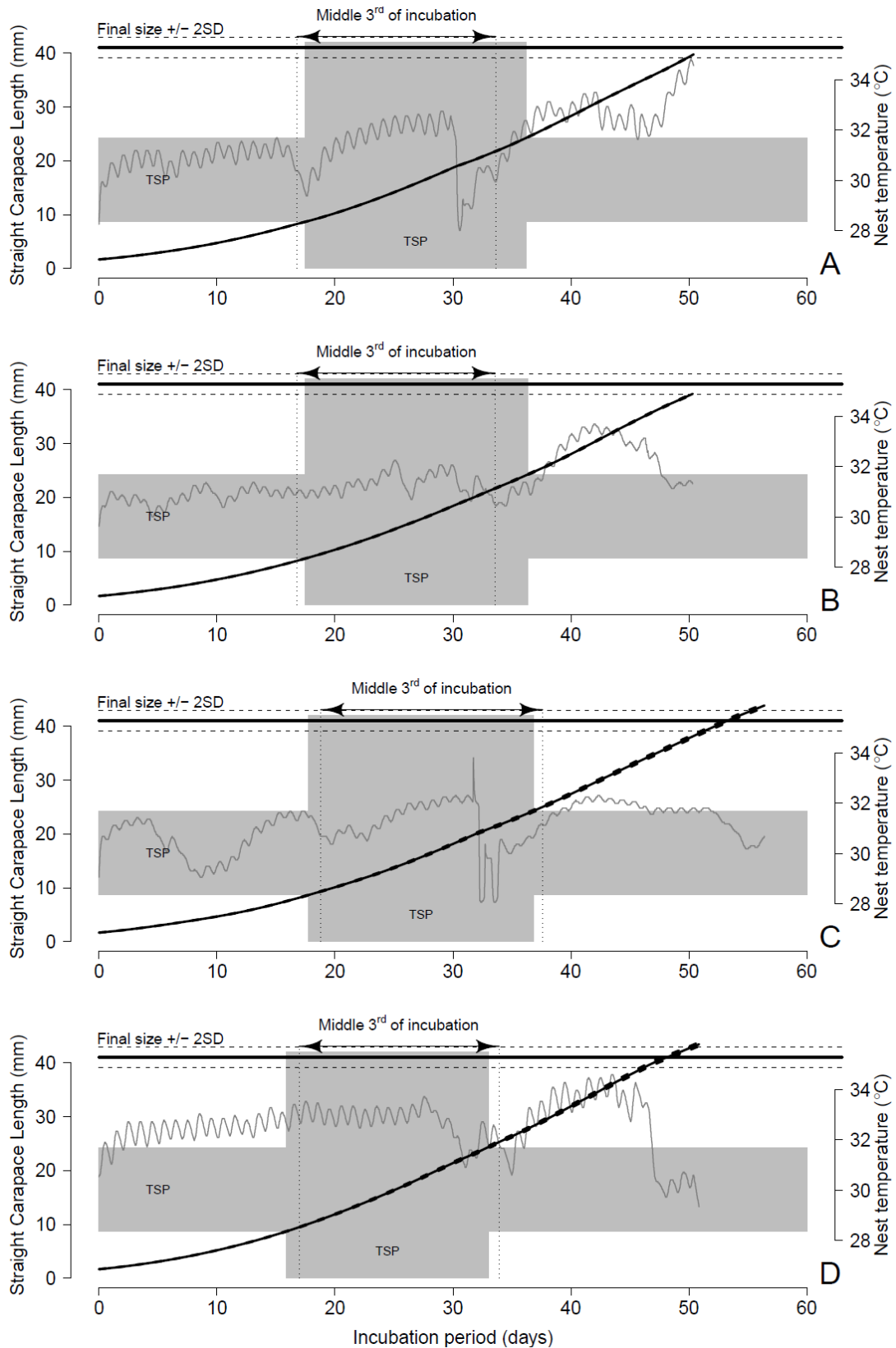


Figure 3: Model of embryonic development for 4 nests. Nests were laid A) December 2014, B) January 2015, C) February 2015, and D) March 2015. For comparison the nest with the most delayed thermosensitive period (TSP) compared to the middle third of incubation were plotted to illustrate the model of embryonic growth (black line). Dotted lines around this curve represent the confidence interval. Grey lines show traces of incubation temperature. The boundaries of the TSP during development (left y axis) and the matching period during incubation (x axis) are indicated by the grey area. The horizontal black line represents the final straight carapace length (SCL) which is 40.97 mm and the horizontal dotted lines show $\pm 2sd=0.96$ mm. Vertical dotted lines represent the boundaries of the middle third of incubation.

We calculated the difference between the timing for the onset of the middle third of incubation and the onset of the TSP (Fig. 4, A) and between the timing for the end of the middle third of incubation and the end of the TSP (Fig. 4, B) for all natural nests. The TSP can start 2 days before the onset of the middle third of incubation or 1.5 days after (Fig. 4, A). For all nests except one, the TSP ends after the end of the middle third of incubation with a delay that reaches from 1 to 3 days for 74% of nests (Fig. 4, B).

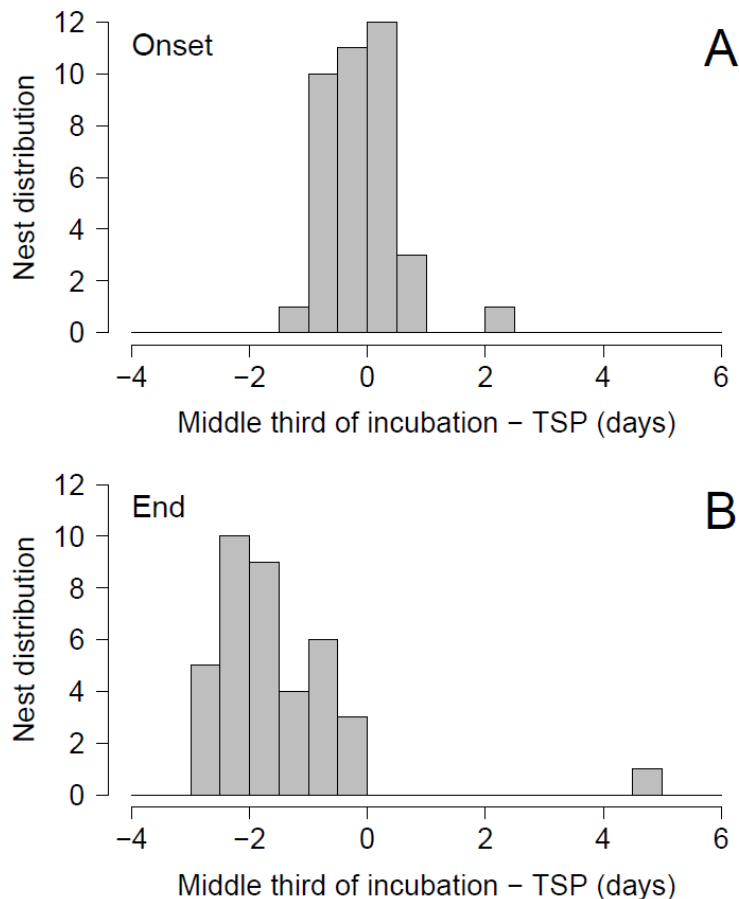


Figure 4: Shifting of the thermosensitive period for sex determination (TSP) in natural condition. Relative frequency of differences between the onset of the middle third of incubation and the onset of the TSP (A) and between the end of the middle third of incubation and the end of the TSP (B) in days for the 38 natural nests.

A difference of 4 days between the duration of the TSP and the duration of the middle third of incubation results in 20% discrepancy if the TSP lasts 20 days and 27% discrepancy if the TSP lasts 15 days.

3.3. Reaction norm of sex ratio at constant temperatures

The logistic model was selected as the best equation of thermal reaction norm of sex ratio for proxies 1 to 6 to be used ($P=29.12$ °C, $S=-0.27$, Transitional Range of Temperature= 29.96 to 28.31 °C, Fig. 5, A), whereas the Hill model was selected as the best equation for the incubation duration proxies (proxy 7) ($P=59.32$ days, $S=0.019$, Transitional Range of whole incubation

durations=62.78 to 56.09 days, Fig. 5, B) as well as for proxy 8 (P=21.31 days, S=0.018, Transitional Range of TSP durations=22.66 to 20.07 days, Fig. 5, C) (Table 2).

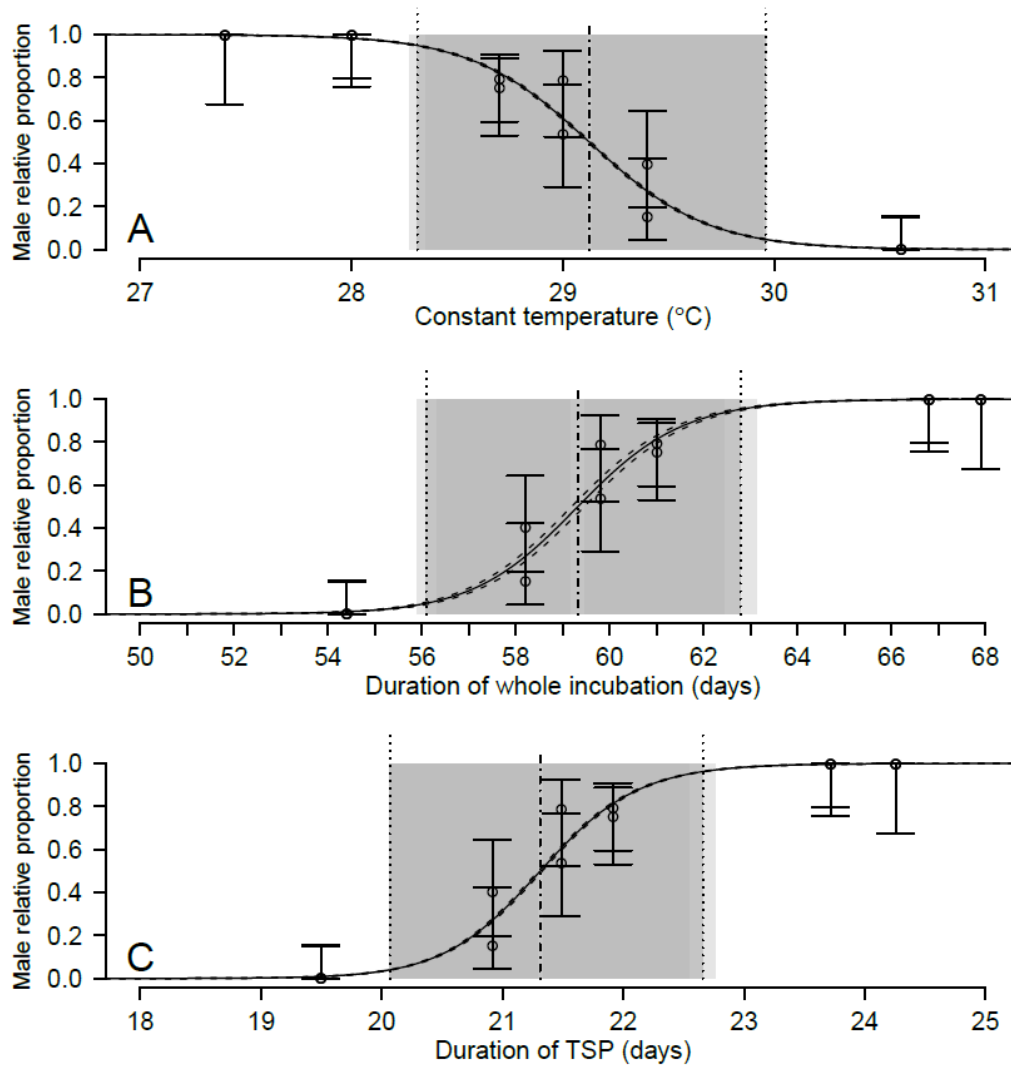


Figure 5: Patterns of temperature-dependent sex determination (TSD). Relationship between true sex ratio (black points are the average sex ratio and error bars show the confidence interval) from laboratory experiments and constant incubation temperature (A), the whole incubation period (B), and the duration of the thermosensitive period (TSP) (C) based on constant temperature experiments (Marcovaldi et al. 1997). The Transitional Range of Temperatures (TRT) is indicated by the grey area in between the horizontal dotted lines. Shades of grey indicate the confidence interval of the boundaries of the TRT.

Equation/model	Incubation durations	TSP durations	Constant temperatures
Logistic	28.15	28.08	28.48 (0.50)
Hill	27.95 (0.52)	27.91 (0.51)	28.50
Richards	34.80	35.00	36.28
Hulin	62.80	63.03	64.54
GSD	163.41	163.41	163.41

Table 2; Model selection for the equation that best describes the relationship between the true sex ratio observed in laboratory conditions and the considered proxy. Lowest AICc values are indicated in bold and the Akaike weight in brackets.

3.4. Sex ratio from temperature-based proxies (proxies 1 – 6)

Sex ratio was predicted for each of the 38 natural nests (Appendix B). Sex ratio of nests laid after the end of November was estimated to be 100% females regardless of the proxy used (Fig. 6). For nests laid before the end of November, sex ratio estimates differed if either CTE_A or CTE_W were used and for the different incubation periods considered. The average sex ratio for all clutches and the dispersion of sex ratio among clutches (lower and upper quantiles at 2.5%) were estimated. On average, more females were estimated if sex ratio was based on CTE_A or CTE_W during the whole incubation period (up to 90% females) rather than CTE_A or CTE_W during the middle third of incubation or the TSP (less than 60% females based on CTE_A and less than 80% females based on CTE_W , Fig. 6, respectively A and B). Importantly, the dispersion of sex ratio among nests is similar when estimated from CTE_A or CTE_W during the middle third of incubation or during the TSP but differs considerably when predicted from either CTE_A or CTE_W during the whole incubation period. During October, sex ratio of clutches estimated from CTE_A during either the middle third of incubation or the TSP shows similar dispersion (10% to 80% females and 20% to 90% females, respectively for the middle third of incubation and the TSP) whereas it ranges from 60% to 100% females when based on CTE_A during the whole incubation (Fig. 6, A). Similarly, sex ratio ranged from 20% to 95% females when based on CTE_W during either the middle third of incubation or the TSP whereas it ranges from 80% to 100% females when estimated from CTE_W during the whole incubation (Fig. 6, B).

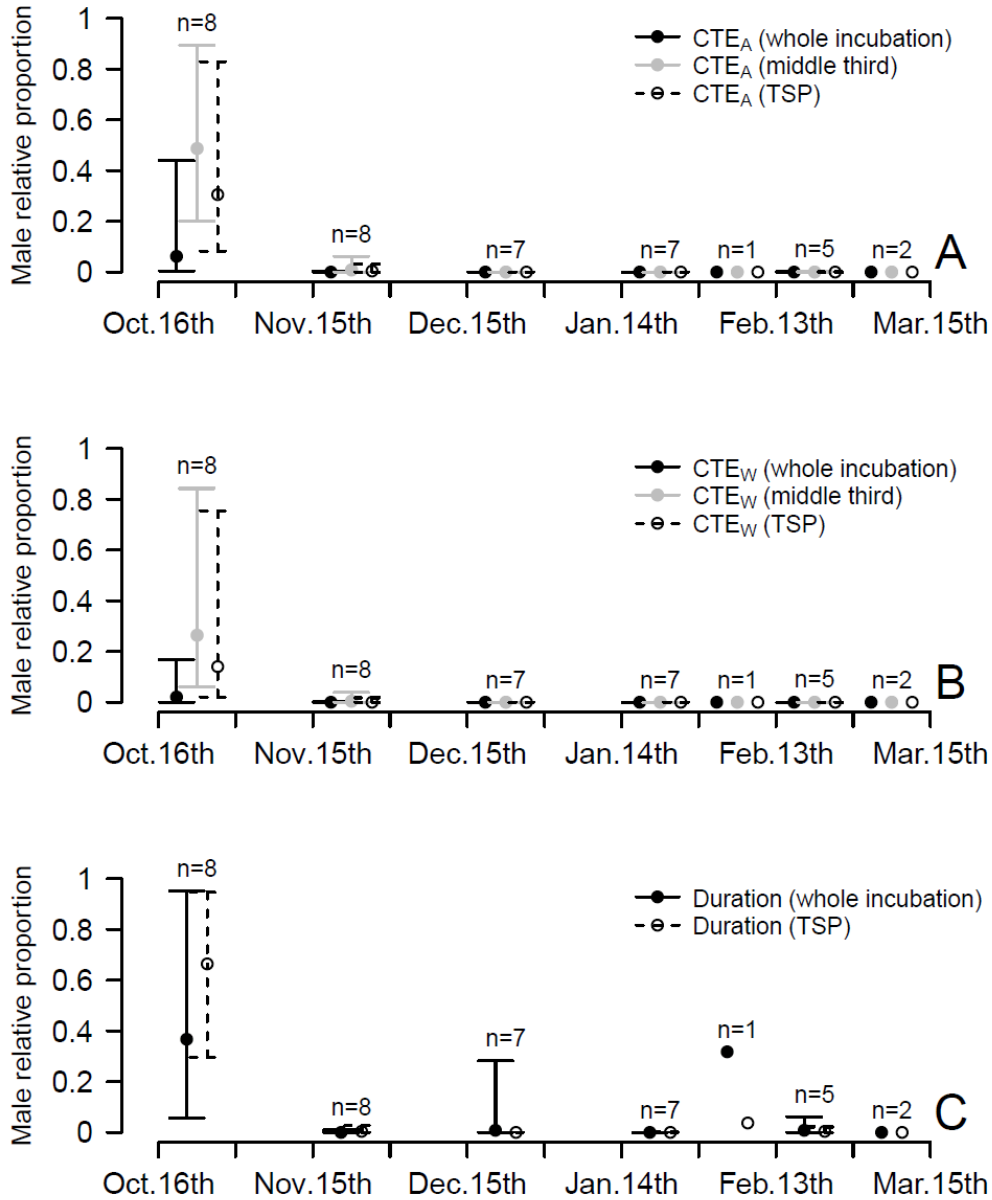


Figure 6: Sex ratio predicted for natural nests incubated at Praia do Forte during the 2014-2015 nesting season. Estimates are based on CTE_A (A), CTE_W (B) and durations of developmental periods (C). Error bars show lower (2.5%) and upper (97.5%) quantiles for sex ratio estimated for each 15-days period (n indicates the number of nest that were laid within the 15-days periods).

The overall difference in sex ratio estimates among the three developmental periods is less pronounced if CTE_W is used rather than CTE_A (Table 3).

Proxy	Mean sex ratio (% of males)	Lower quantile (2.5%)	Upper quantile (97.5%)
CTE _A (whole incubation)	2.05%	<0.1%	16.40%
CTE _A (middle third of incubation)	10.40%	<0.1%	58.90%
CTE _A (middle-third of development)	6.84%	<0.1%	45.40%

CTE _w (whole incubation)	0.79%	<0.1%	8.92%
CTE _w (middle third of incubation)	5.99%	<0.1%	37.00%
CTE _w (middle-third of development)	3.86%	<0.1%	28.10%
Duration-based equivalent in days (whole incubation)	9.97%	<0.1%	86.20%
Duration-based equivalent in days (Middle-third of development)	13.80%	<0.1%	91.60%

Table 3: Average and quantiles for sex ratio predicted for the 38 natural nests according to each proxy.

3.5. Sex ratio from duration-based proxies (proxies 7 and 8)

Whole incubation durations of natural nests ranged from 46.5 to 63.5 days (mean = 52.9 days, sd = 4.2) with a slight decreasing trend along the nesting season (Appendix A). Average sex ratio during the last 15 days of October differs substantially if whole incubation durations are used as a proxy rather than TSP durations (60% and 35% females respectively for whole incubation durations and TSP durations). After mid-November, on average 100% females average is expected regardless of the proxy used (Fig. 6, C). Only one nest was monitored during the first half of February so this difference cannot be discussed. Moreover, the dispersion of sex ratio expected during 15-days periods differed according to the proxy. The dispersion of sex ratio expected from TSP durations is always narrower than expected from whole incubation durations. This is particularly the case during the last 15 days of December when 100% females were predicted from TSP durations whereas 70% to 100% females were predicted from whole incubation durations. The averaged sex ratio for the 38 clutches is expected to be 90% females when whole incubation durations are used as proxies and 86.2% when TSP durations are used. The overall dispersion of sex ratio is expected to range from approximately 100% to 13% females when whole incubation durations are used as compared to 100% to 8.4% when TSP durations are used (Table 3).

3.6. Quantifying differences among methods

Euclidean distance for each pairwise comparison of proxy-specific sex ratio estimates has been calculated. The highest distance is observed for comparison between sex ratio estimates based on CTE_w during the whole incubation and based on the duration of the TSP. The lowest distance is observed for comparison between sex ratio estimates based on CTE_A during the TSP and CTE_w during the TSP (Table 4).

	CTE _A (whole incubation)	CTE _A (middle third of incubation)	CTE _A (middle- third of developme- nt)	CTE _w (middle third of incubation)	CTE _w (middle third of incubation)	CTE _w (middle- third of developme- nt)	Duration- based equivalent in days (whole incubation)
CTE _A (middle third of incubation)	1.13						
CTE _A (middle-third of development)	0.69	0.50					
CTE _w (whole incubation)	0.32	1.33	0.96				
CTE _w (middle third of incubation)	0.61	0.64	0.21	0.90			
CTE _w (middle-third of development)	0.39	0.91	0.42	0.71	0.29		
Duration-based equivalent in days (whole incubation)	1.31	1.29	1.18	1.40	1.25	1.29	
Duration-based equivalent in days (Middle- third of development)	1.64	0.94	1.17	1.80	1.28	1.48	0.96

Table 4: Matrix Euclidean distances of pairwise comparison of each proxy-specific sex ratio estimates.

The dendrogram shows two main branches: one with two leaves that correspond to sex ratio estimates based on duration-based proxies (duration of the whole incubation and duration of the TSP) and another one with six leaves that correspond to sex ratio estimates based on temperature-based proxies (CTE_A and CTE_w during the whole incubation, during the middle third of incubation and during the TSP) (Fig. 7). Two sub-groups can be identified within the branch of temperature-based proxies for sex ratio: one with two leaves which corresponds to CTE_A and CTE_w during the whole incubation period and another one with four leaves that corresponds to CTE_A and CTE_w during the middle third of incubation and during the TSP. The structure of the dendrogram is well supported by data (approximately unbiased bootstrap values between 79% and 100%, Fig. 7).

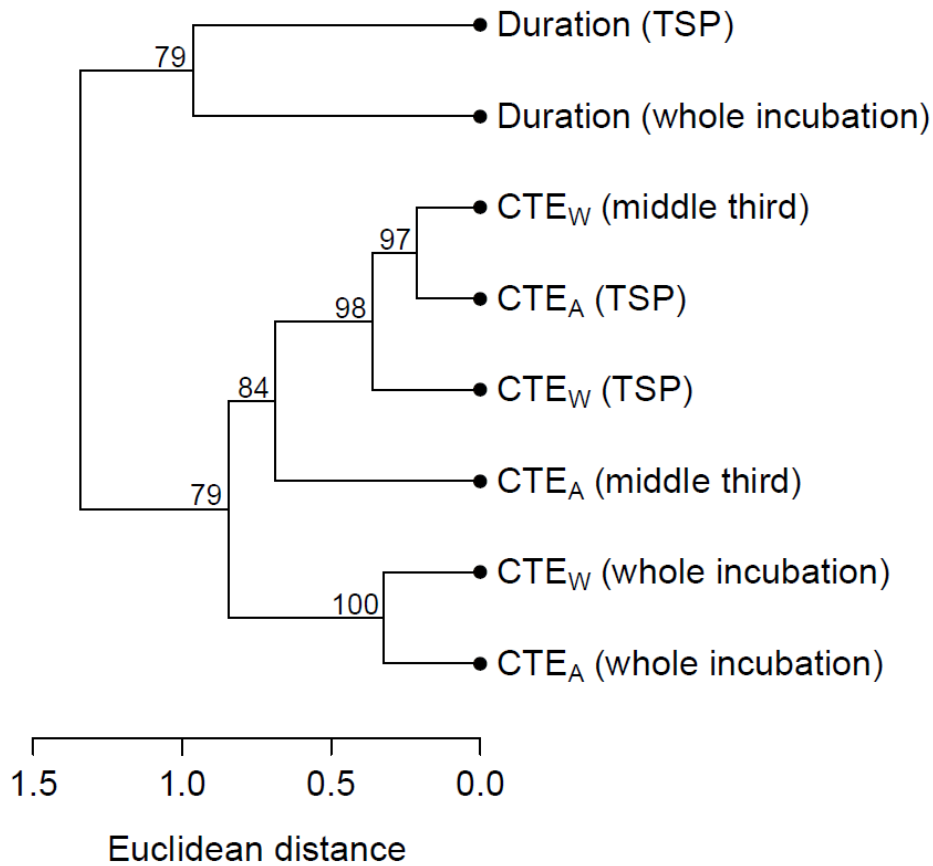


Figure 7: Clustering of sex ratio estimates. Dendrogram based on Euclidean distances of each proxy-specific sex ratio estimates. Approximated unbiased bootstrap values (in percentage) are indicated at nodes.

4. Discussion

Sex ratio estimates differ greatly depending on the proxy being used. A higher proportion of females tend to be estimated for temperature based proxies (CTE_A or CTE_W) when the whole incubation period was used rather than the middle third of incubation or the thermosensitive period for sex determination (TSP). For duration based proxies the whole incubation duration produced a wider range of sex ratio than the TSP durations, being longer, incubation duration are more variable than TSP durations and thus estimate a wider range of sex ratio. Further, temperature experienced during the TSP is more similar among nests whereas temperature experienced out of TSP boundaries may be much more different among nests. Ideally we would compare predictions to the true sex ratio of nests, however for this the sacrifice of hatchlings is needed. Thus, CTE_W during the TSP was used as a reference to discussing differences among methods since it is the closest proxy to the marine turtle mechanism of TSD (Georges et al. 1994, Georges et al. 2005). When compared to estimates from this proxy, the CTE_A during the TSP and the CTE_W during the middle third of incubation gave similar sex ratio estimates

whereas estimates using the CTE_W and CTE_A during the whole incubation period or both duration based proxies were substantially different. Estimates using CTE_A during the middle third of incubation also provided unsuitable discrepancy.

4.1. Temporal scale of proxies

Marine turtle sex ratio is determined only during the TSP which corresponds to the middle third of development (Yntema and Mrosovsky 1982, Georges et al. 2005, The Chu et al. 2008, Woolgar et al. 2013). Thus, calculating sex ratio with temperatures from the whole incubation period is inaccurate. In an attempt to provide more accurate estimates, the middle third of development has been widely used as the middle third of the whole incubation period (Sieg et al. 2011, Maulany et al. 2012, Gomuttapong et al. 2013, Jribi et al. 2013b, King et al. 2013, Jribi and Bradai 2014, Candan and Kolankaya 2016, Önder and Candan 2016, Yalçli Özdilek et al. 2016). However, this is true only when temperature remains constant during incubation. If temperature fluctuates the boundaries of the true TSP during incubation period does not necessarily fall within the middle third of the whole incubation period (The Chu et al. 2008). In nature, various climatic events have an incidence on the temperature within the nest. Temperature can vary stochastically due to precipitations, wind speed or cloud cover and deterministically due to daily and seasonally solar cycles. The extent to which nest temperature is affected by those meteorological and climatic parameters depends on thermal properties of the incubation substratum and the depth at which the nest is buried. Even when marine turtles bury their eggs deep enough to buffer temperature fluctuations, a slight temperature increase or decrease during incubation invalidates methods that approximate the true TSP by the middle third of the whole incubation period. Therefore, it is crucial to determine the moment at which each developmental stage occurs to accurately determine the true TSP boundaries. Monitoring the progression of embryo growth may require the sacrifice of embryos, however alternative methods are now available (Georges et al. 2005, Girondot and Kaska 2014).

4.2. Using thermal reaction norm for growth rate

Caution should also be made to not oversimplify the effect of temperature on sex determination by estimating sex ratio based on the average temperature (CTE_A) during any developmental periods. This make the wrong assumption that embryonic growth rate has no impact on sex determination (Georges 1989, Georges et al. 1994, Georges et al. 2005, Neuwald and Valenzuela 2011). If an embryo of a species with MF TSD pattern is exposed 12 hours at male-producing temperatures and 12 hours at female-producing temperatures, then few cells will proliferate under male-producing temperature and a larger amount at female-producing

temperature. As an alternative, it is suggested to estimate the sex ratio based on the incubation temperature at which half of the development has occurred (developmental median) (Georges 1989, Georges et al. 1994, Georges et al. 2005). This requires to accessing or modelling embryo morphometric. As another alternative, it has been proposed that the duration of the whole incubation can be used as a proxy for sex ratio (Godfrey 1997). This proxy is not invasive and easily measurable in the field. This assumes that the thermal reaction norm for the growth rate of embryos is monotonic within the thermal range experienced by embryos during incubation. Which means that the higher the temperature, the shorter the incubation and, in turn, more feminizing. However, a wide diversity of sex ratio can be observed for the same incubation duration when temperature fluctuates (Georges et al. 1994). Moreover, this method does not enable to get the duration of the TSP. The thermal reaction norm for growth rate of loggerhead population nesting at Praia do Forte is not monotonic with an optimum at 34.5°C. This is consistent with previous observation that turtle embryos incubated at 35°C or higher temperature; it may still develop but suffer of developmental abnormalities or even not develop successfully (Pieau 1978, Valverde et al. 2010). When the thermal reaction for growth rate is non- monotonic within the range of incubation temperature embryos can exhibit the same growth rate for two different temperatures. This has been observed for loggerhead populations in Turkey (Girondot and Kaska 2014) and Australia (Woolgar et al. 2013). It is therefore possible that embryos that have incubated in distinct thermal environment could present the same TSP duration. In such cases, the CTE_w during the TSP is the only reliable proxy to predict sex ratio of TSD species.

4.3. Proxies used to infer sex ratio

It is acknowledged that the mean nest temperature is a poor proxy to predict sex ratio of TSD species under natural fluctuating thermal regimes because more development would occur above the mean than below (Bull and Vogt 1981, Pieau 1982, Mrosovsky et al. 1984, Bull 1985, Georges 1989, Georges et al. 1994, Valenzuela 2001, Georges et al. 2005, Neuwald and Valenzuela 2011) and that the TSP lies in the middle third of development (Yntema 1979, Bull and Vogt 1981). The first type of CTE_w have been formulated by Georges (1989) and have been implemented by some studies, which acknowledges that the true TSP does not necessarily lie during the middle third of incubation in natural conditions (Valenzuela 2001, Georges et al. 2005, The Chu et al. 2008, Neuwald and Valenzuela 2011, Woolgar et al. 2013, Stubbs et al. 2014). At least three models have been proposed to account for thermal reaction norm for the rate of development while calculating a proxy to predict sex ratio: (i) degree.hours, (ii) Dallwitz-Higgins and (iii) Sharpe-DeMichele (Georges et al. 2005). The degree.hours model

assumes that the developmental rate is linear and monotonic within any thermal range which is not relevant for most situations. However, Dallwitz-Higgins and Sharpe-DeMichele models provide two different equations that are able to account for a decrease in developmental rates at high temperatures. These models are very similar (Georges et al. 2005) and have already been used to calculate CTE_W (Stubbs et al. 2014, Tapilatu and Ballamu 2015). Despite an improvement in predicting the sex ratio of TSD species, the most used proxies to predict sex ratio of marine turtles from traces of temperature under natural condition for the last decade (2010 to 2016) are still (i) the duration of the whole incubation period (ii) the CTE_A during the middle third of the whole incubation period (simple average) and (iii) the CTE_A during the whole incubation period (Fig. 8).

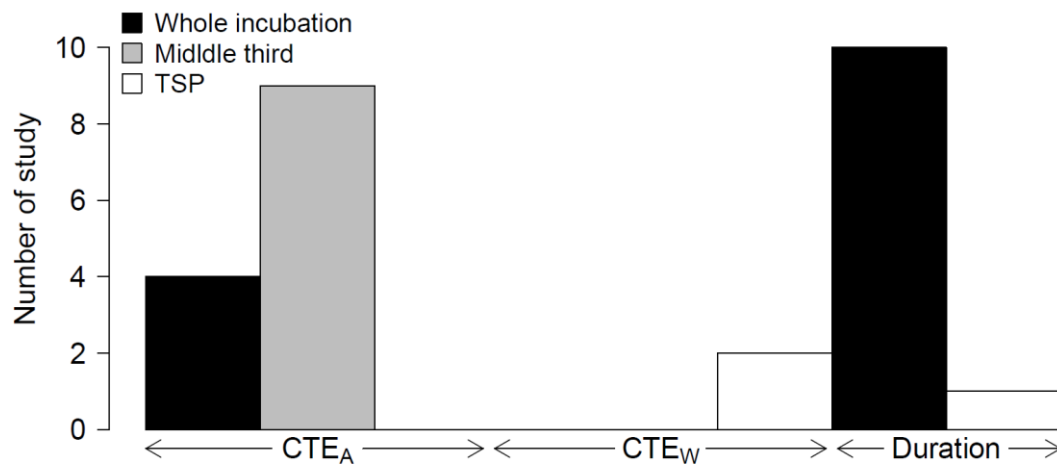


Figure 8: Proxies used in the literature to infer sex ratio. Number of studies that have used each proxy to predict hatchling sex ratio of sea turtles from recorded traces of temperature between 2010 and 2016 (Steckenreuter et al. 2010, Sieg et al. 2011, Katselidis et al. 2012, LeBlanc et al. 2012a, LeBlanc et al. 2012b, Maulany et al. 2012, Fuller et al. 2013, Gomuttapong et al. 2013, Jribi et al. 2013a, King et al. 2013, dei Marcovaldi et al. 2014, Jribi and Bradai 2014, Kılıç and Candan 2014, Simões et al. 2014, Stubbs et al. 2014, Rocha et al. 2015, Sarı and Kaska 2015, Tapilatu and Ballamu 2015, Candan and Kolankaya 2016, dei Marcovaldi et al. 2016, Yalçılı Özdilek et al. 2016).

This is a reflection of the ease to collect information on the incubation duration of nests. Although, the duration of the whole incubation does allow for a rapid assessment of incubation conditions, it must be stressed out that sex ratio estimation based on this proxy is not reliable. However it can give information about nests incubated at higher temperatures and at risk of reaching temperatures at the upper thermal threshold, which can help trigger active responses. Importantly, when selecting the best proxy to use, consideration needs to be undertaken both on the reliability of sex ratio estimate and on the logistical and feasible opportunities for data collection at each site.

4.4. Using proxies to predict future sex ratios

A common goal of studies assessing marine turtle viability under climate change conditions is to predict future sex ratio under different climate scenarios. However, most studies use oversimplified proxies, such as monthly mean temperature as a proxy of nest temperature and consider the middle third of the whole incubation period as the TSP for sex determination, affecting the reliability of these studies. Importantly, we showed that nest temperature time series exhibit specific statistical properties (autoregressive process) that should be taken into account when predicting sex ratio. Consideration should also be given to the fact that other temperature-dependent traits are interacting at the same time. For example, survival rate of embryos before hatching depends on incubation temperature (Howard et al. 2014) and the nesting dynamic of adult females may depend on the sea surface temperature at the feeding and breeding sites through resources availability and energy expenditure (Weishampel et al. 2004, Pike et al. 2006, Mazaris et al. 2009, Weishampel et al. 2010, Ben Hassine et al. 2011). Together, these parameters can amplify, buffer or even neutralize the effect of climate change on embryonic development of marine turtles, altering the sex ratio being produced. Importantly, further studies are needed to explore the sensitivity of embryos to extreme temperatures. Thus, information on temperature-dependent hatching success is required to provide reliable primary sex ratio estimates and thus assess the vulnerability of TSD species under different climate change conditions.

5. Conclusion

Several proxies are used to estimate the sex ratio of marine turtle hatchlings. Here we showed that sex ratio estimates differ greatly according to the proxy used. CTE_w during the TSP is likely the most relevant biological proxy thus likely produces the most reliable sex ratio estimates. In natural conditions, temperature can vary either stochastically or seasonally. Given this, growth rate of embryos is not expected to be constant. Hence, the thermosensitive period for sex determination can start earlier or later and last shorter or longer depending on environmental thermal variations. Thus, the need to adequately determine the TSP and to obtain appropriate estimates of embryo growth rate and the influences of temperature. Ultimately, further information should be obtained on how to model temperature-dependent hatching success in natural conditions in order to improve our understanding of the impact of climate change on the developmental stage of TSD species.

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Supplementary files

APPENDIX A: Proxy values for the 38 natural nests (P1-P8)

Nest ID	Laying date	Proxy 1 (°C)	Proxy 2 (°C)	Proxy 3 (°C)	Proxy 4 (°C)	Proxy 5 (°C)	Proxy 6 (°C)	Proxy 7 (day)	Proxy 8 (day)
1	10/21/2014	29.12	28.20	28.46	29.53	28.29	28.61	59.42	22.28
2	10/22/2014	29.63	29.09	29.21	29.79	29.32	29.45	61.33	22.16
3	10/22/2014	30.13	29.53	29.65	30.40	29.92	30.03	63.46	22.57
4	10/23/2014	30.71	29.38	29.83	31.39	29.66	30.25	58.21	21.59
5	10/25/2014	30.38	29.23	29.54	30.85	29.52	29.89	56.21	21.10
6	10/26/2014	30.08	29.06	29.31	30.51	29.38	29.66	57.33	21.43
7	10/27/2014	30.18	29.30	29.51	30.50	29.73	29.94	56.38	20.94
8	10/28/2014	30.07	29.06	29.38	30.51	29.57	29.95	57.33	21.25
9	11/25/2014	31.57	30.98	31.19	32.06	31.09	31.34	50.29	18.55
10	11/25/2014	31.24	30.41	30.66	31.77	30.54	30.84	49.29	18.63

11	11/25/2014	30.58	29.84	30.03	30.98	29.98	30.20	53.42	19.93
12	11/25/2014	31.21	30.97	31.13	31.63	31.14	31.33	54.50	19.40
13	11/26/2014	31.25	30.51	30.84	31.83	30.72	31.10	51.29	18.94
14	11/26/2014	30.81	30.05	30.30	31.34	30.18	30.49	53.38	19.81
15	11/27/2014	31.01	30.32	30.52	31.45	30.46	30.70	53.25	19.67
16	11/28/2014	32.09	31.35	31.64	32.62	31.59	31.94	51.33	18.71
17	12/18/2014	31.18	31.15	31.26	31.72	31.25	31.36	49.50	17.94
18	12/21/2014	33.05	33.37	32.85	33.20	33.44	33.02	58.54	17.17
19	12/22/2014	31.86	31.81	31.92	32.33	31.94	32.03	50.50	18.00
20	12/23/2014	32.33	32.22	32.34	32.85	32.34	32.44	49.25	17.60
21	12/23/2014	31.58	31.27	31.26	31.89	31.35	31.33	50.42	18.47
22	12/25/2014	32.32	32.13	32.25	32.80	32.17	32.29	47.46	17.20
23	12/27/2014	31.94	31.99	32.01	32.18	31.98	32.00	52.29	18.43
24	01/21/2015	31.45	31.32	31.27	31.71	31.35	31.28	50.33	18.37
25	01/21/2015	32.30	32.33	32.26	32.46	32.35	32.28	48.50	17.44
26	01/22/2015	32.03	31.97	31.84	32.24	31.96	31.83	48.54	17.70
27	01/26/2015	32.27	31.88	31.89	32.43	31.85	31.86	46.46	17.28
28	01/27/2015	32.91	32.37	32.41	33.10	32.35	32.40	47.33	17.27
29	01/27/2015	31.80	31.35	31.43	31.76	31.35	31.42	51.50	18.76
30	01/28/2015	32.57	31.95	32.08	32.86	31.97	32.14	48.46	17.60
31	02/12/2015	31.00	31.30	31.29	31.11	31.36	31.35	58.46	20.02
32	02/13/2015	31.08	31.38	31.36	31.14	31.44	31.42	55.38	19.23
33	02/15/2015	32.34	31.96	31.96	32.68	32.04	32.04	52.50	18.87
34	02/16/2015	31.10	31.06	31.08	31.27	31.12	31.13	56.42	19.86
35	02/23/2015	31.87	31.90	32.19	32.53	32.02	32.36	51.17	17.87
36	02/25/2015	31.66	32.03	32.07	31.88	32.11	32.16	51.38	17.87
37	03/03/2015	31.47	31.88	31.89	31.62	31.97	31.98	49.29	17.37
38	03/11/2015	32.57	32.74	32.73	32.68	32.73	32.71	50.88	17.76

APPENDIX B: Proportion of males predicted for the 38 natural nests according to each proxy

Nest ID	Laying date	Proxy 1	Proxy 2	Proxy 3	Proxy 4	Proxy 5	Proxy 6	Proxy 7	Proxy 8
1	10/21/2014	0.503	0.965	0.916	0.186	0.953	0.863	0.521	0.913
2	10/22/2014	0.137	0.53	0.416	0.0813	0.323	0.234	0.853	0.887
3	10/22/2014	0.0259	0.184	0.13	0.00984	0.0529	0.0354	0.972	0.955
4	10/23/2014	0.00326	0.282	0.0719	<0.001	0.126	0.0166	0.269	0.666
5	10/25/2014	0.0107	0.4	0.18	0.00192	0.193	0.059	0.0552	0.371
6	10/26/2014	0.0306	0.551	0.339	0.00644	0.278	0.126	0.142	0.574
7	10/27/2014	0.0215	0.342	0.198	0.00673	0.0995	0.0495	0.0639	0.284
8	10/28/2014	0.0315	0.559	0.281	0.00659	0.165	0.0476	0.142	0.464
9	11/25/2014	<0.001	0.00122	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
10	11/25/2014	<0.001	0.00951	0.00384	<0.001	0.00589	0.00203	<0.001	<0.001
11	11/25/2014	0.00527	0.068	0.036	0.00121	0.0422	0.0199	0.00396	0.0284
12	11/25/2014	<0.001	0.00127	<0.001	<0.001	<0.001	<0.001	0.0113	0.00703
13	11/26/2014	<0.001	0.00652	0.00198	<0.001	0.00308	<0.001	<0.001	0.002
14	11/26/2014	0.00222	0.0341	0.0139	<0.001	0.0211	0.00696	0.00381	0.0206
15	11/27/2014	0.00112	0.0129	0.00634	<0.001	0.00773	0.00331	0.00336	0.0143
16	11/28/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.00104
17	12/18/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
18	12/21/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.333	<0.001
19	12/22/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
20	12/23/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
21	12/23/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
22	12/25/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
23	12/27/2014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.00129	<0.001
24	01/21/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
25	01/21/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
26	01/22/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

27	01/26/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
28	01/27/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
29	01/27/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.00121
30	01/28/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
31	02/12/2015	0.00113	<0.001	<0.001	<0.001	<0.001	<0.001	0.316	0.0355
32	02/13/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0259	0.00443
33	02/15/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0016	0.00162
34	02/16/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0663	0.0237
35	02/23/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
36	02/25/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
37	03/03/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
38	03/11/2015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Chapter 3: Differences in the climatic debts of loggerhead sea turtles nesting populations

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Abstract: Future climatic changes are expected to profoundly affect biodiversity and ecosystem functioning. Shifting of species' phenology by initiating reproductive events earlier in response to warming temperatures is one of the most striking effect currently observed in wild populations. Sea turtles are oviparous reptiles that exhibit temperature dependent sex determination and of which nesting phenology is linked to ambient temperature. These organisms have long generation time and high degree of site fidelity which may preclude any rapid genetic adaptation or range shifts to keep tracking their climate envelope. Therefore, shifting the nesting season could be the most effective short-term response to buffer or

neutralize detrimental effects of warming temperatures at nesting beaches. We calculated the shifting of nesting phenology required to keep producing a primary sex ratio that lies within the range currently produced for 7 loggerhead populations across a wide range of latitudes. We also calculated the shifting of nesting phenology required to keep producing viable hatchlings. We found that temperature dependent plasticity in nesting phenology may not help all populations to keep up with warming temperatures. Our results emphasize the need to calibrate population-specific models of nest temperature, embryo development and nesting phenology to properly assess vulnerability of sea turtles under climate change constraints.

Keywords: Climate change, phenotypic plasticity, phenology, sea turtle, *Caretta caretta*, temperature, embryo, sex ratio

1. Introduction

Current climate changes affect species' abundance, distribution, phenology, physiology and behavior (McCarty 2001, Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Parmesan 2006, Rosenzweig et al. 2007, Bellard et al. 2012, Poloczanska et al. 2013, Garcia et al. 2014). Such modifications at population and species levels will undoubtedly scale toward community and ecosystem levels by disrupting trophic interactions (Gilman et al. 2010, Walther 2010, Angert et al. 2013). Species with high heritable genetic variation and short generation time are expected to rapidly respond to selection by microevolution on determinant traits (Bradshaw and Holzapfel 2006, 2008, Visser 2008, Pen et al. 2010, Somero 2010, Visser et al. 2010). In contrast, species with relatively longer generation time might face difficulties to adapt genetically to keep up with the current rate of biotic and abiotic changes, although some species of birds and mammals may benefit sufficient heritable variation on phenotypically plastic traits (Nussey et al. 2005, Bradshaw and Holzapfel 2006, Visser 2008). For others, phenotypic plasticity in physiology and behavior may, to some extent, compensate for rapid environmental shifts to keep tracking their climate envelop (Chevin et al. 2010, Huey et al. 2012, Refsnider and Janzen 2012). Many species are already shifting spatially to higher latitudes or altitudes and temporally by initiating reproductive events earlier or later in the season (Root et al. 2003, Both et al. 2004, Parmesan 2006, Rosenzweig et al. 2007). Such phenotypic plasticity may be adaptive if it enables a species to match with its resources or maladaptive if it creates a mismatch with its resources (Both et al. 2006, Charmantier et al. 2008, Both 2010, Visser et al. 2012). The extent of the response may vary at intraspecific level depending on population-specific environmental conditions or local adaptations (Visser and Both 2005, Both et al. 2006, Mazaris et al. 2013, Rutschmann et al. 2015).

Ectotherms are expected to be profoundly affected by changes in their thermal environment because virtually all their life history traits depend on ambient temperature which, in turn, affects fitness of individuals (Deutsch et al. 2008, Tewksbury et al. 2008, Huey et al. 2009). Temperature can even be viewed as an ecological resource for ectotherms (Magnuson et al. 1979) and especially for oviparous reptiles with temperature dependent sex determination (TSD) because it constrains embryonic development and sex ratio which may, in turn, jeopardize population viability.

Sea turtles are long-lived migratory ectotherms that exhibit TSD (with females produced at high temperatures and males at low temperatures) (Yntema and Mrosovsky 1980). They are also known for natal homing behavior and high degree of nest site fidelity (Miller 1997). The latter traits might preclude for rapid large-scale spatial shifts toward more suitable thermal conditions for egg development (Bowen et al. 1994, Bowen and Karl 2007) which could induce highly female biased sex ratio and possibly low rate of embryo survival in the context of global warming (Hawkes et al. 2009, Poloczanska et al. 2009, Fuentes et al. 2011, Hamann et al. 2013). Microevolution of the pivotal temperature (i.e. the temperature at which 50% of each sex is expected) is unlikely because this threshold temperature is highly conserved among populations (Hulin et al. 2009), which suggest strong genetic constraints, and because long generation time will not facilitate a rapid genetic selection of individuals that might deviate. Indeed, heritability of pivotal temperature seems to be very low in turtles (McGaugh and Janzen 2011, Refsnider and Janzen 2015). Therefore, moving the nesting season temporally might be the more efficient short-term option for sea turtles to cope with contemporary rate of thermal changes. Earlier onset of the nesting season and earlier occurrence of the peak of the nesting season have already been observed for a fresh water turtle (*Chrysemys picta*) (Schwanz and Janzen 2008), for some populations of the loggerhead sea turtle (*Caretta caretta*) (Weishampel et al. 2004, Pike et al. 2006, Hawkes et al. 2007, Mazaris et al. 2008, Weishampel et al. 2010, Mazaris et al. 2013, Lamont and Fujisaki 2014, Patel et al. 2016) and the green sea turtle (*Chelonia mydas*) (Weishampel et al. 2010) in response to warmer temperature experienced by adult females at either nesting areas or foraging areas. However, whether this phenotypic plasticity in nesting behavior will enable sea turtles to overcome thermal changes at nesting beaches still requires investigations. Importantly, to what extent sea turtles would have to shift their nesting phenology to keep producing both sexes or, at least, viable hatchlings, is an issue that has not been addressed before. Here we apply the concept of “climatic debt” previously described for communities of bird and butterfly at a spatial scale (Devictor et al. 2012) to several populations

of the loggerhead sea turtle at a temporal scale. We aim to quantify their “climatic debt” by calculating the shift in nesting phenology required to keep up with temperature changes at nesting beaches.

The primary sex ratio of sea turtles, i.e. the proportion of males (or females) of hatchlings that successfully emerged from nests at a particular nesting beach, depends on (i) the effect of incubation temperature during the thermosensitive period for sex determination (TSP) and (ii) the proportion of embryos that survived during development (hatching success) and successfully emerged from nests (emergence rate). Hatching success and emergence rate of sea turtles depend, in part, on incubation temperature (Santidrian Tomillo et al. 2012, Horne et al. 2014, Howard et al. 2014, Santidrián Tomillo et al. 2014, Santidrian Tomillo et al. 2015b). Temperature within the sand varies seasonally as does the number of nest in incubation. Therefore, the dynamic of the nesting season, i.e. the progression of the number of nest being laid throughout the season, must be taken into account to properly predict the number of males and females that may potentially reach the sea. Ideally, the primary sex ratio would be calculated as the number of males (or females) divided by the total amount of eggs laid corrected by nest-specific hatching success and emergence rate. Mechanistic models to predict the sex ratio of a nest in natural conditions (Fuentes et al. 2017, Girondot et al. In press) and the dynamic of a nesting season (Girondot 2010, Ben Hassine et al. 2011) are already available and have been applied in the present study. However, the physiological processes underlying temperature-dependent hatching success and emergence rate yet need to be mathematically described. Nevertheless, an embryo of sea turtle that incubates at constant temperature outside the range of 25-35°C cannot develop (Ackerman 1997) and a statistical approach revealed that 0% of hatching success is expected for nests of the loggerhead sea turtle (*Caretta caretta*) of which mean temperature during incubation exceeds 35.1°C (Horne et al. 2014). Here we consider 35°C as being the critical thermal threshold over which embryo development is likely to fail. In order to investigate potential failure in embryonic development we developed a thermal index (THI) of nest temperature that accounts for the number of nests under incubation during a particular nesting season. Meanwhile, we developed a sex ratio index (SRI) that accounts for the number of nests laid during a particular nesting season. We calculated these 2 indices based on (i) reconstructed daily minimum and maximum nest temperature, (ii) modelled embryonic development and (iii) modelled number of nests throughout a nesting season.

First, mean daily nest temperature is reconstructed based on a correlative model that links the temperature recorded within natural nests and climate temperature such as air temperature

above the incubating nests and sea surface temperature in front of the nesting beach (Girondot and Kaska 2014b, Monsinjon et al. In press). This enable producing long term time series of nest temperature using outputs of climate models that provide reanalysis of historical climate temperature at a regional scale. Diel fluctuations of temperature within nests (daily minimum and maximum) are reconstructed by applying the average daily amplitude observed in natural nests. This step is of great concern because thermal fluctuations are known to have profound effects on embryonic development of ectotherms (Les et al. 2009, Paaijmans et al. 2013, Bowden et al. 2014, Horne et al. 2014) and more specifically on sexual fate for species with TSD (Valenzuela 2001, Georges et al. 2004, Georges et al. 2005, Schwanz and Proulx 2008, Warner and Shine 2010, Neuwald and Valenzuela 2011, Georges 2013). Second, embryonic growth (in term of carapace size) is modelled based on population-specific thermal reaction norm for growth rate combined with a growth function (Girondot and Kaska 2014a, Fuentes et al. 2017, Girondot et al. In press, Monsinjon et al. In press). When iteratively applied to temperature time series of reconstructed nest temperature, this approach enables extracting information about embryo development such as the duration of incubation and the temperature within the TSP for any dates throughout the season. Sex ratio is estimated by applying the average temperature weighted by the differential growth during the TSP (a constant temperature equivalent) (Fuentes et al. 2017) to the population-specific thermal reaction norm for sex ratio estimated using constant temperature experiments previously carried out in laboratory conditions. Third, the pattern of the nesting season in term of nest number is inferred based on the number of nests counted during monitoring surveys (Girondot 2010, Ben Hassine et al. 2011). More details about nest temperature reconstruction, embryonic growth model, sex ratio estimation and nesting phenology model are given in the Materials and Methods.

We forecasted THI and SRI according to region-specific scenarios for future changes in air temperature above nesting beaches and global scenarios for future changes in ocean temperature (IPCC 2014). Those indices were first calculated assuming the period of the nesting season will not shift until 2100. Thereafter, we shifted the pattern of the nesting season each day from 1 to 365 days earlier and we calculated the resulting THI and SRI. Finally, we quantified the “climatic debts” in term of temporal shift required to keep up with temperature changes for THI to remain under the critical thermal threshold and for SRI to remain within the current population-specific range of sex ratio.

Temperature within nests was recorded among 4 Regional Management Units (RMUs) of the loggerhead sea turtle (Fig. 1) which were previously defined according to genetic and satellite

data in order to facilitate research and conservation of sea turtles (Wallace et al. 2010). 3 nesting sites were investigated in the Mediterranean RMU (Turkey, Libya, Cyprus), 8 nesting sites were investigated in the Atlantic North West RMU (6 in Florida and 2 in Georgia). 2 nesting sites were investigated in the Atlantic South West RMU (Brazil). 1 nesting site was investigated in the Indian South West RMU (South Africa). These data were used to detect population-specific thermal reaction norms for growth rate within each RMU. The number of nest throughout several nesting seasons was monitored for 7 of these nesting sites: Dalyan (Turkey), Black Beard Island (Georgia), Wassaw Island (Georgia), Boca Raton (Florida), Praia do Forte (Brazil), Rio de Janeiro (Brazil) and Bhanga Nek (South Africa). We identified the sex ratio likely to be produced during nesting seasons at these 7 nesting sites and we quantified their “climatic debt” according to scenarios for future changes in thermal incubation conditions.

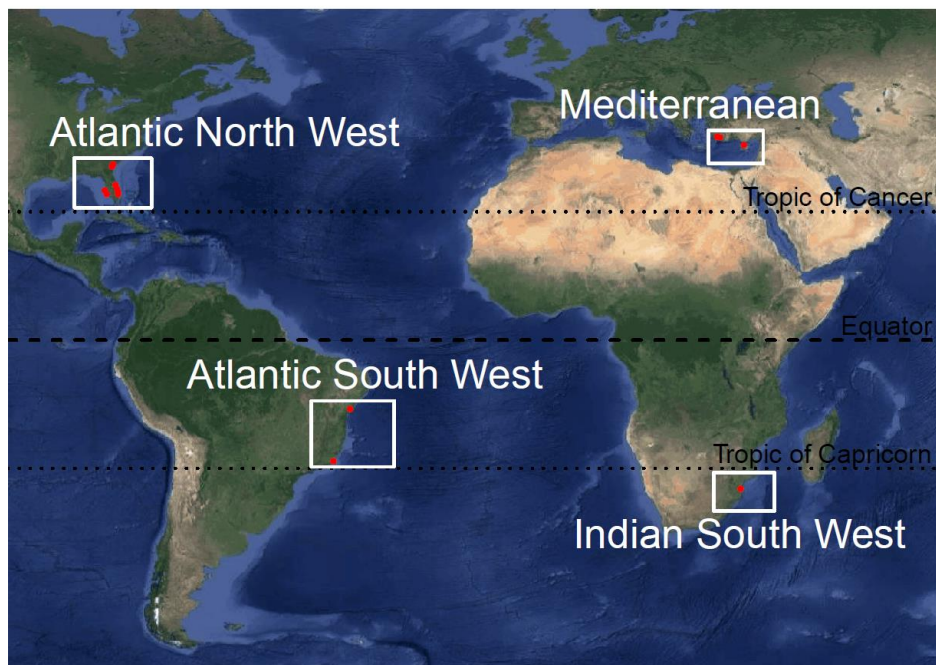


Figure 1: Location of nesting sites where nest temperature was recorded (red dots). White squares encompass nesting populations that belong to the same RMU. The number of nest was counted throughout several nesting seasons in Turkey at Dalyan (36°47'39"N and 28°37'13"E), in Georgia at Black Beard (31°28'58"N and 81°12'54"W) and Wassaw (31°53'26"N and 80°58'15"W), in Florida at Boca Raton (26°21'59"N and 80° 4'3"W), in Brazil at Praia do Forte (12°30'38"S and 37°58'28"W) and Rio de Janeiro (21°39'47"S and 41° 1'55"W) and in South Africa at Bhanga Nek (26°59'33"S and 32°52'2"E). Black dashed line indicates the Equator and black dotted lines indicate the tropics.

2. Materials and methods

2.1. Forecasting nest temperature

We modelled past nest temperature at nesting sites based on a previously published method (Girondot and Kaska 2014b, Monsinjon et al. In press). Hereafter we briefly describe the method and emphasize some modifications.

Nest temperature correlates with air temperature (AT) and sea surface temperature (SST) in front of the beach (Girondot and Kaska 2014b). We collected such data from the European Centre for Medium-Range Weather Forecasts (ECMWF) data sets, which provide temperature every 6 hrs (UTC) for several decades at 0.1° resolution (Dee et al. 2011). SST and AT at a height of 2 m were extracted from the 1st January 1979 to the 31st December 2016 at geographic coordinates closest to the location of nesting sites. We modelled the average daily temperature using a generalized linear mixed model with Gaussian distribution and identity link function:

$$NT_{j,i} = \mu + \alpha \cdot SST_{i-dSST} + \beta \cdot AT_{i-dAT} + \gamma \cdot d_i + \epsilon_j$$

Where $NT_{j,i}$ is the daily average temperature of the nest j on day i ($i = 0$ for nest deposition), μ is a constant term, α and β are the coefficients associated with the linear relationship between NTx and SST and AT lagged by $dSST$ and dAT days, respectively. Here, the analysis was performed for $dSST$ and dAT from 0 to 2 days and we selected the best combination based on the lowest AIC (Burnham and Anderson 2002). The combination with the lowest $dSST + dAT$ was retained when several combinations had similar AIC support ($\Delta AIC < 2$). Metabolic heating, which is the increase of temperature within the nest as compared to adjacent sand temperature, may also affect temperature within egg chamber. We accounted for such effect by integrating the parameter γ which describes the maximum difference of temperature between sand and nest at the end of incubation with d_i being the proportion of incubation time for the nest (0 is for day 0 and 1 is for the end of incubation). The model is built with a random effect on nest identity, which includes its position on the beach and a temporal autocorrelation structure of order 1 (AR1 model). We chose this this temporal autocorrelation structure because mean daily nest temperature can be viewed as an autoregressive process of first order, i.e. temperature at day d is correlated with temperature at day $d-1$ (Fuentes et al. 2017). Meanwhile, we calculated the average daily amplitudes of temperatures (AmpT) using daily maximum (from noon to noon) and daily minimum (from twelve to twelve) (Eccel 2010) for all days and all nests independently for each nesting sites. We also calculated the average time of the day when daily maximum and daily minimum occur (respectively, $Ti.Max$ and $Ti.Min$). After site-specific long term time series (1979-2016) of mean daily nest temperature were reconstructed, we replaced daily mean by daily maximum ($mean+AmpT/2$) and daily minimum ($mean-AmpT/2$) that we set at $Ti.Max$ and $Ti.Min$ respectively. All parameters described above are summarized in Supplementary files (Table S1).

We forecasted nest temperature based on region-specific scenarios of changes in air temperature and global scenario of changes in ocean temperature (IPCC 2014). We chose an optimistic scenario (RCP4.5) that predicts a rising radiative forcing that will stabilize at $+4.5 \text{ W.m}^{-2}$ after 2100 as compared to preindustrial values assuming a decrease in emissions of CO_2 after 2040 (Moss et al. 2010). We also chose to make predictions according to the more extreme scenario (RCP8.5). This pessimistic scenario predicts a radiative forcing that will reach $+8.5 \text{ W.m}^{-2}$ by 2100 as compared to preindustrial values and keep rising afterward assuming no decrease in emissions of CO_2 (Moss et al. 2010). We extracted such data from KNMI (Koninklijk Nederlands Meteorologisch Instituut) Climate Explorer website (https://climexp.knmi.nl/plot_atlas_form.py). In short, the interface allows the user to select the region (here defined by IPCC Working Group 1), the dataset (here Coupled Model Intercomparison Project 5 for IPCC's Fifth Assessment Report), the variable (here near-surface temperature), the scenario of Representative Concentration Pathway (RCP) in greenhouse gas concentration (here RCP4.5 and RCP8.5) and the period to be compared with to obtain anomalies (here anomalies as compared with 1979-2016 period). We retrieved projected temperature anomalies at the following regions: North-East Brazil, Eastern-North-America, Southern-Africa and South-Europe-Mediterranean. We add projected (region-specific) increases in temperature to air temperature time series previously extracted from ECMWF data sets. We also extracted projected increase in temperature for the oceans at the scale of the world. We add the projected increase in temperature to sea surface temperature time series previously extracted from ECMWF data sets. Temperature anomalies are summarized in Supplementary files (Table S2). Nest temperature is then reconstructed following the method described above (using parameters summarized in Supplementary files (Table S1) but this time using forecasted time series of air temperature and sea surface temperature. These new nest temperature time series can be viewed as forecasts for the period from 2062 to 2100.

2.2. Modelling embryonic development

The progression of the size of an embryo according to temperature experienced within the nest can be modelled based on the thermal reaction norm for growth rate using an equation that describes the effect of temperature on the rate of growth along with an equation that describes the progression of the size of an embryo during incubation period (Georges et al. 2005, Girondot and Kaska 2014a). This method has already been implemented to estimate the thermal reaction norm for growth rate of 3 loggerhead nesting populations (Girondot and Kaska 2014a, Fuentes et al. 2017, Girondot et al. In press, Monsinjon et al. In press) using a 4-parameters equation of which principles stem in the thermodynamic description of the effect of temperature on the rate

of biological reactions (Sharpe and DeMichelle 1977, Schoolfield et al. 1981) combined with a modified version of Gompertz model that was initially used to describe the dynamics of tumor growth (Laird 1964). Hereafter we briefly describe these equations.

The model integrates in a single framework (i) the embryo growth modelled using the Gompertz equation (eq. 1) (Laird 1964) and (ii) the dependency of the growth rate on temperature (i.e. the thermal reaction norm for growth rate) (eq. 2) (Sharpe and DeMichelle 1977, Schoolfield et al. 1981).

The embryo growth model in term of straight carapace length (SCL) is described as follows:

$$X(t) = K \exp\left(\ln\left(\frac{X(0)}{K}\right) \exp(-r(T) t)\right) \text{ [Equation 1]}$$

$X(0)$ being the size at time 0, K being the asymptotic metric and $r(T)$ being the growth rate depending on temperature $r(T)$ (see eq. 2). The values of $X(0)$ and K are fitted using data from developmental table (developmental stages, days of incubation and straight carapace length) previously obtained for the loggerhead sea turtle in Turkey (Kaska and Downie 1999) as per Girondot et al. (In press).

The thermal reaction norm for growth rate is described as follows:

$$r(T) = \frac{\rho_{(298\text{ K})} \frac{T}{298} \exp\left[\frac{\Delta H_A^\ddagger}{R} \left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_H}{R} \left(\frac{1}{T_{1/2H}} - \frac{1}{T}\right)\right]} \text{ [Equation 2]}$$

$r(T)$ being the rate of embryonic growth at temperature T (time⁻¹), R being the universal gas constant (J.K⁻¹.mol⁻¹), $T_{1/2H}$ being the temperature in Kelvin at which the enzymes are half active and half inactive due to high-temperature effect, ΔH_H being the change in enthalpy associated with the high-temperature inactivation of the enzymes (J.mol⁻¹), ΔH_A^\ddagger being the enthalpy of activation of the reaction catalyzed by the enzymes (J.mol⁻¹) and $\rho_{(298\text{ K})}$ being the developmental rate at 298 Kelvin (24.85°C) assuming no enzyme inactivation (time⁻¹).

The 4 parameters of equation 3 that best describe the final SCL of embryos at the end of incubation are fitted using maximum likelihood with a Gaussian distribution of SCL and an identity link. Matrix of variance-covariance for fitted parameters of equation 3 was estimated using the inverse of the Hessian matrix (matrix of second order derivatives for all couples of parameters) in order to calculate the confidence interval of $r(T)$ based on delta-method (Bates and Watts 1988).

Basically, the model requires (i) traces of temperature recorded within nests from the moment when eggs were laid to the moment when hatchlings emerged from the nest and (ii) measurements of the SCL of hatchlings.

Temperature was recorded using temperature data loggers placed in the middle of the clutches while females were laying their eggs (≈ 45 cm). Data loggers were excavated the day when hatchling emerged from the nest or several days later. We kept traces of temperature within the period of incubation, i.e. between the laying date and the hatching date. When certain data loggers were much colder than actual temperature inside the egg chamber, we omitted the first 6 hours following the first recorded temperature so that we keep temperatures consistent with the actual range of temperature experienced by embryos during incubation. Temperature was recorded at several nesting sites within the following Regional Management Units (RMUs) of the loggerhead sea turtle: Mediterranean, Atlantic North West, Atlantic South West and Indian South West. We used already published temperature data for the Mediterranean RMU: in Turkey at Dalyan (Girondot and Kaska 2014b, a, Monsinjon et al. In press) and Fethiye (Kaska et al. 2006), in Cyprus at Alagadi (Godley et al. 2001) and in Libya near Sirte (Monsinjon et al. In press). For the Atlantic North West RMU, temperature was recorded in Florida at Sarasota (12 nests in 2002), Sanibel (4 nests in 2002, 3 nests in 2010 and 5 nests in 2011), Melbourne (4 nests in 2002 and 5 nests in 2003), Hutchinson Island (8 nests in 2002 and 1 nest in 2004), Juno Beach (5 nests in 2002) and Boca Raton (11 nests in 2002, 2 nests in 2003, 3 nests in 2004, 2 nests in 2010, 6 nests in 2011, 1 nest in 2012, 3 nests in 2013, 9 nests in 2014, 7 nests in 2015 and 11 nests in 2016) and in Georgia at Black Beard Island (39 nests in 2003) and Wassaw Island (27 nests in 2004). In 2016 at Boca Raton, 17 other clutches were translocated in a different area of the beach and were split for a moisture experiment not presented here (half of the eggs incubated under natural conditions while the others received a daily dose of water that mimicked rainfalls). No embryo hatched from 3 sub-clutches so we kept the resulting 31 sub-clutches that we added to Boca Raton data set of nest temperature. For the Atlantic South West RMU, temperature was recorded in Brazil near Rio de Janeiro (12 nests in 2015 and 5 nests in 2016) and we used published data of nest temperature recorded at Praia do Forte, Brazil (Fuentes et al. 2017). In the original publication for data from Praia do Forte, some constant temperature incubation experiments carried out in laboratory conditions were added to fit the thermal reaction norm for growth rate. Here we considered only nests that incubated in natural conditions. For the Indian South West RMU, temperature was recorded in South Africa near Bhanga Nek (5 nests in 2008, 7 nests in 2009, 15 nests in 2010 and 12 nests in 2011).

The SCL of 970 hatchlings was measured at Black Beard Island, Georgia (44 in 2001, 246 in 2002 and 680 in 2003 (mean = 44.13 mm and sd = 1.64 mm). The SCL of 949 hatchlings was measured at Wassaw Island, Georgia (90 in 2000, 180 in 2002, 200 in 2003 and 480 in 2004 (mean = 44.80 mm and sd = 1.79 mm). The SCL of 202 hatchlings was measured at Boca Raton, Florida (110 in 2013 and 92 in 2016) (mean = 43.10 mm and sd = 1.86 mm). No hatchlings were measured at other nesting sites in Florida (Sarasota, Sanibel, Melbourne, Hutchinson Island, Juno Beach) so we used data from Boca Raton for those. The SCL of 132 hatchlings was measured at Bhanga Nek, South Africa, in 2011 (mean = 43.24 mm and sd = 1.99 mm). We took SCL measures from a previously published study for the population nesting at Praia do Forte, Brazil (mean = 40.97 mm and sd = 0.96 mm) (Fuentes et al. 2017). No hatchlings were measured at Rio de Janeiro, Brazil, so we used data from the closest population in Praia do Forte, Brazil. We took SCL measures from a previously published study for the population nesting at Dalyan, Turkey (mean = 39.33 mm and sd = 1.92 mm) (Girondot and Kaska 2014a). No hatchlings were measured neither at Fethiye, Turkey, nor at Alagadi, Cyprus, so we used data from the closest population in Dalyan, Turkey. No hatchlings were measured at Sirte, Libya, so we used data obtained from hatchlings measured in Tunisia (mean = 38.30 mm and sd = 1.19 mm) as per Monsinjon et al. (In press).

To detect potential differences in thermal reaction norms for growth rate among nesting populations from the same RMU, we applied a similar approach as per Monsinjon et al. (In press). In short, the thermal reaction norm for growth rate is estimated for two populations (i) independently for both populations (hypothesis 1: both populations exhibit different reaction norms) and (ii) after data sets of nest temperature were grouped in a single one (hypothesis 2: both populations exhibit similar reaction norms). The sum of AICs of both models estimated independently (hypothesis 1) is then compared with the AIC of the model estimated with both data sets grouped together (hypothesis 2). Here we calculated the AIC corrected for finite simple size (AICc) and the Akaike weight, i.e. the relative support of both hypotheses (Burnham and Anderson 2002). We kept the hypothesis 1 (reaction norms are different) only if this hypothesis was strongly supported (Akaike weight > 0.9). Before comparing the closest populations one another within the same RMU, we first grouped those which belongs to the same subdivided management unit (demographically isolated nesting population) (Shamblin et al. 2014).

Once population-specific thermal reaction norms were estimated, we modelled embryonic development at nesting sites based on long term time series of reconstructed nest temperature.

If we consider a nest being laid at a date i , the progression of embryo size (SCL) is modelled until it reaches, at the date $i+n$, the final SCL of a completely developed embryo known for the nesting population. This allowed to extracting information such as how long incubation would last (n) and what would the temperature be within the thermosensitive period for sex determination (TSP) (see below for defining TSP boundaries within incubation). The model is run again at the date $i+1$.

The TSP occurs between embryonic stages 21 and 26 for sea turtles (Miller 1985) which roughly corresponds to the middle third of incubation at constant temperature as generally observed in most reptiles with TSD (Pieau and Dorizzi 1981, Bull 1987, Desvages et al. 1993, Girondot et al. In press). The SCL at the onset of the TSP and the SCL at the end of the TSP are respectively 21% and 71% of the SCL of a completely developed embryo for the loggerhead sea turtle (Girondot et al. In press).

2.3. Estimating sex ratio

The loggerhead sea turtle exhibits a male/female pattern of TSD (pattern TSDIa) which corresponds to the production of males at low temperatures and females at high temperatures (reviewed in Wibbels et al. 2003). Here we inferred the thermal reaction norm for sex ratio of loggerhead populations nesting in Mediterranean, Atlantic North West, Atlantic South West and Indian South West RMUs based on constant temperature experiments previously carried out in laboratory conditions. Data for Mediterranean are from Greece (Mrosovsky et al. 2002). Data for Atlantic North West are from Florida (Mrosovsky 1988), North Carolina (Mrosovsky 1988) and Georgia (Yntema and Mrosovsky 1980, 1982). Data for Atlantic South West are from Brazil (Marcovaldi et al. 1997). Data for Indian South West are from South Africa (Maxwell 1987). The logistic equation that describes the thermal reaction norm for sex ratio (Girondot 1999) is given below (eq. 3):

$$sr(T) = \frac{1}{1+e^{\left(\frac{P-T}{S}\right)}} \text{ [Equation 3]}$$

Where $sr(T)$ is the sex ratio (here the proportion of males) according to constant temperatures T . P is the pivotal temperature (i.e. the constant temperature at which both sexes are expected to be produced in equal proportion) and S is the parameter that controls the steepness of the transition between male producing temperatures and female producing temperatures. These parameters were fitted using maximum likelihood based on constant temperature experiments described above.

The simple mean temperature during either period of incubation is known to be a poor predictor of sex ratio because the effect of temperature on sex determination may be mediated by gonadal growth which also depends on temperature (Georges et al. 2005, Neuwald and Valenzuela 2011). Indeed, embryos that spend equal time at masculinizing and feminizing temperatures would develop into females because more cells proliferated during the feminizing period rather than during the masculinizing one. A constant temperature equivalent (CTE) was developed to account for such effect by calculating the temperature at which half of the development occurred (Georges 1989, Georges et al. 1994) and has recently been improved to account for a nonlinear development within incubation (Fuentes et al. 2017). Basically, this new CTE (called CTE_{Growth} thereafter) is the mean temperature weighted by the differential growth during the thermosensitive period for sex determination (TSP). The equation of the CTE_{Growth} is given below (eq. 4):

$$CTE_{Growth} = \frac{\sum_{i=k}^{N-1} T_i \times G_i}{\sum_{i=k}^{N-1} G_i} \text{ [Equation 4]}$$

T_i is the temperature at the moment i of incubation with k being the first recorded temperature during the period of interest (here the first temperature recorded during the TSP). G_i is the growth of embryos in term of straight carapace length (SCL) between two records (i.e. the difference between the embryonic size at time $i+1$ and i). N is the last recorded temperature during the period of interest (here the last temperature recorded during TSP). We produced sex ratio estimates based on population-specific thermal reaction for sex ratio using this proxy (CTE_{Growth}).

2.4. Unravelling the dynamic of the nesting season

The number of nest was surveyed daily by patrols at nesting beaches during a particular period of the year which is specific to monitoring program. We omitted nest attempts (false crawls) if the data set contained such information. Nests were counted from the 1st April to the 30th September for 6 nesting seasons (2010-2015) at Dalyan, Turkey. Nests were counted from the 1st April to the 31st August for 3 nesting seasons (2001-2003) at Black Beard Island and for 5 nesting seasons (2000-2004) at Wassaw Island, Georgia. Nests were counted from the 1st March to the 31st October for 16 nesting seasons (2001-2016) at Boca Raton, Florida. Nests were counted from the 1st September to the 31st May of the following year for 26 nesting seasons (1989-2014) at Praia do Forte and for 14 nesting seasons (2001-2014) at Rio de Janeiro, Brazil. Nests were counted from the 1st October to the 30th April of the following year for 32 nesting seasons (1980-2011) at Bhangha Nek, South Africa.

We used a set of equations that describe the underlying dynamic of a nesting season based on time series of nest counts (from Girondot 2017). Basically, a nonlinear function with 7 parameters is fitted using maximum likelihood with negative-binomial distribution for each ordinal day t of the year (eq. 5):

$$\begin{cases} t < B \rightarrow \text{MinB} \\ t \in [B, P - F/2] \rightarrow \left(\left(1 + \cos(\pi(P - F/2 - t)(P - F/2 - B)) \right) / 2 \right) (\text{Max} - \text{MinB}) + \text{MinB} \\ t \in [P - F/2, P + F/2] \rightarrow \text{Max} \\ t \in [P + F/2, E] \rightarrow \left(\left(1 + \cos(\pi(t - P + F/2)(E - P + F/2)) \right) / 2 \right) (\text{Max} - \text{MinE}) + \text{MinE} \\ t > E \rightarrow \text{MinE} \end{cases}$$

[Equation 5]

These parameters describe adequately the pattern of a nesting season: a minimum number of nest when turtles are not active before (MinB) and after (MinE) the active season (respectively at day B and E) and a maximum number of nest (Max) at the ordinal day when the peak of activity occurs (P) around which the curve can flattens out (F). The dispersion around the mean is controlled by the negative-binomial parameter Theta (see Girondot 2017 for more details). Here we assumed the curve is not flat around the peak ($F = 0$). First, we allowed all parameters to vary across seasons except Theta in order to obtain season-specific description of nesting activity at a particular nesting site. Second, we fitted parameters without allowing them to vary across seasons in order to obtain a global description of nesting activity. The latter were used to provide a visual picture of the average nesting pattern as compared to seasonal variations in sex ratio.

2.5. Calculating thermal index and sex ratio index

We calculated the thermal index (*THI*) as being the average nest temperature during a particular season weighted by (i) the number of nest laid and (ii) the duration of incubation. The equation is given below (eq. 6):

$$THI = \frac{\sum_{i=k}^{N-1} T_i \times Nest_i \times Inc_i}{\sum_{i=k}^{N-1} Nest_i \times Inc_i} \text{ [Equation 6]}$$

T_i is the temperature at the moment i of the season with k being the temperature at the first date of the season. $Nest_i$ is the number of nests laid at the moment i of the season. Inc_i is the duration of incubation for nests laid at the moment i of the season. N is the temperature at the last date of the season. We consider 0% of hatching success if *THI* exceeds 35°C.

We calculated the sex ratio index (*SRI*) as being the average sex ratio during a particular nesting season weighted by the number of nest laid. The equation is given below (eq. 7):

$$SRI = \frac{\sum_{i=k}^{N-1} SR_i \times Nest_i}{\sum_{i=k}^{N-1} Nest_i} \text{ [Equation 7]}$$

SRI is the sex ratio (male proportion) at the moment *i* of the season with *k* being the sex ratio at the first date of the season. *Nest_i* is the number of nests laid at the moment *i* of the season. *N* is the sex ratio at the last date of the season.

Past indices were calculated only for seasons when nests were counted and using nest number modeled using season-specific description of the nesting activity.

Thereafter, we projected future indices according to climate scenarios using our forecasts of nest temperatures, incubation durations, sex ratio and using the nest number modeled based on the average description of the nesting activity that was previously looped and applied to all available seasons (i.e. indices are calculated each season for 38 seasons which would correspond to the period from 2062 to 2100).

3. Results

We estimated population-specific thermal reaction norms for growth rate (Fig. 2). The thermal reaction norm for growth rate differs substantially between both populations nesting in the Atlantic South West RMU but did not differ among populations within other RMUs (result not shown). Populations nesting in Mediterranean (Turkey, Libya and Cyprus) exhibit a non-monotonic pattern where the growth rate increases with temperature until a maximum reached at 32°C and the growth rate slightly decreases up to this temperature (Fig. 2, A). Populations nesting in Atlantic North West (Florida and Georgia) exhibit a monotonic pattern where the growth rate increases steadily with temperature (Fig. 2, B). Populations nesting in Atlantic South West (Brazil) exhibit contrasted patterns (Fig. 2, C). The population nesting at Rio de Janeiro exhibit a bell-shaped pattern where the growth rate increases sharply with temperature to reach a maximum at 29.8°C and decreases considerably up to this temperature whereas the growth rate for the population nesting at Praia do Forte increases steadily with temperature to reach a maximum at 33°C and decreases mildly up to this temperature (Fig. 2, C). The Population nesting in Indian South West (South Africa) exhibits a monotonic pattern where the growth rate increases steadily with temperature (Fig. 2, B).

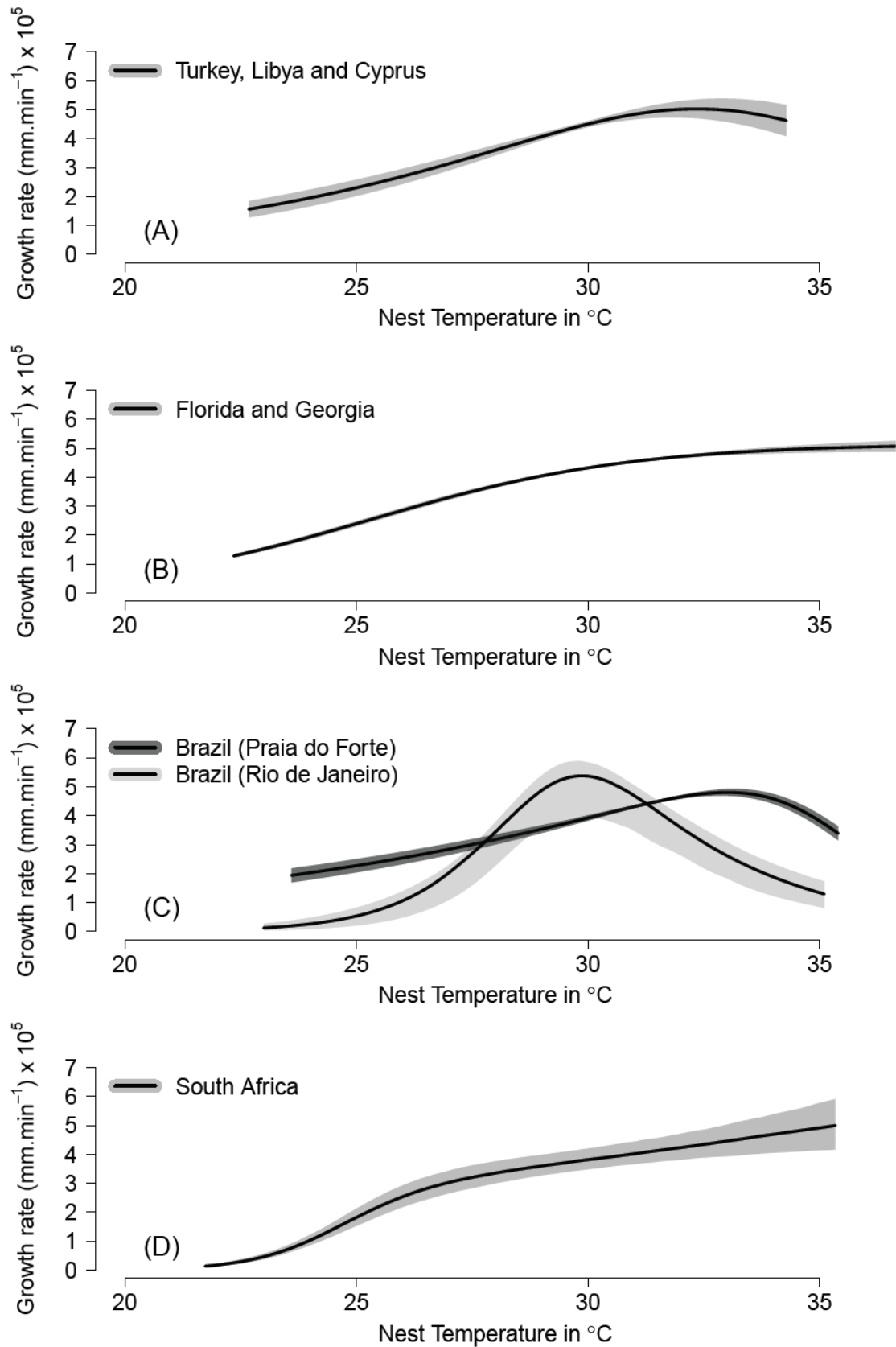


Figure 2: Thermal reaction norms for growth rate. Expected growth rate (black solid lines) is shown along the range of temperature recorded within nests at nesting sites in Mediterranean (A), Atlantic North West (B), Atlantic South West (C) and Indian South West (D) RMUs. Grey areas show the confidence interval at 95%.

We appropriately modelled nest temperature accounting for diel fluctuations for the 7 populations of which nest number was surveyed during nesting seasons (Supplementary files: Fig. S1). We produced long term time series (from 1979 to mid-2017) of daily minimum and maximum nest temperature (one for each nesting site) along which we ran our embryo growth model based on population-specific thermal reaction norms. For any days, we retrieved information about embryo development such as the duration of incubation and the average temperature weighted by the differential embryo growth within the TSP.

To produce sex ratio estimates, we inferred population-specific thermal reaction norms for sex ratio based on previous incubation experiments carried out at constant temperature in laboratory conditions (Fig. 3). The pivotal temperature (P) is 29.23°C for populations nesting in Mediterranean with a transitional range of temperature (TRT) ranging from 28.46°C to 29.99°C (Fig. 3, A). P = 28.97°C for populations nesting in Atlantic North West with a TRT ranging from 27.26°C to 30.69°C (Fig. 3, B). P = 29.12°C for populations nesting in Atlantic South West with a TRT ranging from 28.31°C to 29.94°C (Fig. 3, C). P = 29.24°C for populations nesting in Indian South West with a TRT ranging from 28.77°C to 29.71°C (Fig. 3, D).

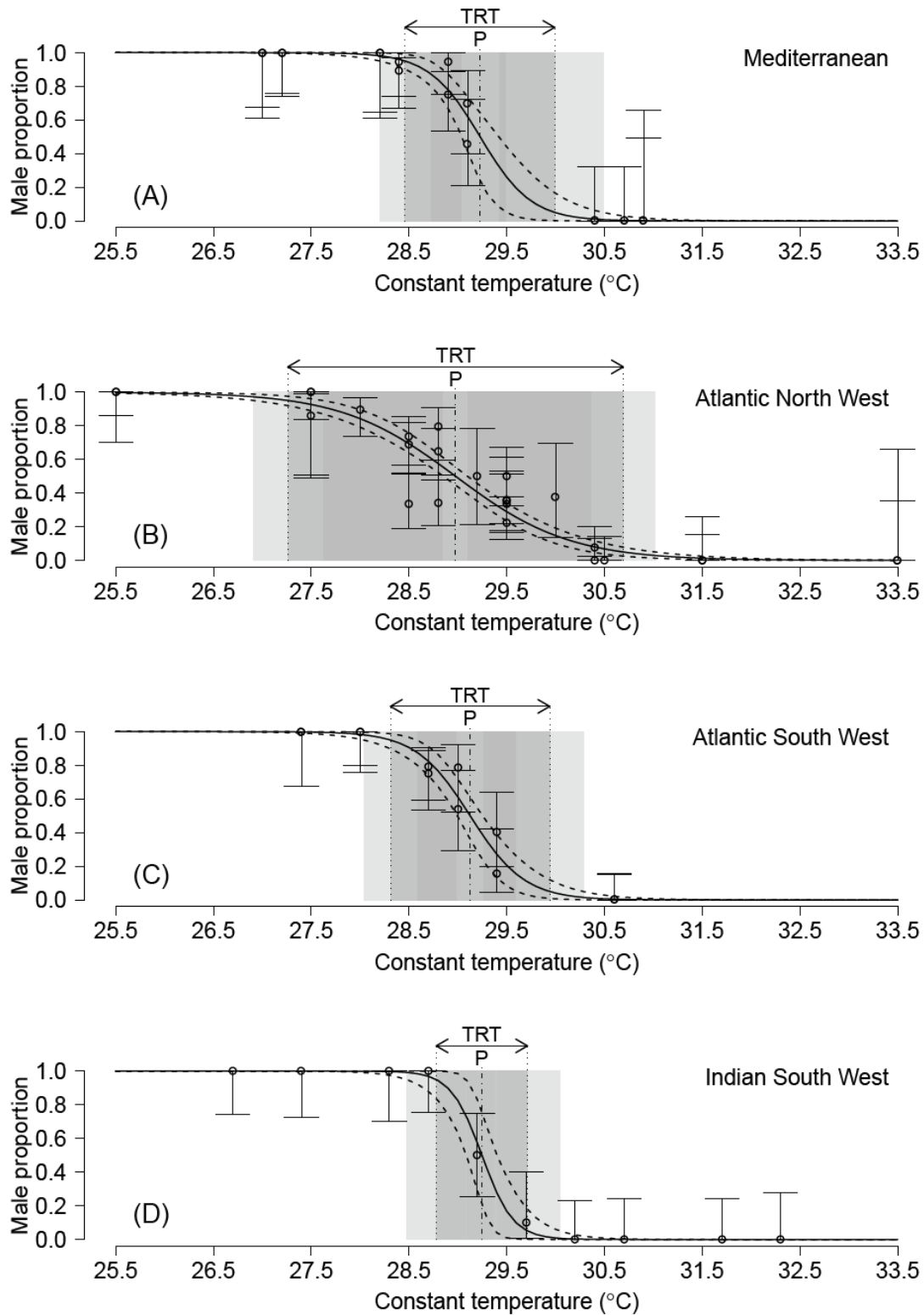


Figure 3: Thermal reaction norms for sex ratio. Expected sex ratio (black solid lines) based on constant temperature experiments previously carried out in laboratory conditions for populations nesting in Mediterranean (A) (Mrosovsky et al. 2002), Atlantic North West (B) (Yntema and Mrosovsky 1980, 1982, Mrosovsky 1988), Atlantic South West (C) (Marcovaldi et al. 1997) and Indian South West (D) (Maxwell 1987). Confidence interval at 95% is represented by black dotted lines. Observed sex ratio at several constant incubation temperatures is shown (black points are the average sex ratio and error bars show the confidence interval of observations). The Transitional Range of Temperatures (TRT) is indicated by the grey area in between the horizontal dotted lines. The Pivotal temperature (P) is indicated by the horizontal dotted line within the TRT. Shades of grey indicate the overlapping confidence intervals of the TRT and P.

We converted time series of average temperature weighted by the differential embryo growth within the TSP into time series of sex ratio estimates based on population-specific thermal reaction norms for sex ratio. Thereafter we assessed the seasonal pattern of sex ratio for each population by calculating the sex ratio expected for any day of the year along which we show the average seasonal pattern of nesting activity (previously standardized to match the range of sex ratio) (Fig. 4). At Dalyan (Turkey) the nesting season starts when the sex ratio is likely to shift from 100% males to a more balanced sex ratio, the peak of nesting occurs when both sexes can be produced in quasi-equal proportions and the season ends when the sex ratio is likely to shift from mixed sexes to 100% males (Fig. 4, A). It worth noting that the expected sex ratio during the nesting season at this nesting site is likely to be highly variable across years (Fig. 4, A). At Black Beard Island and Wassaw Island (Georgia), the nesting season spans the period of the year when a female-biased sex ratio is likely to be produced (Fig. 4, B and 4, C respectively), albeit the skew might be less pronounced at Wassaw Island (Fig. 4, C). It should be noted that inter-years variability of sex ratio during the nesting season is also relatively high at these two nesting sites (Fig. 4, B and 4, C), albeit a bit less variable at Black Beard Island (Fig. 4, B). At Boca Raton (Florida), the nesting season starts when some males may still be produced and lasts during the period when almost only females are expected to be produced across all years (Fig. 4, D). A similar case is observed at Praia do Forte (Brazil) where some males may be produced when the season starts and almost only females expected to be produced throughout the rest of the nesting season (Fig. 4, E). At Rio de Janeiro (Brazil), the nesting season starts when the expected sex ratio is shifting from a male-biased sex ratio to a more balanced sex ratio, the nesting peak occurs when the sex ratio is shifting from a balanced sex ratio to a slightly female-biased sex ratio and the nesting season ends before the sex ratio might be highly skewed toward females (Fig. 4, F). It worth noting that the sex ratio within the nesting season is highly variable across years at this nesting site (Fig. 4, F). At Bhangha Nek (South Africa) the nesting season starts when the sex ratio is likely to shift from 100% males to a female biased sex ratio, the peak of nesting occurs when mostly females are expected to be produced and the season ends before the sex ratio is likely to shift from 100% females to 100% males (Fig. 4, G). It is worth noting that the expected sex ratio during the nesting season at this nesting site is likely to be highly variable across years (Fig. 4, G).

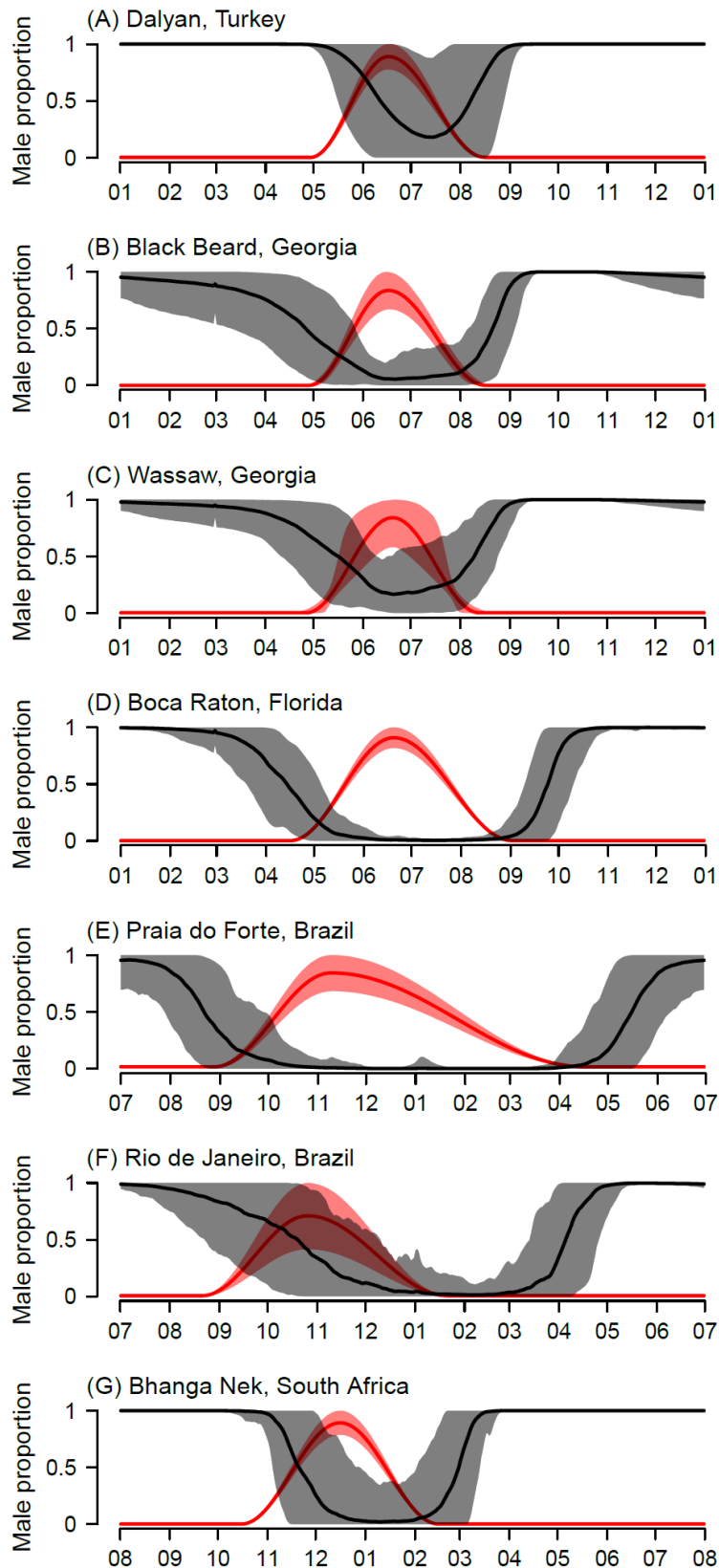


Figure 4: Seasonal variations in sex ratio and nesting activity. Expected mean sex ratio (black lines) for any days throughout the season and its standard deviation (grey area) are shown for populations nesting at Dalyan (Turkey) (A), at Black Beard Island (Georgia) (B), at Wassaw Island (Georgia) (C), at Boca Raton (Florida) (D), at Praia do Forte (Brazil) (E), at Rio de Janeiro (Brazil) (F) and at Bhanga Nek (South Africa) (G). Standardized fitted nesting activity (progression of number of nest throughout the season) is indicated by red lines and the confidence interval at 95% is indicated by red shaded areas. Note that the first day on the x axis (values indicate the month of the year) differs according to the nesting site.

Past thermal index (THI) was higher at Praia do Forte (THI = 31.01°C) and Boca Raton (THI = 31.74°C) as compared to other nesting sites (THI = 29.42°C, 29.65°C, 28.97°C, 29.37°C and 29.49°C, respectively at Dalyan, Black Beard, Wassaw, Rio de Janeiro and Bhanga Nek) (Fig. 5, A). According to the more optimistic scenario (RCP4.5), THI might increase sharply at Boca Raton (+2.43°C) and Black Beard (+2.36°C) as compared to Dalyan (+0.32°C) and Rio de Janeiro (+0.97°C) while other nesting sites might face intermediate increments (+1.81°C, +1.68°C and +1.56°C respectively at Wassaw, Praia do Forte and Bhanga Nek) (Fig. 5, A). According to the more pessimistic scenario (RCP8.5), THI at Boca Raton and Praia do Forte might cross the critical threshold over which no embryo is expected to develop successfully (respectively THI = 37.10°C and 35.06°C) whereas other nesting sites might still remain under this critical threshold (THI = 33.98°C, 32.98°C, 32.70°C, 32.60°C and 30.87°C, respectively at Black Beard, Bhanga Nek, Rio de Janeiro, Wassaw and Dalyan) (Fig. 5, A). The population nesting at Boca Raton would have to shift its phenology at least 23 days earlier to still produce some viable embryos and the population nesting at Praia do Forte would have to shift its phenology at least 3 days earlier to still produce some viable embryos (Table 1).

Past sex ratio index (SRI) was highly female-biased at Praia do Forte (SRI = 0.01) and Boca Raton (SRI = 0.02) whereas it was less skewed toward females at Black Beard (SRI = 0.18), Bhanga Nek (SRI = 0.18), Dalyan (SRI = 0.23), Rio de Janeiro (SRI = 0.21) and Wassaw (SRI = 0.37) (Fig. 5, B). According to the more optimistic scenario (RCP4.5), the SRI might decrease substantially at Wassaw (-0.33), Black Beard (-0.17), Bhanga Nek (-0.17), Rio de Janeiro (-0.11) and Dalyan (-0.11) whereas the SRI at Praia do Forte and Boca Raton might decrease only slightly (respectively, -0.01 and -0.02) (Fig. 5, B). According to the more pessimistic scenario (RCP8.5), the SRI might be considerably skewed toward females at Dalyan and Wassaw (SRI = 0.02 and 0.01 respectively) while other nesting sites might produce only females (SRI < 0.005). (Fig. 5, B). According to the scenario RCP4.5, populations nesting at Dalyan and Rio de Janeiro may keep producing a sex ratio that lies within their respective past range of SRI by shifting their nesting phenology only few days earlier (respectively, 4 and 3 days) whereas others population would have to shift up to 3 weeks earlier (27, 30, 47, 53 and 61 days, respectively at Boca Raton, Bhanga Nek, Black Beard, Wassaw and Praia do Forte) (Table 1). According to the scenario RCP8.5, the population nesting at Praia do Forte could not keep producing a sex ratio that lies within its past range of SRI and other populations would have to shift their phenology up to 3 weeks earlier (25, 59, 66, 72, 75 and 82 days, respectively at Dalyan, Boca Raton, Black Beard, Wassaw, Bhanga Nek and Rio de Janeiro) (Table 1).

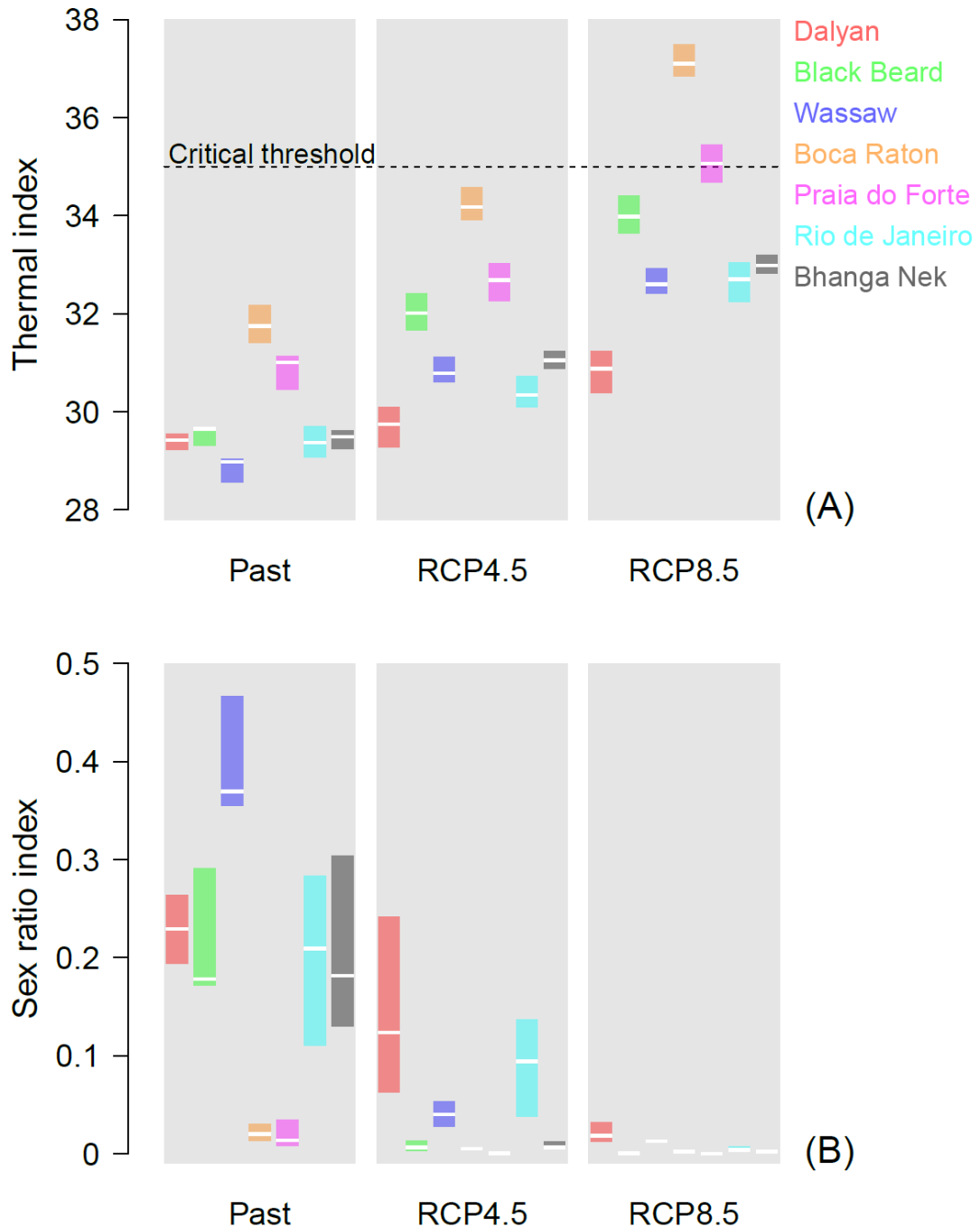


Figure 5: Thermal index (A) and sex ratio index (B). Colored areas show lower and upper quantiles (respectively, at 25% and 75%) and white dashes indicate quantile at 50%. Past indices were calculate based on 6 nesting seasons at Dalyan (Turkey), 3 nesting seasons at Black Beard Island (Georgia), 4 nesting seasons at Wassaw (Georgia), 16 nesting seasons at Boca Raton (Florida), 26 nesting seasons at Praia do Forte (Brazil), 14 nesting seasons at Rio de Janeiro (Brazil) and 32 nesting seasons at Bhanga Nek. Indices were projected according to 2 scenarios for future Representative Concentration Pathway (RCP) in greenhouse gas concentration (RCP4.5 and RCP8.5) and were calculated based on forecasted nest temperature and sex ratio with the average pattern of nesting phenology looped for 38 seasons. Black dashed line indicates the upper (critical) thermal threshold set at 35°C in this study.

Nesting site	Index	RCP4.5	RCP8.5
Dalyan	SRI	4	25
	THI	0	0
Black Beard	SRI	47	66
	THI	0	0
Wassaw	SRI	53	72
	THI	0	0
Boca Raton	SRI	27	59
	THI	0	23
Praia do Forte	SRI	61	none
	THI	0	3
Rio de Janeiro	SRI	3	82
	THI	0	0
Bhanga Nek	SRI	30	75
	THI	0	0

Table 1: Shifting of nesting phenology required to keep up with temperature. White rows indicate the minimum number of days at which the nesting phenology must be shifted earlier for the sex ratio index (SRI) to lie within the range of SRI (quantiles at 25% and 75%) that occurred in the past. Grey rows indicate the number of days at which the nesting phenology must be shifted earlier for the thermal index (THI) to remain under the critical threshold (35°C). Shifting of nesting phenology is presented according to 2 scenarios of Representative Concentration Pathway (RCP) of greenhouse gas: an optimistic scenario (RCP4.5) and a pessimistic scenario (RCP8.5).

4. Discussion

4.1. Primary sex ratio

Our results suggest that loggerhead nesting populations located at low latitudes are nesting during the period when almost only females are expected to be produced whereas nesting populations located at high latitudes are nesting during the period when both sexes can be produced with males more likely expected during the first part of the nesting season and mostly females during the second part of the nesting season. The reason why low-latitudes nesting populations are nesting during a female producing period while both sexes could be produced during other periods of the year remains unclear. If such bias in primary sex ratio scale toward adult sex ratio, detrimental effects at population level can occur such as reduced fertility rates (Bell et al. 2009) or loss of genetic diversity through reduction of effective population size (Hamann et al. 2010). For instance, sex ratio is one of the main parameters that may jeopardize population viability of the leatherback sea turtle (*Dermochelys coriacea*) in the face of climate changes (Saba et al. 2012). However, whether this highly female biased primary sex ratio is likely to persist through age classes is still unknown and may vary within and among sea turtle species (Hamann et al. 2010). Nevertheless, males can breed annually with multiple females while females do not return to breeding grounds every year which may lead to a more balanced operational sex ratio and, in turn, buffer negative consequences of any female biased adult sex ratio (Hays et al. 2010, Wright et al. 2012). Moreover, a study proposed that temperature dependent sex determination can confer a short-term demographic advantage by producing

more females with rising temperature which could enhance population growth rate (Santidrian Tomillo et al. 2015a). However, this is not likely to help in the current rate of climate change for the leatherback sea turtle (Santidrian Tomillo et al. 2015a) and this will inevitably decrease effective population size which can induce genetic erosion and thus lower potential to respond to selection pressures (Hamann et al. 2010).

According to the more optimistic scenario (RCP4.5) for future thermal changes at nesting beaches, we calculated that loggerhead populations nesting at Dalyan (Turkey) and Rio de Janeiro (Brazil) would have to shift their nesting phenology at least 4 and 3 days earlier, respectively, to offset potential changes in primary sex ratio. The nesting season of the loggerhead sea turtle occurs earlier in response to warmer sea surface temperature several months before the nesting season at either foraging or nesting sites (Weishampel et al. 2004, Pike et al. 2006, Hawkes et al. 2007, Mazaris et al. 2008, Mazaris et al. 2009, Weishampel et al. 2010, Mazaris et al. 2013, Lamont and Fujisaki 2014, Patel et al. 2016). One of the steepest response was found in Mediterranean where loggerheads start the nesting season 8.5 days earlier in response to 1°C increase in sea surface temperature at breeding areas (Mazaris et al. 2008). Given that the average increase in sea surface temperature may reach 1.189°C according to the scenario RCP4.5, this would make loggerheads initiating the nesting season 10.10 days earlier. Therefore, populations nesting at Dalyan (Turkey) and Rio de Janeiro (Brazil) may be able to neutralize the effect of warming temperatures. However, this is not likely to be the case for the 5 other populations that would require shifting their nesting phenology up to 3 weeks earlier to keep producing a primary sex ratio that lies within the range currently produced. According to the more pessimistic scenario (RCP8.5) this would make loggerheads initiating the nesting season 24.57 days earlier which may not be sufficient for any populations to keep producing their current range of primary sex ratio. Also, it should be noted that populations nesting at low latitudes are globally less sensitive to temperature for starting the nesting season earlier (Mazaris et al. 2013) which may weaken even more their potential to buffer the effect of warming temperature at nesting beaches.

In this study, we assumed that populations would have to advance their nesting phenology in the future to keep producing a primary sex ratio that resemble the one currently produced. However, the primary sex ratio currently produced may not be sustainable for all populations. The range of primary sex ratio within which no detrimental effects are expected (genetically and demographically) for a population of sea turtle to sustain remains unknown. We urge further research to build model of population dynamics that integrates a genetic Allee effect

linked to potential biases in sex ratio. This would give insights in critical sex ratio thresholds according to different scenarios of initial genetic diversity.

4.2. Hatchling production

Hatchling production is a determinant demographic parameter for population viability of sea turtles (Mazaris 2005) and is related to incubation temperature (Howard et al. 2014). According to the more optimistic scenario (RCP4.5), average temperature within nest under incubation throughout a nesting season may remain under the upper thermal threshold (here 35°C). However, the expected hatching success could be already very low if mean incubation temperature exceeds 33°C (Miller 1997, Howard et al. 2014, Jourdan and Fuentes 2015, Santidrian Tomillo et al. 2015b). Given that, populations nesting at Boca Raton (Florida) and Praia do Forte (Brazil) (low latitudes) may face low embryo survival according to the optimistic scenario. This is consistent with the observation that hatching success is already relatively lower at nesting sites located at low latitudes than at nesting sites located at high latitudes (Pike 2014). According to the more pessimistic scenario (RCP8.5), average temperature within nests under incubation might exceed the 35°C upper threshold which suggest that no embryo would be able to develop under such thermal conditions. We calculated that the population nesting at Boca Raton (Florida) and Praia do Forte (Brazil) would have to start nesting at least 23 and 3 days earlier, respectively, to keep producing some viable hatchlings. Loggerhead populations nesting in the Atlantic coast of Florida were shown to advance their nesting phenology from approximately 2.5 days (Weishampel et al. 2010) to 7 days (Pike et al. 2006) in response to 1°C increase in sea surface temperature offshore the nesting site. Giving a 2.891°C increase in sea surface temperature according to the more pessimistic scenario (RCP8.5), this would allow populations to advance their nesting phenology from 7.22 to 20.23 days earlier. In this scenario, such temperature-dependent plasticity in the nesting phenology will not help loggerhead population nesting at Boca Raton (Florida) to keep up with warming temperatures. Regarding the population nesting at Praia do Forte (Brazil), it should be sufficient to neutralize the effect of warming temperatures. However, such plasticity in nesting phenology still needs to be investigated for loggerhead populations nesting in Brazil.

4.3. Keeping up with a warming world

Sea turtles survived dramatic thermal changes in past climates (Hamann et al. 2007, Hawkes et al. 2009). Temperature dependent sex determination could have enhanced resilience of species in in the past (Silber et al. 2011). However, the exact mean by which sea turtles adapted and whether they will be able to adapt in the future remain unclear. Here we showed that shifting

the nesting phenology earlier may not be sufficient for all loggerhead populations to offset the effect of warming temperatures at nesting beaches and especially for those located at low latitudes. Indeed, shifting the nesting phenology earlier may not help a fresh water turtle either (Schwanz and Janzen 2008, Telemeco et al. 2013). We did not address whether nesting later in the season or extending the duration of the nesting season could buffer the effect of warming temperatures. Any delay in the nesting phenology in response to warmer temperature for the loggerhead sea turtle has not been reported yet. Some loggerhead populations were shown to shorten their nesting season in response to warmer temperature (Pike et al. 2006, Weishampel et al. 2010) while the opposite response was also found (Hawkes et al. 2007, Lamont and Fujisaki 2014). Further studies should investigate the potential of extending the nesting season in buffering the effect of warming temperature by considering population-specific sensitivity to temperature. However, we believe this would not help mitigating the effect of warming temperature at nesting beaches to the same extent as shifting the nesting phenology earlier because warming temperature are expected to induce an extension of the female producing period and perhaps shorten the period within which embryo can successfully develop.

Plasticity in nest site choice could also successfully buffer the effect of warming temperatures in reptiles (Doody et al. 2006, Doody and Moore 2010) as long as different thermal conditions within habitats are available and accessible (Doody and Moore 2010, Refsnider and Janzen 2012). For instance, shade cover may provide sufficient thermal variation for a freshwater turtle to select thermal microhabitats for nesting (Refsnider et al. 2013). Digging deeper nests could also dampen thermal fluctuations and result in cooler incubation temperature but this is unlikely for sea turtles which are already constrained by their morphology (Miller 1997). Variation in sand color at nesting beaches can also provide thermal microhabitats (Hays et al. 2001). In the present study, we reproduce thermal heterogeneity of temporal and fine spatial scale (within the nesting beach) but we did not account for potential thermal heterogeneity at regional scale (i.e. if individual change nesting beach). Further studies are required to provide biologically relevant characterization of thermal heterogeneity at the scale of nesting beaches (Hamann et al. 2007).

Rapid spatial shift of nesting sites at a regional scale is unlikely for sea turtle due to their natal homing behavior and high degree of nest site fidelity (Miller 1997). However, some loggerhead females were found to attempt nesting hundreds of kilometers apart from their nesting sites in Australia (Limpus 2008) and it is likely that sea turtles survived past climate changes by relocating nesting sites (Hamann et al. 2007). Such establishment of new nesting sites could be

the only way to keep up with a changing climate for sea turtle populations that will be or currently are nesting at unsuitable nesting sites (Hawkes et al. 2009, Fuentes et al. 2013) as long as new suitable nesting sites are available (Katselidis et al. 2012, Pike 2013).

4.4. Assessing vulnerability of sea turtles

Assessing the vulnerability of a species to climate changes requires to link knowledges about population-specific (i) exposure to environmental changes, (ii) sensitivity to these changes and (iii) adaptive capacity (potential response to selection pressures, phenotypic plasticity and population dynamics) (Butt et al. 2016). Relevant conservation strategies can be provided only if those parameters are considered together (Butt et al. 2016). A study developed a framework for assessing the vulnerability of sea turtle nesting beaches accounting for exposure, sensitivity and adaptive capacity to warming incubation temperatures, sea level rise and cyclonic activity (Fuentes et al. 2011). Here we showed that exposure, sensitivity and adaptive capacity (here by shifting of the nesting phenology) to thermal changes in incubation conditions may vary greatly among loggerhead populations. We also found that shifting of the nesting phenology earlier may not be enough for all populations to buffer the effect of warming temperatures. This emphasize the need to calibrate population-specific models to forecast primary sex ratio.

Nesting phenology and hatching success both influence the number of males and females hatchlings that may reach the sea. Only few studies attempted to account for differential survival of both sexes by identifying the relationship between mean incubation temperatures and hatching success or emergence rate (Santidrián Tomillo et al. 2014, Hays et al. 2017). Embryo survival depends on the magnitude of the thermal stress and the time spent up to a critical threshold (Howard et al. 2014). Therefore, the mean temperature during incubation may not be appropriate to predict hatching success because it does not capture biologically-determinant thermal variation. Promising studies about heat tolerance of loggerhead embryos will probably help developing a model based on cellular processes underlying embryo survival in response to temperature (Tedeschi et al. 2015, Tedeschi et al. 2016). Variation in moisture levels within nests is another factor that influences hatching success (Santidrian Tomillo et al. 2015b). Further studies must take this parameter into account to forecasting hatching success of sea turtles as the rate of precipitation is expected to be modified in the future (IPCC 2014).

In our study we did not account for sea level rise, cyclonic activity and coastal development which may also jeopardize availability of suitable nesting sites (Fuentes et al. 2009, Fuentes et al. 2011, Fuentes et al. 2013). Resilience of sea turtle populations also depends on population demography and genetic diversity. Importantly, further researches are needed to identify

whether sea turtles may benefit sufficient heritable variation in determinant traits such as nesting behavior, embryo growth rate and embryo thermal tolerance.

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Supplementary files

Table S1: Parameters used to reconstruct nest temperature

Nesting site	Lag.AIR	Lag.SST	Intercept	Coef.AIR	Coef.SST	Coef.MH	AmpT	Ti.Min	Ti.Max
Dalyan	1	1	22.5	0.059	0.103	4.55	0.36	547	1380
Black Beard	1	0	4.3	0.209	0.683	2.86	1.09	663	1390
Wassaw	1	1	18.3	0.270	0.094	2.39	0.98	647	1380
Boca Raton	1	0	-3.4	0.331	0.870	1.56	0.84	829	1
Praia do Forte	1	0	10.9	0.191	0.516	2.14	0.61	809	3
Rio de Janeiro	1	2	19	0.287	0.078	2.95	1.02	609	1400
Bhanga Nek	1	1	19.5	0.168	0.168	3.08	0.84	637	1410

Table S1: Parameters used to reconstruct nest temperature. Daily mean nest temperature is related with air temperature (AT) dAT days before (Lag.AIR), with sea surface temperature (SST) dSST days before (Lag.SST) and with a linear relationship with the time of incubation that describes temperature changes due to metabolic heating (MH). Estimated coefficients of the generalized linear mixed model (GLMM) are indicated (Intercept = μ , Coef.SST = α , Coef.AIR = β , and Coef.MH = γ). The average daily amplitude of temperature (daily maximum – daily minimum for all days and for all nests) is indicated (AmpT) in °C. The average time during the day when the minimum and the maximum temperatures occur (Ti.Min and Ti.Max, respectively) are indicated in number of minutes after midnight.

Table S2: Changes in temperature used to forecast nest temperature

Area	RCP2.6	RCP4.5	RCP6.0	RCP8.5
South-Europe-Mediterranean	1.172	2.121	2.375	3.934
Eastern-North-America	1.239	2.411	2.670	4.512
North-East-Brazil	0.928	1.769	2.175	3.568
Southern-Africa	0.993	1.965	2.439	3.910
World-Sea	0.500	1.189	1.456	2.891

Table S2: Changes in temperature used to forecast nest temperature. This table summarizes region-specific projected near-surface temperature anomalies that were retrieved from KNMI (Koninklijk Nederlands Meteorologisch Instituut) Climate Explorer website (https://climexp.knmi.nl/plot_atlas_form.py). Temperature increases is indicated for each scenario of future Representative Concentration Pathway (RCP) in greenhouse gas concentration (IPCC 2014). White rows indicate temperature anomalies we added to air temperature data and the grey row indicates temperature anomalies we added to sea surface temperature data.

Figure S1: Fit quality of nest temperature

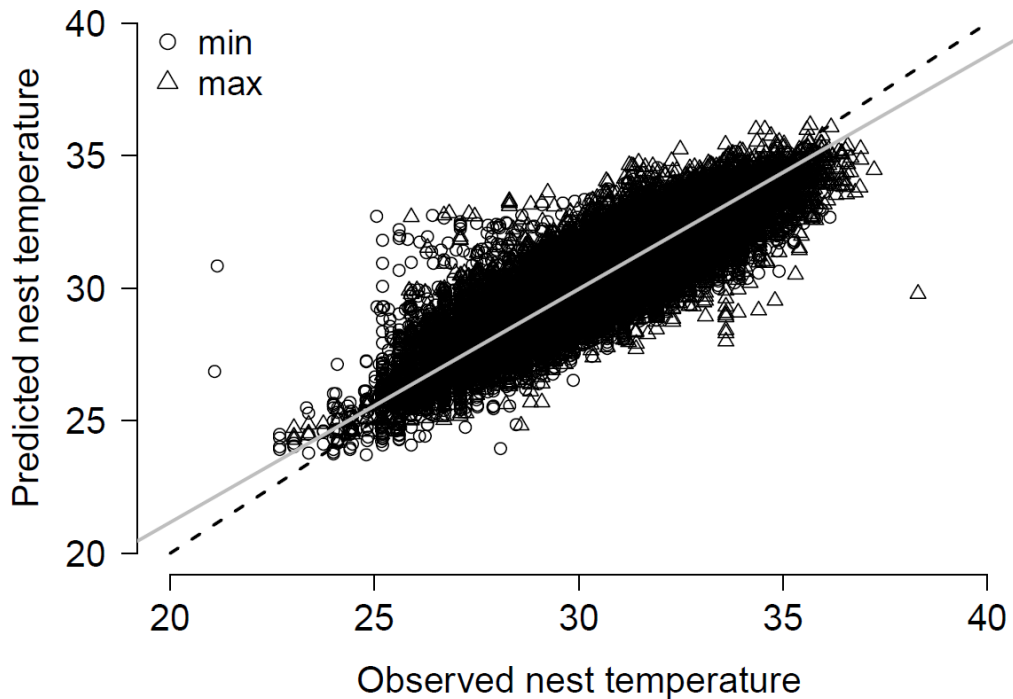


Figure S1: Fit quality of nest temperature. Predicted and observed daily minimum (circles) and daily maximum (triangles) temperatures within natural nests that incubated at Dalyan (Turkey), Black Beard and Wassaw Islands (Georgia), Boca Raton (Florida), Praia do Forte and Rio de Janeiro (Brazil) and Bhang Nek (South Africa). Black dashed line indicates the line of equality and grey solid line indicate the least rectangles line.

Chapter 4: Nesting phenology of the loggerhead sea turtle in response to temperature and resource availability

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Abstract: It is now well accepted that climate changes affect the geographical distribution and the timing of seasonal events, i.e. the phenology, of species and will ultimately change ecosystems functioning. The main trend among species is an earlier onset of phenological events. Ectotherms are expected to be strongly affected by changes in their thermal environment because most of their life-history traits are temperature-dependent. Sea turtles are globally threatened and may face important changes in their phenology. Most studies on sea turtle phenology often report an earlier onset of the nesting season in response to warmer temperatures as well as a modification in the duration of the nesting season. However, inconsistencies remain among and within species in the direction and the strength of their response to temperature. Moreover, some authors suggest that nesting phenology is triggered by an environmental cue before migration whereas others suggest that environmental conditions at the nesting site are more important. We investigated a loggerhead population nesting in Brazil and we found that an earlier onset of the nesting season was triggered by temperature at two foraging sites while the duration of the nesting season depends on net primary production at two other foraging sites. These emphasizes the need to account for food availability to understand underlying processes of the dynamic of the nesting season of sea turtles.

Keywords: Climate change, phenotypic plasticity, *Caretta caretta*, temperature, net primary production, migratory organism, ectotherm

1. Introduction

Species may respond to a changing environment by shifting their spatial distribution, their temporal occurrence or modifying their physiology (Bellard et al. 2012). Shifts in some species' geographical range toward either higher latitudes or upward in elevation in response to warmer temperatures are already observed in both marine and terrestrial ecosystems (Parmesan et al. 1999, McCarty 2001, Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Perry et al. 2005, Parmesan 2006, Rosenzweig et al. 2007, Poloczanska et al. 2013). Such modifications may have profound impacts on community composition and ultimately ecosystem functioning by altering trophic interactions (McCarty 2001, Hoegh-Guldberg and Bruno 2010, Walther 2010).

If a species' potential to disperse is constrained either morphologically or by the fragmentation of habitats, modifying its phenology, i.e. the timing of seasonal events, provides a short-term response to biotic and abiotic changes (Walther et al. 2002). Changes in phenology may be governed by genetic adaptation (Bradshaw and Holzapfel 2006) or phenotypic plasticity (Charmantier et al. 2008, Chevin et al. 2010). Such a change may be adaptive when it allows species to match with the period when resources are available or maladaptive when it creates a mismatch between the species' period of activity and its main resource (McCarty 2001, Walther et al. 2002, Visser and Both 2005, Walther 2010). In that way, fitness of individuals is closely related to their ability to track environmental changes. Hence, phenology is an important trait that must be investigated to anticipating species' risk of extinction and if so providing efficient conservation procedures. To what extent species are able to shift their phenology (genetically or phenotypically) and whether it allows for tracking suitable conditions in a changing environment (adaptive or maladaptive) is an urgent issue to address in the context of contemporary climate changes (IPCC 2014). The main trend currently observed in response to warmer temperatures is an earlier initiation of reproduction events in some species of birds, amphibians, reptiles, insects, and an earlier initiation of the growing season for some species of plants (albeit the opposite response has also been documented) (McCarty 2001, Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Weishampel et al. 2004, Parmesan 2006).

Ectothermic organisms, such as reptiles, amphibians, fishes and insects are expected to be strongly affected by climate changes because virtually all their life history traits are tightly

related to environmental temperature (Deutsch et al. 2008, Angilletta et al. 2009, Huey et al. 2009, Sinervo et al. 2010, Huey et al. 2012). Species with short generation time, such as insects, may be able to cope with a rapid change in thermal regimes through genetic adaptation (microevolution) as long as populations benefit from sufficient genetic variation. Conversely, species with long generation time may struggle to keep pace with a similar rate of change solely by microevolution and even if their temperature-dependent traits are heritable. For those organisms, such as most reptiles, phenotypic plasticity might be the best strategy to compensate for rapid and unpredictable changes in their thermal environments. Some reptiles exhibit temperature-dependent sex determination (TSD), that is, the sexual fate of individuals is irreversibly determined by temperature during incubation of eggs. Most chelonians and only some lizards exhibit TSD whereas it is the rule among sphenodontians and crocodylians (Valenzuela et al. 2013). This phenomenon has the potential for skewing the sex ratio and ultimately alter population dynamics as well as reducing its effective size. Moreover, embryo survival of egg-laying species is also closely related to temperature during incubation and some species are probably already at the edge of their thermal limits (Doody and Moore 2010). Therefore, species with TSD are expected to be vulnerable in the face of climate change (Mitchell and Janzen 2010). Nevertheless, those species may buffer the effect of warming temperature through maternal behavioral plasticity such as nest site choice and the timing of nesting (Doody et al. 2006, Schwanz and Janzen 2008, Refsnider and Janzen 2012) as long as environmental temperatures are sufficiently heterogeneous.

Sea turtles are among those long-lived ectotherms with TSD and 6 out of the 7 species are currently threatened (IUCN 2016). These migratory organisms forage in certain areas and travel long distances to their nesting site where they may lay several clutches during one season. Thereafter they return to their foraging site where they can spend more than 2 years before the next reproductive event (Solow et al. 2002). The length of intervals between two clutches is related to the temperature they experience offshore nesting rookeries (Sato et al. 1998, Hays et al. 2002, Cheng and Wang 2009). Weishampel et al. (2004) have shown that the median date of the nesting season is negatively related to sea surface temperature at the nesting site for a Floridian population of the loggerhead sea turtle (*Caretta caretta*). Nesting earlier was associated with a shorter nesting season for this population (Pike et al. 2006). This pattern is observed for loggerhead populations nesting in the east coast of Florida (Weishampel et al. 2004, Pike 2009, Weishampel et al. 2010) whereas warmer temperatures are associated with an extension of the nesting season in the Gulf of Mexico (Lamont and Fujisaki 2014), in North

Carolina (Hawkes et al. 2007), and in Mediterranean (Mazaris et al. 2013). In Mediterranean, an earlier onset of the nesting season was associated with warmer temperature at breeding, foraging and nesting sites (Mazaris et al. 2008, Mazaris et al. 2009, Ben Hassine et al. 2011, Mazaris et al. 2013, Patel et al. 2016). Two hypotheses have been previously formulated to explain the variation in the timing of the onset of the nesting season. Turtles start migrating according to an environmental cue perceived at foraging sites and nest soon after they arrive at their nesting site (Mazaris et al. 2009) (called “foraging sites hypothesis” in the present study) or they arrive early at the nesting site and start laying their eggs in response to an environmental cue perceived at this location (Pike 2009) (called “nesting sites hypothesis” in the present study). In any cases, the underlying physiological process of such phenotypic plasticity in nesting behavior and whether it provides an adaptive strategy remain unclear (Lamont and Fujisaki 2014, Neeman et al. 2015a). The fact that the relationship between the duration of the nesting season and the temperature contrasts greatly among populations suggests a complex site-specific interplay between biotic and abiotic factors.

To date, no study has investigated the potential phenological response of loggerhead nesting populations to variation in resource availability. Here we aim to identify whether the onset and the duration of the nesting season are triggered by sea surface temperature and resource availability (using net primary production as a proxy of resources) at either nesting sites or foraging sites for the loggerhead population nesting at Praia do Forte, Brazil.

2. Materials and methods

2.1. Foraging sites and nesting data

The nesting beach is located at Praia do Forte in the Bahia State, Brazil (Fig. 1). The nesting activity spans the period from September to March, with a peak in November and December (Marcovaldi and Chaloupka 2007, Lima et al. 2012). Marine turtle nesting activity were monitored daily during nesting seasons from 1989 to 2015 (26 nesting seasons) by TAMAR staff beach patrols (for more information about TAMAR see Marcovaldi and de Marcovaldi 1999). Some adult females were tracked during their interesting period using satellite transmitters. Eight potential foraging sites were identified (Fig. 1).

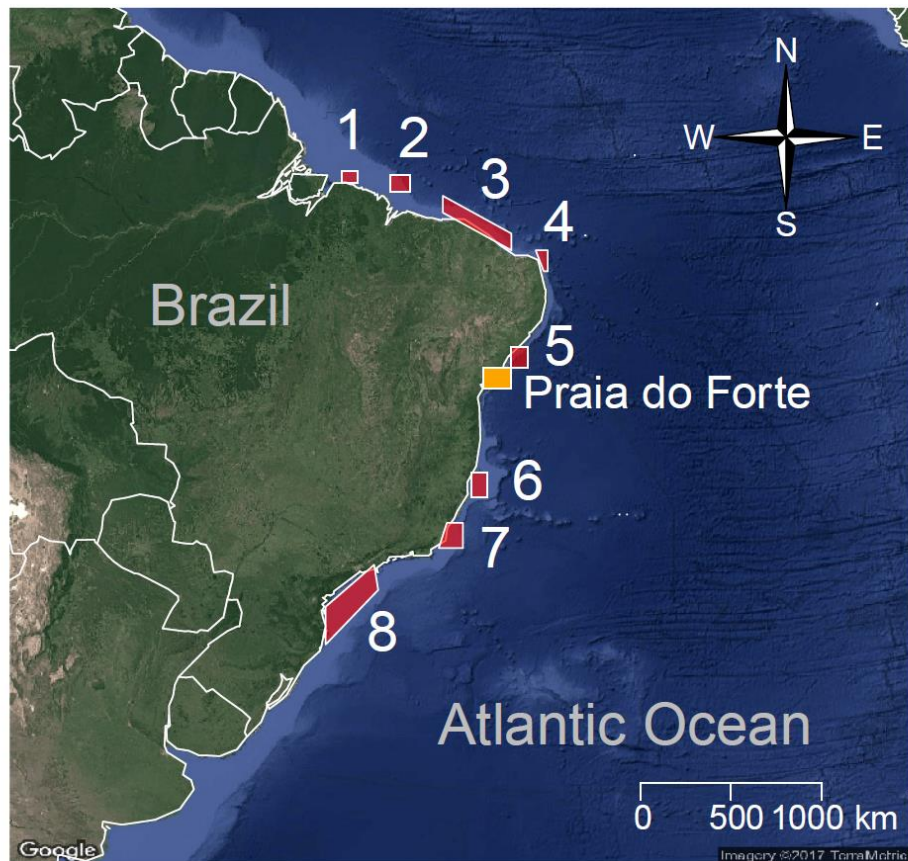


Figure 1: Location of the nesting site and foraging sites. Red areas represent potential foraging sites and the orange area represent the nesting site. Sea surface temperature and net primary production time series were extracted within these locations.

2.2. Sea surface temperature and net primary production

We extracted sea surface temperature (SST in °C) and net primary production (NPP in $\text{mg Carbon}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) at the potential foraging sites and offshore the nesting site. SST time series (each 6 hours) were retrieved from the European Centre for Medium-range Weather Forecasts website (<http://apps.ecmwf.int/datasets/data/interim-full-daily/levtype=sfc/>) which provide a global reanalysis of climate temperature on earth at a 0.75° spatial resolution from the 1st January 1979 to nowadays (ERA-Interim project). NPP time series (each 8-day) were retrieved from the Ocean Productivity website (<http://www.science.oregonstate.edu/ocean.productivity/standard.product.php>) which provide estimates from the 4th July 2002 to nowadays. These estimates were calculated using the Vertically Generalized Production Model (Behrenfeld and Falkowski 1997) based on surface chlorophyll concentrations, sea surface temperature and photosynthetically active radiation (these 3 parameters were taken from MODIS and SeaWifs projects). SST time series were averaged for each 8-day to match NPP data.

2.3. Unravelling the dynamic of the nesting season

Some authors used the first nest observed as an estimate of the onset of the nesting season and the duration of the period in between the first and the last nests as an estimate of the duration of the nesting season (Pike et al. 2006, Hawkes et al. 2007, Mazaris et al. 2008, Mazaris et al. 2009, Pike 2009, Lamont and Fujisaki 2014, Patel et al. 2016). However, this method is inappropriate because the first and the last nests may be sporadic events and therefore does not reflect the actual initiation of nesting as well as the duration of the overall season. To overcome this problem, we estimated the dates of the onset of the nesting season and the duration of the nesting season following the methods in Ben Hassine et al. (2011) (see also Girondot et al. 2015). This study used a set of equations that describe the underlying dynamic of the nesting season based on time series of nest counts (Girondot 2010). These equations are implemented on R package “phenology”. Basically, the parameters of a nonlinear function with 6 parameters are fitted using maximum likelihood with negative-binomial distribution against daily nest counts. These parameters describe adequately the pattern of a nesting season: a minimum number of nest (Min) when turtles are not active, a maximum number of nest (Max) at the ordinal day when the peak of activity occurs (Peak), the length of the season before the peak (LengthB), the length of the season after the peak (LengthE) and the negative-binomial parameter that describes the dispersion around the mean (Theta) (eq. 1) (but see Girondot 2010 for more detailed). Either parameters were allowed to vary across seasons except Theta which is a characteristic of the species and site.

$$\begin{cases} t < \text{Peak} - \text{LengthB} \rightarrow \text{Min} \\ t \in [\text{Peak} - \text{LengthB}, \text{Peak}] \rightarrow \left(\frac{1 + \cos(\pi(\text{Peak} - t)(\text{LengthB}))}{2} \right) (\text{Max} - \text{Min}) + \text{Min} \\ t \in [\text{Peak}, \text{Peak} + \text{LengthE}] \rightarrow \left(\frac{1 + \cos(\pi(t - \text{Peak})(\text{LengthE}))}{2} \right) (\text{Max} - \text{Min}) + \text{Min} \\ t > \text{Peak} + \text{LengthE} \rightarrow \text{Min} \end{cases}$$

[Equation 1]

2.4. Detecting environmental cues

We restricted nesting data from 2002 to 2015, which results in 12 nesting seasons, in order to match with available estimates of NPP. The dates of the onset of nesting seasons were converted in number of days after the 1st January of the year when the nesting season starts (ordinal days). The duration of nesting seasons was calculated as the number of days between the date of the onset and the date of the end of the nesting season pattern inferred by the model described above.

The onset of the nesting seasons may relate to temperature and resource availability at foraging sites (“foraging sites hypothesis”) or offshore the nesting site (“nesting sites hypothesis”). To test the “foraging sites hypothesis”, we calculated the average SST and NPP at the 8 foraging sites independently during any period p (8, 16, 24 and 32 days) and for any lag l (from 8 to 232 days each p days respectively) before the onset of the nesting season. This results in 992 set of predictive variables. Given the high number of potential relationships, we followed the Random forest approach (Ho 1995, 1998), using R package “VSURF”. Random forest is a classification algorithm that performs well in discriminating among predictive variables when the number of variables is much larger than the number of observations and gives measures of variable importance. This method involves stochastic discrimination so we ran the algorithm 100 times and counted the number of time each variable was returned. When several signals (with different p and l) were found for a certain foraging site, we retained the set of predictive variables that had the maximum counts over the 100 simulations. The resulting sets of variables were incorporated in a generalized linear model with a Gaussian link function and we applied a stepwise model selection method based on the lowest AICc, that is the AIC corrected for finite sample size (Burnham and Anderson 2002). To test the “nesting sites hypothesis”, we calculated the average SST and NPP at the nesting site during any period p (8, 16, 24 and 32 days) and for any lag l (from 8 to 232 days each p days respectively) before the onset of the nesting season. This results in 108 set of predictive variables. We conducted the same procedure as described above to discriminate among variables.

We applied the same approach regarding to the duration of the nesting season. However, the duration of the nesting season may also relate to SST and NPP experienced by turtles at the nesting site while they are laying their eggs (an alternative way to test the “nesting sites hypothesis”). To address this hypothesis, we built a generalized linear model with a Gaussian link function using both SST and NPP averaged during the nesting season. Thereafter, we selected the best model based on the lowest AICc.

NPP values were log transformed before being analyzed in order to stabilize the variance. The dynamic of the nesting season could also relate with population demography (Robinson et al. 2014). To test whether the onset and the duration of the nesting season depend on a demographical parameter, we build a generalized linear model with a Gaussian link function with the number of nest per season as predictive variable. Thereafter we compare all models based on their AICc and we considered models with $\Delta AICc < 2$ as competing models. All statistical tests and models were conducted using R software.

3. Results

3.1. Long term trends in nesting phenology

The onset of the nesting season occurred slightly earlier in recent years at a rate of 0.22 days per year, albeit not significantly ($F_{1,24} = 0.67$, $R^2 = 0.02$, $P = 0.42$, $n = 26$ seasons, Fig. 2, A). The duration of the nesting season was significantly extended at a rate of 3.11 days per year ($F_{1,24} = 44.88$, $R^2 = 0.65$, $P < 0.001$, $n = 26$ seasons, Fig. 2, B). The duration of the nesting season was not correlated with the onset of the nesting season (Pearson's coefficient of correlation = -0.39, $t = -2.09$, $df = 24$, $P = 0.047$, $R^2 = 0.15$) but instead it was correlated with the peak of the nesting (Pearson's coefficient of correlation = 0.49, $t = 2.74$, $df = 24$, $P = 0.011$, $R^2 = 0.24$) and the end of the nesting season (Pearson's coefficient of correlation = 0.94, $t = 13.18$, $df = 24$, $P < 0.001$, $R^2 = 0.88$).

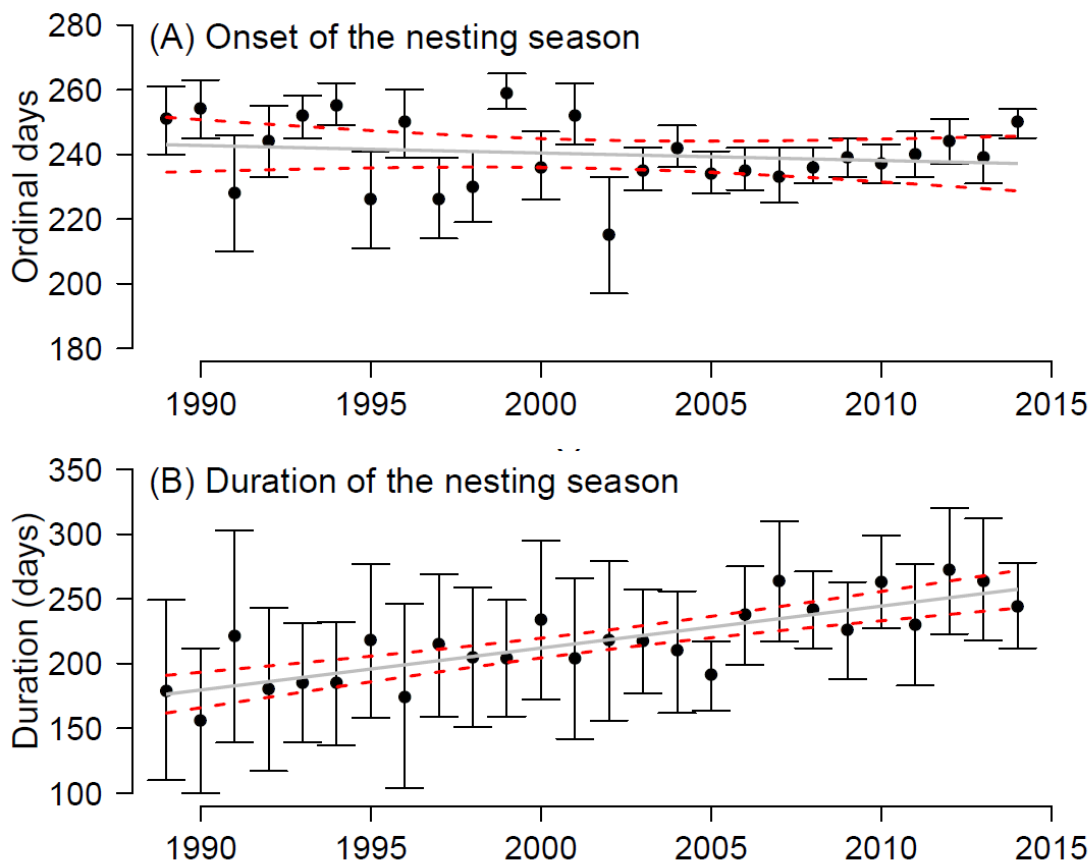


Figure 2: Long term trends in nesting phenology. Progression of the onset of the nesting season (A) and the duration of the nesting season (B) are represented. Black points represent the estimated mean and error bars show the confidence interval at 5%. The grey lines represent the linear regression according to seasons and the confidence interval is indicated by red dotted lines.

3.2. Onset of the nesting season

Two variables were retained to explain the onset of the nesting season according to the “Foraging sites hypothesis” (AICc = 59.27, Table 1): SST at foraging site 7 (between 104 and 128 days before the onset of the nesting season, Fig. 3, A) and SST at foraging site 1 (between

200 and 216 days before the onset of the nesting season, Fig. 3, B). According to this model, the onset of the nesting season is shifted earlier when SST is warmer at both foraging sites. When explaining the onset of the nesting season according to the “Nesting sites hypothesis”, we selected SST (between 112 and 120 days before the onset of the nesting season). The model built according to the “Foraging sites hypothesis” (AICc = 59.27, Table 1) outperformed the one built according to the “Nesting sites hypothesis” (AICc = 71.83, Table 1). The model based on demography did not differ from the null model (Table 1). The selected model performed well in predicting the actual onset of the nesting season as well as its anomaly (i.e. earlier or later when seasons are compared one another) (Fig. 4, A).

Parameter	Hypothesis	Model	AICc
Onset of the season	Foraging sites	SST1[200-216]+SST4[64-72]+SST7[104-128]	63.73
		SST1[200-216]+SST7[104-128]	59.27
	Nesting site	SST[128-156]+SST[112-124]+SST[112-120]	79.34
		SST[112-124]+SST[112-120]	74.79
		SST[112-120]	71.83
Demography	Number of nests	74.58	
Null		75.39	
Duration of the season	Foraging sites	NPP2[32-40]+NPP3[136-144]+NPP4[72-88]	112.54
		NPP2[32-40]+NPP4[72-88]	107.86
	Nesting site (before the season)	NPP[104-128]+NPP[56-72]+NPP[120-136]+SST[160-168]	122.31
		NPP[56-72]+NPP[120-136]+SST[160-168]	116.14
	Nesting site (within the season)	SST[Onset-End]+NPP[Onset-End]	113.83
		SST[Onset-End]	117.40
NPP[Onset-End]		113.08	
Demography	Number of nests	111.90	
Null		114.58	

Table 1: Summary of models for the onset and the duration of the nesting season. Numbers next to the variables indicate the foraging sites where the cue is detected (see Fig. 1). The period within which variables were averaged is indicated in brackets. The AICc of selected models are indicated in bold.

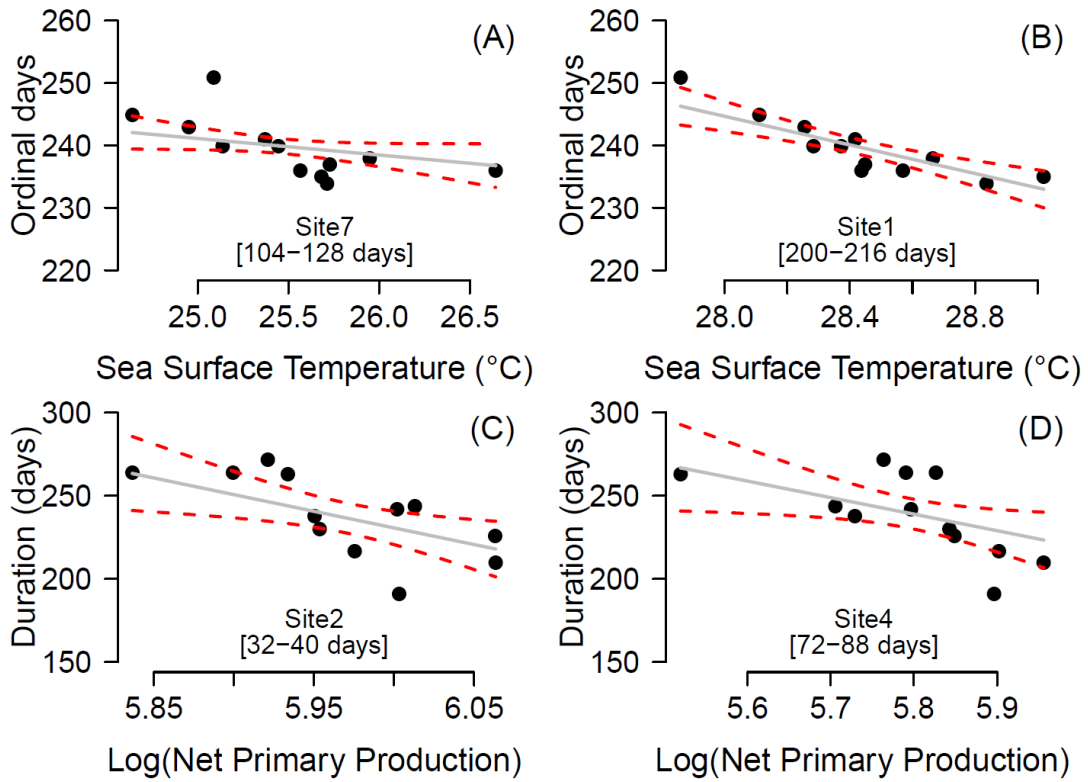


Figure 3: Phenological responses to environmental cues. The onset of the nesting season relates to sea surface temperature at foraging sites 7 (A), foraging site 1 (B). The duration of the nesting season relates with net primary production at foraging site 2 (C) and foraging site 4 (D). Grey lines represent the fit of respective models and the confidence interval at 5% is indicated by red dashed lines. The period during which the environmental cue was found is indicated in brackets.

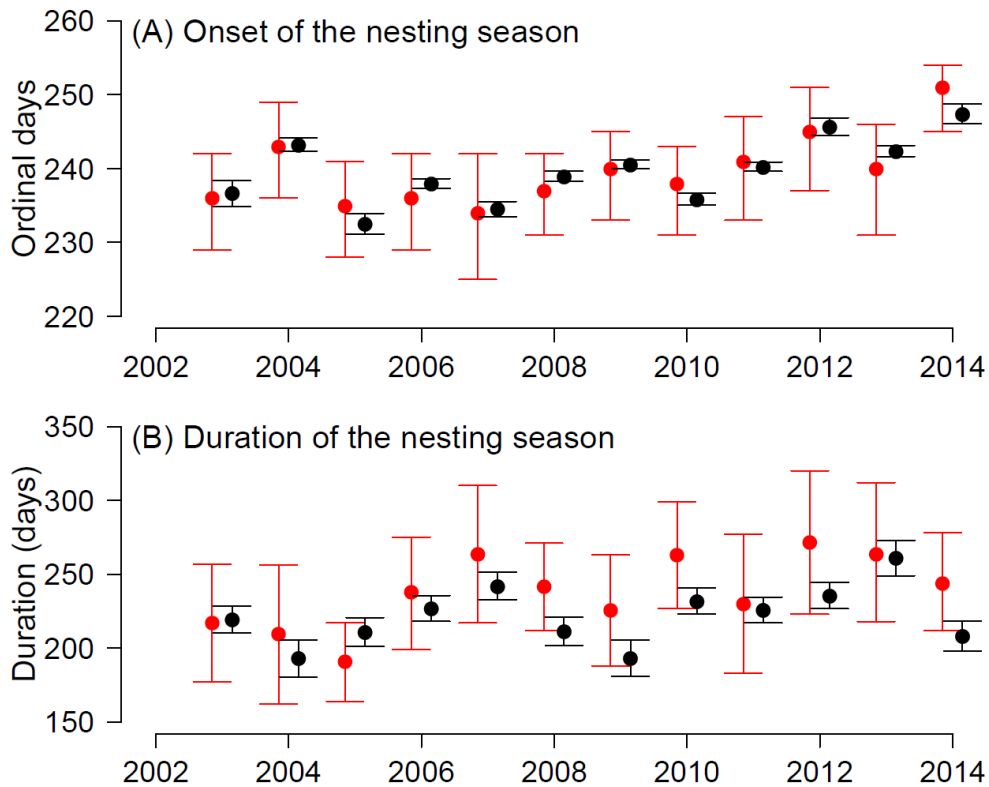


Figure 4: Goodness of predictions. Red points indicate the actual mean for the onset of the nesting season (A) and the duration of the nesting season (B). Black points represent values predicted by our model. Error bars show the confidence interval at 5%.

3.3. Duration of the nesting season

Two variables were retained to explain the duration of the nesting season according to the “Foraging sites hypothesis” (AICc = 107.86, Table 1): NPP at foraging site 2 (between 32 and 40 days before the onset of the nesting season, Fig. 3, C) and NPP at foraging site 4 (between 72 and 88 days before the onset of the nesting season, Fig. 3, D). According to this model, the duration of the nesting season decreases when NPP increases at both foraging sites. When explaining the duration of the nesting season according to the “Nesting sites hypothesis” with a cue sought before the season, we selected NPP (between 56 and 72 days before the onset of the nesting season), NPP (between 120 and 136 days before the onset of the nesting season) and SST (between 160 and 168 days before the onset of the nesting season). When explaining the duration of the nesting season according to the “Nesting sites hypothesis” with a cue sought within the season, we selected NPP. The model built according to the “Foraging sites hypothesis” (AICc = 107.86, Table 1) outperformed the one based on demography (AICc = 111.90, Table 1). Models based on the “Nesting sites hypothesis” did not differ from the null model (Table 1). The selected model performed well in predicting the actual duration of the nesting season (Fig. 4, B).

4. Discussion

4.1. Nesting phenology of sea turtles

Nesting phenology of sea turtles has recently received much consideration. These marine vertebrates show abilities to shift their nesting phenology in response to temperature. However, the way they respond to this environmental cue still shows discrepancies among and within species. The green turtles (*Chelonia mydas*) nest earlier in response to warmer temperatures in the east coast of Florida (Weishampel et al. 2010) or do not respond (Pike 2009) whereas the opposite response was reported in the south-west Indian ocean (Dalleau et al. 2012). The loggerhead sea turtles (*Caretta caretta*) nest earlier in response to warmer temperatures in the east coast of Florida (Weishampel et al. 2004, Pike et al. 2006, Pike 2009, Weishampel et al. 2010), in the Gulf of Mexico (Lamont and Fujisaki 2014), in North Carolina (Hawkes et al. 2007) and in Mediterranean (Mazaris et al. 2008, Mazaris et al. 2009, Patel et al. 2016). However, the strength of the response varies greatly among populations probably according to a latitudinal gradient (Mazaris et al. 2013). The leatherback sea turtles (*Dermochelys coriacea*) also seems to respond differently among populations (Neeman et al. 2015a) and a study suggested that the nesting season is rather shaped by demographical parameters because old breeders and neophyte breeders exhibit different behaviors (Robinson et al. 2014). Whether the

duration of the nesting season is extended or shortened in response to warmer temperature is also inconsistent among populations. Pike et al. (2006) and Weishampel et al. (2010) reported a shortening of the nesting season for loggerheads nesting in the east coast of Florida whereas the opposite pattern was observed in North Carolina (Hawkes et al. 2007) and in the Gulf of Mexico (Lamont and Fujisaki 2014). It remains unclear whether these different ways to respond to temperature (even mediated by food availability) are due to site-specific environmental conditions or population-specific strategies to cope with environmental changes. Sea turtles are migratory organisms that spend at least one year at their foraging sites (where they may respond to a cue to start migrating) and travel to their nesting site (where they may respond to a cue to start nesting). This consideration must be taken into account because turtles may experience very different environmental conditions at both areas. Only few authors attempted to discriminate whether the most important environmental cue that triggers nesting phenology is found at either nesting site or foraging area. Mazaris et al. (2009) found that temperature at the nesting site contribute much more in triggering the nesting phenology of loggerhead sea turtles (“Nesting sites hypothesis”). This suggest that adult females come earlier at the nesting site perhaps to mate with males and wait for optimal conditions (Pike 2009, Katselidis et al. 2012, Schofield et al. 2013). Sea turtles are ectotherms so higher temperature would then accelerate their metabolism and thus the development of their first clutch leading to an earlier onset of the nesting season (or reversely). However, Neeman et al. (2015) reported that thermal conditions at foraging sites are more determinant in triggering the nesting phenology of leatherback sea turtles (“Foraging sites hypothesis”). Our results are consistent with this hypothesis. This suggest that adult females start migrating in response to an effect of temperature (either directly or mediated by food availability) and begin to nest soon after their arrival at their nesting site. Temperature may be either positively or negatively linked to turtles’ preys depending on site-specific trophic interactions and a direct effect of temperature on their preys’ survival. In addition, the rate at which turtles may catch their preys depends on their metabolism rate which depends on temperature.

4.2. Effect of temperature and food availability

Our results suggest that temperature at two foraging sites triggers the onset of the nesting season and that food availability at two foraging sites determines the duration of the nesting season of the loggerhead sea turtle nesting at Praia do Forte, Brazil.

Warmer temperatures at two foraging sites were associated with an earlier onset of the nesting season. Foraging populations responded the same way to temperature but according to different

periods. The closest foraging population responded to temperature almost 3 months before the nesting season while the farthest responded to temperature nearly 6 months before the nesting season. This suggests that those populations, even at opposite directions apart the nesting site, are the first cohorts arriving synchronously at the nesting beach. This has important conservation implication because females that arrive first may encounter more suitable conditions for the incubation of their eggs (Katselidis et al. 2012). Further investigations must confirm the relevance of these 2 foraging sites. Nevertheless, this finding highlights the need to seek a cue at different periods when investigating multiple potential foraging sites.

In the present study, the duration of the nesting season was not correlated with an earlier onset of the nesting season (Pike et al. 2006) but instead with a delay in the peak and a delay in the end of the nesting season. We found that food availability (using net primary production as a proxy) at two foraging sites drives the duration of the nesting season. The duration of the nesting season decreases when food is more abundant. This suggests that populations foraging at these locations are the last cohorts that arrive at the nesting site. We speculate that net primary production is the limiting factor creating a “Bottom-up forcing” that constrains departure of adult females at those specific sites. Food could be limiting at these locations so that populations arrive sooner during years when food is available which would shorten the duration of the nesting season. Those foraging populations may still be seeking food while the nesting season already started and arrive later at the nesting site which would result in an extension of the nesting season during less-productive years. However, this hypothesis requires further investigations because sea turtles would normally spend another year at their foraging sites if they did not accumulate enough reserves (Solow et al. 2002, Saba et al. 2007). Our results show that net primary production affect the nesting phenology of this loggerhead population and might be the reason why some studies reported contrasted response of the duration of the nesting season among population when looking solely at the effect of temperature (Pike et al. 2006, Hawkes et al. 2007, Weishampel et al. 2010, Lamont and Fujisaki 2014). Therefore, this important ecological parameter should be considered to project sea turtles’ response to climate change (Saba et al. 2007, Saba et al. 2012, Neeman et al. 2015b).

4.3. Other potential factors

Some authors suggested that population demography might also affect the overall pattern of the nesting season because experienced breeders and neophyte breeders may nest at different periods (Lamont and Fujisaki 2014, Robinson et al. 2014). This loggerhead population is currently recovering thanks to conservation efforts carried out by TAMAR. Therefore, we

might expect the co-occurrence of experienced breeders and neophyte breeders. We tested the relationship between the total amount of nest per season (used as a proxy of population size) and the onset of the nesting season as well as the duration of the nesting season. This hypothesis was not strongly supported for this nesting population neither for the onset of the nesting season nor for the duration of the nesting season.

Large scale climate indices such as El Niño-Southern Oscillation (ENSO), North Atlantic Oscillation (NAO), Atlantic Multidecadal Oscillation (AMO) and Pacific Decadal Oscillation (PDO) are often good predictors of temperature and resource availability. Nest number per season is related to ENSO for leatherback populations in the Pacific (Saba et al. 2007), to AMO for loggerhead populations in the Pacific as well as to PDO in the Pacific (Van Houtan and Halley 2011) and to AMO for hawksbill sea turtle (*Eretmochelys imbricata*) in the Gulf of Mexico (Monte-Luna et al. 2012). The median nesting date (a proxy of nesting peak) of a leatherback population in the Pacific does not respond to such climate index whereas the standard deviation of nests distribution (a proxy of the duration of the nesting season) does (Robinson et al. 2014). We did not address this issue for loggerheads nesting in Brazil but we encourage further studies to do so. In addition, the phenology of some migratory birds seems to respond to photoperiodicity and may provide an adaptive response to climate changes (Coppack et al. 2003, Visser et al. 2010). To our knowledge, this type of response has never been investigated in sea turtles and thus provides an interesting avenue for future research.

5. Conclusion

In conclusion, we found that temperature is the most important cue that triggers the departure of loggerheads adult females to their nesting site. This is more consistent with the hypothesis that females start nesting soon after their arrival rather than arriving early and wait for optimal conditions. However, whether this is the rule for the loggerhead sea turtle or this behavior is population-specific still need to be confirmed. An important finding is that food availability at certain foraging sites seems to constraint the duration of the nesting season. Again, whether this is the rule for this species or this phenomenon is specific to this population still need to be investigated. Nevertheless, this important ecological parameter should be taken into account to properly assess the vulnerability of sea turtles in the face of climate changes.

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Chapter 5: Thermal reaction norm for sexualization, the missing link between temperature and sex ratio for reptiles with temperature-dependent sex determination

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Abstract: Species with temperature dependent sex determination can experienced biased sex ratio in natural conditions which questions their vulnerability in the face of contemporary climate change and the adaptive significance of such sex determining mechanism. Several indirect methods, often based on laboratory experiments, are used to predict the sex ratio of natural clutches. However, no study has validated the use of such methods. Here we propose a novel method based on the thermal reaction norm for sexualization and compare it with previous methods. Using data of hatchling sex ratio and temperature obtained in the field for the loggerhead sea turtle (*Caretta caretta*), we show that the thermal reaction norm for sexualization enable producing relevant sex ratio estimates whereas previous methods tends to overestimate the actual proportion of males.

Keywords: Climate change, phenotypic plasticity, embryonic development, growth rate, ectotherm, incubation

1. Introduction

Since temperature-dependent sex determination (TSD) was first described (Charnier 1966), the evolutionary and ecological consequences have fascinated at least two generations of researchers without finding definitive answers. When TSD occurs, instead of being determined by sexual chromosomes, i.e. genotypic sex determination (GSD), sexual phenotype depends on environmental temperature experienced during ontogeny. This kind of sex phenotypic plasticity is observed in some teleost fishes (Shen and Wang 2014) and is widespread among non-avian

reptiles with all studied crocodylians exhibiting TSD along with the two extant species of tuatara, most chelonians and many lizards (Ewert et al. 1994, Lang and Andrews 1994, Viets et al. 1994, Deeming 2004, Ewert et al. 2004, Harlow 2004, Janzen and Krenz 2004).

For those species, sex is determined by temperature during a particular period of incubation called the thermosensitive period for sex determination (TSP) which corresponds to the middle third of incubation at constant temperatures in all reptiles with TSD studied so far (Bull and Vogt 1981, Pieau and Dorizzi 1981, Yntema and Mrosovsky 1982, Bull 1987, Mrosovsky and Pieau 1991, Wibbels et al. 1991, Desvages et al. 1993, Merchant-Larios et al. 1997, Young et al. 2004, Shine et al. 2007, Burke and Calichio 2014, Girondot et al. In press). During this period, gonads differentiate into either testes or ovaries depending on the endogenous level of steroid hormones (Raynaud and Pieau 1985, Shoemaker-Daly et al. 2010). Although several candidate genes were recently found (Rhen et al. 2015, Yatsu et al. 2015, Schroeder et al. 2016, Yatsu et al. 2016), the gene regulatory network underlying the gonadal fate in reptiles remains elusive in part because different master-switch genes may have been co-opted among species throughout evolution (Bachtrog et al. 2014). Recent studies suggest evolutionary transition between TSD and GSD may occur easier than previously thought (Sarre et al. 2004, Quinn et al. 2011, Schwanz et al. 2013). Indeed, GSD and TSD co-occur within a species of lizard (*Niveoscincus ocellatus*) (Pen et al. 2010) and within a population of another lizard (*Pogona vitticeps*) (Holleley et al. 2015). The latter suggest that sex reversal may provide the proximate mechanism that triggers evolutionary shifts between GSD and TSD in reptiles (Holleley et al. 2016).

When frequency-dependent selection on sex occurs, individuals that produce the rare sex are selected, which ultimately favors individuals that allocate equally in both sexes (Fisher 1930). However, species with TSD might experience biased population sex ratio which have question the adaptive significance of this sex determining mechanism for a long time. The prevalent hypothesis proposed that TSD should be selected if temperature affects fitness of individuals differentially in both sexes, i.e. TSD is a condition-dependent sex allocation strategy (Charnov and Bull 1977). This hypothesis found support in only two short-lived species of lizards (Warner and Shine 2008, Pen et al. 2010) probably because measuring lifetime fitness of individuals is challenging. Nevertheless, a recent promising study proposed that TSD is selected over GSD if age at maturity differs in both sexes and incubation temperature affects annual survival equally in both sexes (Schwanz et al. 2016).

Sex ratio is an important demographical parameter that governs population dynamics of species with TSD (Girondot et al. 2004). Indeed, biased operational sex ratio lead to an Allee effect that decreases effective population size (number of males and females ready to reproduce) which reduce genetic variation and, in turn, increases genetic drift (Girondot et al. 2004). Therefore, knowing the sex ratio of species with TSD is an urgent task for assessing species' vulnerability in the context of contemporary climate change (Brook et al. 2000, Ewen et al. 2001, Stubbs et al. 2014). Determining the sex ratio of populations can be challenging, especially for species that lack heteromorphic sex chromosomes, such as most species with TSD, and when offspring lack sexually dimorphic external characteristics, such as most reptiles (Wyneken et al. 2007).

The most commonly used methods to estimate sex ratio in natural conditions are based on either mean incubation temperatures or incubation durations. However, those proxies are known to be poor predictors of sex ratio (Georges 1989, Georges et al. 1994, Valenzuela 2001, Georges et al. 2004, Schwanz and Proulx 2008). The estimated sex ratios based on these proxies also differ greatly according to the exact procedure used for the estimation (Fuentes et al. 2017). As obviously there is only one "true" sex ratio, we can conclude that most of the ecological or evolutionary studies published so far used wrong sex ratios. Of course, it precludes to a correct understanding of causes and consequences of temperature-dependent sex determination. The problem in using such proxy stems from the fact that the magnitude of thermal fluctuation (daily and seasonally) experienced in natural conditions also affects embryonic development and, in turn, hatchling phenotype of ectotherms (Les et al. 2009, Warner and Shine 2010, Niehaus et al. 2012, Georges 2013, Paaijmans et al. 2013, Bowden et al. 2014, Horne et al. 2014). This is because thermal reaction norms (i.e. the phenotypic expression of a genotype across a range of temperature) for biological rates, such as embryonic growth, are often nonlinear and not monotonic even within the range of temperatures experienced in nature (Georges et al. 2004, Georges et al. 2005, Warner and Shine 2010, Neuwald and Valenzuela 2011). To overcome this problem, Georges (1989) proposed to calculate a constant temperature equivalent (CTE) but no general method for estimation has been validated so far. Moreover, temperature exerts its effect on sex determination during a specific thermosensitive period (TSP) which lies during the middle third of development (Yntema 1979, Bull and Vogt 1981) but does not match the middle third of the incubation period (a common shortcut to infer the TSP) when temperature varies (Valenzuela 2001, Georges et al. 2004, Georges et al. 2005, The Chu et al. 2008, Neuwald and Valenzuela 2011, Woolgar et al. 2013, Stubbs et al. 2014, Fuentes et al. 2017, Girondot et al. In press).

Though sex ratio prediction of reptiles with TSD received an extensive theoretical improvement, any method to estimate sex ratio has never been validated by comparing with actual sex ratio under natural conditions. This is particularly challenging for threatened species such as sea turtles because sacrificing embryos to examine gonads histologically is difficult to justify and available non-lethal method of sexing, such as laparoscopy, is time consuming (Wyneken et al. 2007). Here we take up this challenge by comparing sex ratio estimates based on existing methods with the actual sex ratio of hatchlings (identified using laparoscopy) of the loggerhead sea turtle (*Caretta caretta*) at 6 nesting beaches in Florida (Fig. 1). In addition, we develop a novel method based on the thermal reaction norm for sexualization (i.e. the development of sexual characters of the gonad) thus providing the missing link between temperature and the sex ratio of reptiles with TSD.



Figure 1: Map of nesting sites (red points) in Florida where nest temperature and sex ratio were investigated.

2. Materials and methods

2.1. Nest temperature and sex ratio data

Temperature was recorded within 133 nests from the moment when nests were laid and until hatchlings emerged at 6 nesting beaches in Florida (Fig. 1) between 2002 and 2016 using temperature data loggers placed in the middle of the clutch at approximately 45 cm depth

(Supplementary files, Table S1). Some hatchlings were kept and raised several months until they reached a sufficient size to assess their gonads using laparoscopy following method in Wyneken et al. (2007). Those post-hatchlings were then released offshore. Ten hatchlings were kept from 122 out of the 133 clutches and 35 hatchlings were kept for 2 nests for another experiment not presented here (Nest 63 and 65, Supplementary files, Table S1). Sex of hatchlings was not assessed for embryos that died before they would have reached a sufficient size. In 2016, 17 clutches laid at Boca Raton were translocated in a different area of the beach and were split for a moisture experiment not presented here (half of the eggs incubated under natural conditions while the others received a daily dose of water that mimicked rainfalls). Sex ratio was not assessed for hatchlings from 3 out of these 34 sub-clutches because no embryo hatched. Temperature data loggers were placed in the fridge before being placed into the nest so we removed the first records (first 6 hours period) to keep temperature time series that actually reflect temperature experienced by freshly laid eggs.

2.2. Modelling embryonic development

The progression of the size of an embryo according to temperature experienced within the nest can be modelled based on the thermal reaction norm for growth rate using an equation that describes the effect of temperature on the rate of growth along with an equation that describes the progression of the size of an embryo during incubation period (Georges et al. 2005, Girondot and Kaska 2014). This method has already been implemented to estimate the thermal reaction norm for growth rate of 3 loggerhead nesting populations (Girondot and Kaska 2014, Fuentes et al. 2017, Girondot et al. In press, Monsinjon et al. In press). Calibrating this model requires traces of temperature recorded within nests and measures of the straight carapace length (SCL) of hatchlings. The SCL of 202 hatchlings was measured at Boca Raton nesting beach (110 in 2013 and 92 in 2016) to inform the final SCL for Floridian loggerhead populations. Based on our 133 temperature time series recorded in natural nests and SCL measurements, we fitted the model's parameters using maximum likelihood with a Gaussian distribution of SCL (mean = 43.10 mm and sd = 1.86 mm) and an identity link.

2.3. Targeting TSP boundaries within incubation

The TSP occurs between embryonic stages 21 and 26 for sea turtles (Miller 1985) which roughly corresponds to the middle third of incubation at constant temperatures (Bull and Vogt 1981, Pieau and Dorizzi 1981, Yntema and Mrosovsky 1982, Bull 1987, Wibbels et al. 1991, Desvages et al. 1993, Merchant-Larios et al. 1997, Young et al. 2004, Shine et al. 2007, Burke and Calichio 2014, Girondot et al. In press). The SCL at the onset and the end of the TSP are

respectively 21% and 71% of the SCL of a hatchling (here 43.10 mm) for the loggerhead sea turtle (Kaska and Downie 1999). Therefore, the TSP starts at the moment of incubation when embryos reach a SCL of 9.05 mm (43.10×0.21) and lasts until embryos reach a SCL of 30.60 mm (43.10×0.71).

2.4. Thermal reaction norm of sex ratio

The loggerhead sea turtle exhibits a male/female pattern of TSD (pattern TSDIa) which corresponds to the production of males at low temperatures and females at high temperatures (reviewed in Wibbels et al. 2003). Here we inferred the thermal reaction norm for sex ratio of loggerhead populations nesting in Florida based on constant temperature experiments previously obtained in Florida (Mrosovsky 1988). Only 4 constant temperatures were investigated in Florida by Mrosovsky (1988) so we added data obtained from two closely related populations nesting in Georgia (Yntema and Mrosovsky 1980, 1982, Mrosovsky 1988) and North Carolina (Mrosovsky 1988). We fitted the parameters of a logistic equation to describe the thermal reaction norm for sex ratio using maximum likelihood (Girondot 1999). This equation is valid only for constant incubation temperatures.

2.5. Calculating proxies for temperatures to estimate sex ratio

Different methods have been proposed to convert time series of temperatures from natural nest into a constant-temperature equivalent (CTE) that can be used into the equation relating sex ratio into constant temperature (see above).

CTE can be estimated based on the recorded temperatures during the middle third of incubation (named thereafter CTE_MT). Given that it is now acknowledged that the TSP does not lie during the middle third of incubation when temperature varies (Valenzuela 2001, Georges et al. 2005, The Chu et al. 2008, Neuwald and Valenzuela 2011, Woolgar et al. 2013, Stubbs et al. 2014, Fuentes et al. 2017, Girondot et al. In press), CTE can also be calculated during the thermosensitive period of development defined using a model of embryo growth (see above) (named thereafter CTE_TSP).

The method used to aggregate time series of temperatures into a CTE can use a simple average temperature during either period of incubation (CTE_MT_A or CTE_TSP_A). However, average temperature is known to be a poor predictor of sex ratio because the effect of temperature on sex determination may be mediated by gonadal growth which also depends on temperature (Georges et al. 2005, Neuwald and Valenzuela 2011). Indeed, embryos that spend equal time at masculinizing and feminizing temperatures would develop into females because more cells proliferated during the feminizing period rather than during the masculinizing one.

A constant temperature equivalent (CTE) was developed to account for such effect by calculating the temperature under which half of the development occurred (Georges 1989, Georges et al. 1994) and has recently been improved to account for a nonlinear development within incubation (Fuentes et al. 2017). Basically, this new CTE (called CTE_{Growth} thereafter) is the mean temperature weighted by the growth of embryo at each time step. The equation of the CTE_{Growth} is given below (eq. 1):

$$CTE_{Growth} = \frac{\sum_{i=k}^{N-1} T_i \times G_i}{\sum_{i=k}^{N-1} G_i} \text{ [Equation 1]}$$

T_i is the temperature at the moment i of incubation with k being the first recorded temperature during the period of interest (here the first temperature recorded during the TSP). G_i is the growth of embryos in term of straight carapace length (SCL) between two records (i.e. the difference between the embryonic size at time $i+1$ and i). N is the last recorded temperature during the period of interest (here the last temperature recorded during TSP).

A recent study pointed out that gonadal growth and sex determination of mice are independent processes (Diaz-Hernandez et al. 2015). Therefore, we can test this hypothesis using the same concept than for the CTE_{Growth} but based rather on the differential sexualization which would arise from the thermal reaction norm for sexualization. The equation that describes this new type of CTE (called CTE_{Sexualization} thereafter) is given below (eq. 2):

$$CTE_{Sexualization} = \frac{\sum_{i=k}^{N-1} T_i \times S_i}{\sum_{i=k}^{N-1} S_i} \text{ [Equation 2]}$$

S_i being the extent of sexualization of embryos between two records (i.e. the difference between the extent of sexualization at time $i+1$ and i) and other parameters being the same as described above.

The same study proposed that in turtles with TSD, female producing temperatures promote differentiation of ovaries “due to faster cell proliferation and higher levels of estrogens than testis developing at male-promoting temperature” (Diaz-Hernandez et al. 2015). Therefore, the sex ratio might depend on a combined effect of both the gonadal growth and the sexualization. To test this hypothesis, we developed a third type of CTE (called CTE_{Sexual+Growth} thereafter) by calculating the average temperature weighted by the differential extent of sexualization and the differential growth. The equation is described as follows (eq. 3):

$$CTE_{Sexual+Growth} = \frac{\sum_{i=k}^{N-1} T_i \times S'_i \times G_i}{\sum_{i=k}^{N-1} S'_i \times G_i} \text{ [Equation 3]}$$

2.6. Inferring the thermal reaction norm for sexualization

The shape of the thermal reaction norm for sexualization (S or S' in equations 2 and 3) is based on the same equation than for the growth rate, i.e. using the Sarpe-DeMichele's model modified by Schoolfield et al. (1981). These reaction norms used 4 parameters and measured the strength of sexualization according to temperature. For each $CTE_{Sexual+Growth}$ or $CTE_{Sexualization}$, the estimated sex ratio for each nest could be calculated using the equation of the thermal reaction norm of sex ratio established previously for this population (see above). We fitted the 4 parameters of these equations that maximized the likelihood of the observed sex ratio within the model.

2.7. Comparing methods to predict sex ratio

Sex ratio estimates were produced using 5 proxies for the 133 nests for which temperature was recorded. The 1st proxy is the mean temperature during the middle third of incubation (CTE_{MT_A}) and the 2nd one is the mean temperature during the TSP (CTE_{TSP_A}). The 3rd proxy is based on the CTE_{Growth} during the TSP (called thereafter CTE_{TSP_Growth}). The 4th proxy is calculated using $CTE_{Sexualization}$ during the TSP based (called thereafter $CTE_{TSP_Sexualization}$) and the 5th proxy is calculated using $CTE_{Sexual+Growth}$ during the TSP (called thereafter $CTE_{TSP_Growth+Sexualization}$).

Subsequently, we calculated the Akaike Information Criterion corrected for finite sample size (AICc) (Burnham and Anderson 2002) for either methods (proxies' values are summarized in supplementary files, Table S2) using nests for which sex ratio was successfully assessed (124 out of 133 nests, see supplementary files, Table S1). Methods that required estimating the thermal reaction norm for sexualization were subjected to 4 extra parameters as compared to others. Models are ranked based on the AICc and the Akaike weight which gives a measure of the relative support of each model (Burnham and Anderson 2002).

3. Results

3.1. Embryonic development and TSP boundaries in natural conditions

We modelled the progression of embryo size in term of straight carapace length (SCL) for 133 natural nests for which temperature was recorded in order to identify the period within incubation where the thermosensitive period (TSP) occurs. An example of the embryo growth model according to nest temperature for a nest picked up randomly in our dataset is presented in Figure 2. The TSP occurred in between embryo SCL of 9.05 mm and 30.06 mm (grey area in front of the left axis) which matches with the period of incubation in between day 16.27 and day 33.38 (grey area in front of the x axis) for this particular nest. We can notice here that the

TSP ends 1.04 days later than the end of the middle third of incubation (the period defined in between vertical dotted lines without accounting for embryonic stages) (Fig. 2).

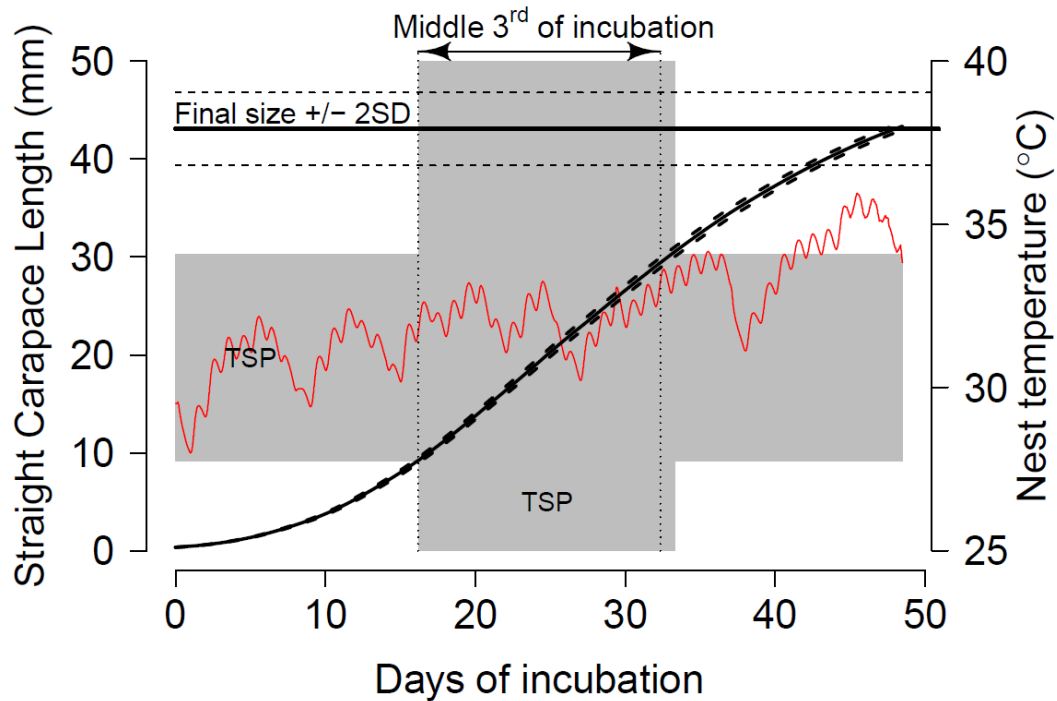


Figure 2: Example of the model of embryonic growth in term of size in mm (black line) and its confidence interval at 95% (black dotted lines). The red line shows traces of incubation temperature. The boundaries of the TSP during development (left y axis) and the matching period during incubation (x axis) are indicated by the grey area. The horizontal black line represents the final straight carapace length (SCL) which is 43.10 mm (Horizontal dotted lines represent $\pm sd = 1.86$ mm). Vertical dotted lines represent the boundaries of the middle third of incubation.

3.2. Thermal reaction norms

The thermal reaction norm for sex ratio was estimated by fitting parameters of the logistic equation according to constant temperature experiments in laboratory conditions. A sex ratio of 0.5 is expected at $P = 28.97^{\circ}\text{C}$ and the slope of the transition between male producing temperatures and female producing temperature, $S = -0.58$. Both sexes can be produced in variables proportions in between 27.26°C and 30.69°C , namely the transitional range of temperatures (TRT) (Fig. 3).

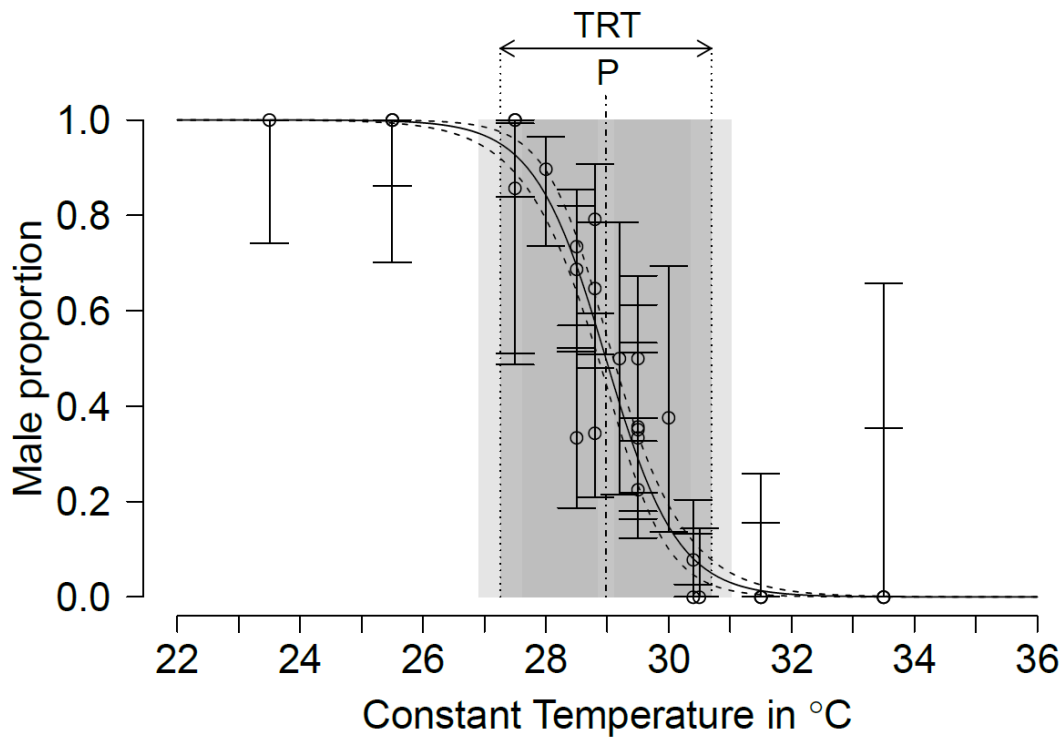


Figure 3: The thermal reaction norm for sex ratio (or also called the pattern of temperature-dependent sex determination) of loggerhead populations nesting in the Atlantic North West (Georgia, Florida, and North Carolina). Relationship between actual sex ratio and constant incubation temperature (black points are the average sex ratio and error bars show the confidence interval of observations) from laboratory experiments (Yntema and Mrosovsky 1980, 1982, Mrosovsky 1988). The Transitional Range of Temperatures (TRT) is indicated by the grey area in between the horizontal dotted lines. The Pivotal temperature (P) is indicated by the horizontal dotted line within the TRT. Shades of grey indicate the confidence intervals of the TRT and P.

The thermal reaction norm for growth rate was estimated by fitting the 4 parameters of the Sharpe-DeMichele equation that best describes final size of a completely developed embryo through a growth model according to temperature time series recorded within nests. The growth rate increases with temperature and reaches its maximum at 35°C with a growth rate at 35°C being 3.125 times higher than the growth rate at 22.5°C (Fig. 4, “Growth”).

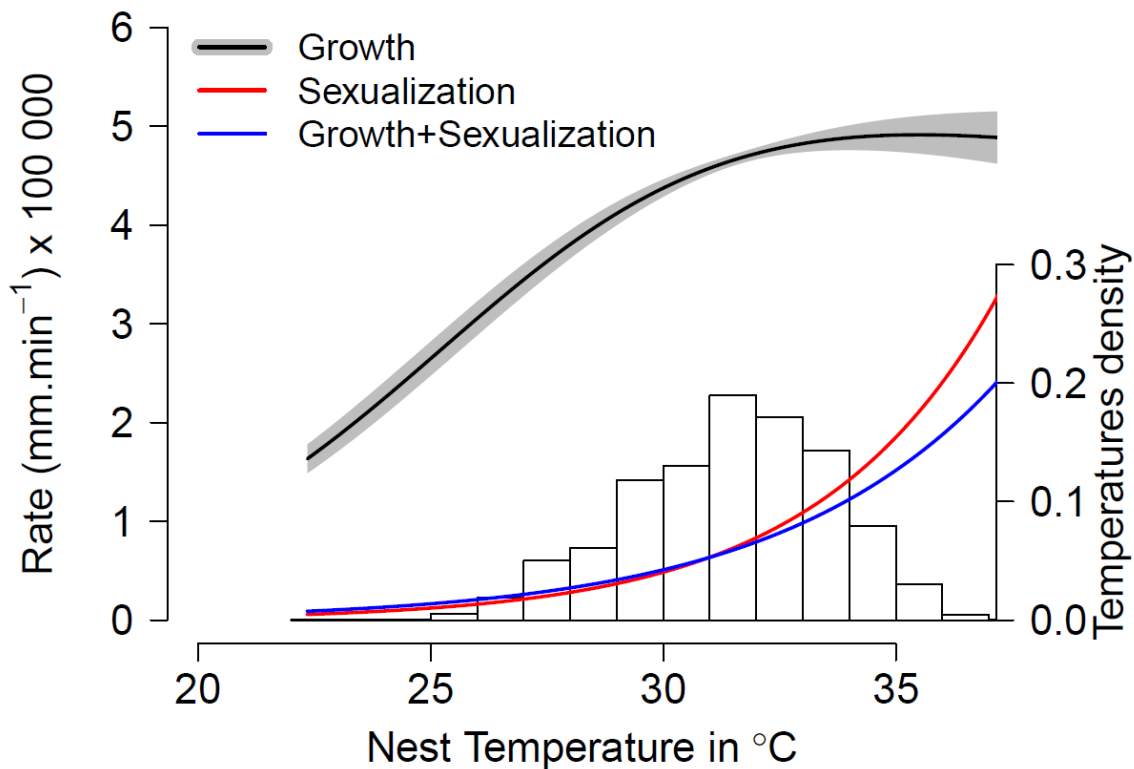


Figure 4: Thermal reaction norm for growth rate (black) and the confidence interval (grey). Thermal reaction norm for sexualization estimated via CTE_TSP_Sexualization (red) or via the CTE_TSP_Growth+Sexualization (blue). The histogram shows the density of temperatures recorded within nests.

The thermal reaction norm for sexualization was estimated using two methods. We estimated the 4 parameters of the Sharpe-DeMichele equation that best describes the observed sex ratio according to temperature time series recorded within nests during the TSP via either the $CTE_{Sexualization}$ (Fig. 4, “Sexualization”: sex ratio depends on sexualization only) or the $CTE_{Sexual+Growth}$ (Fig. 4, “Growth+Sexualization”: sex ratio depends on sexualization and gonadal growth). Both methods yield similar shapes of the thermal reaction norm for sexualization with a monotonic pattern increasing quasi-exponentially between 22.5°C and 37°C.

3.3. Comparison among methods to predict sex ratio

The fit quality of each of the 5 proxies is presented in Figure 5. The best models involve the thermal reaction norm for sexualization inferred via either the $CTE_{Sexual+Growth}$ (Fig. 5, A: AICc = 247.00; Akaike weight = 0.41) or the $CTE_{Sexualization}$ (Fig. 5, B: AICc = 273.31; Akaike weight = 0.58). As expected, the method that produced the worst AICc is the one that uses the mean temperature during the middle third of incubation (Fig. 5, E: AICc = 354.87; Akaike weight < 0.005) followed by the mean temperature during the TSP (Fig. 5, D: AICc = 301.11; Akaike weight < 0.005) and the CTE weighted by embryo growth (Fig. 5, C: AICc = 284.59; Akaike

weight < 0.005). According to this dataset, methods that use the thermal reaction norm for sexualization were able to adequately predict the actual sex ratio in average within the entire range of sex ratio (from 0% of males to 100% of males) whereas other methods tend to overestimate the proportion of males as long as the actual proportion of males increases.

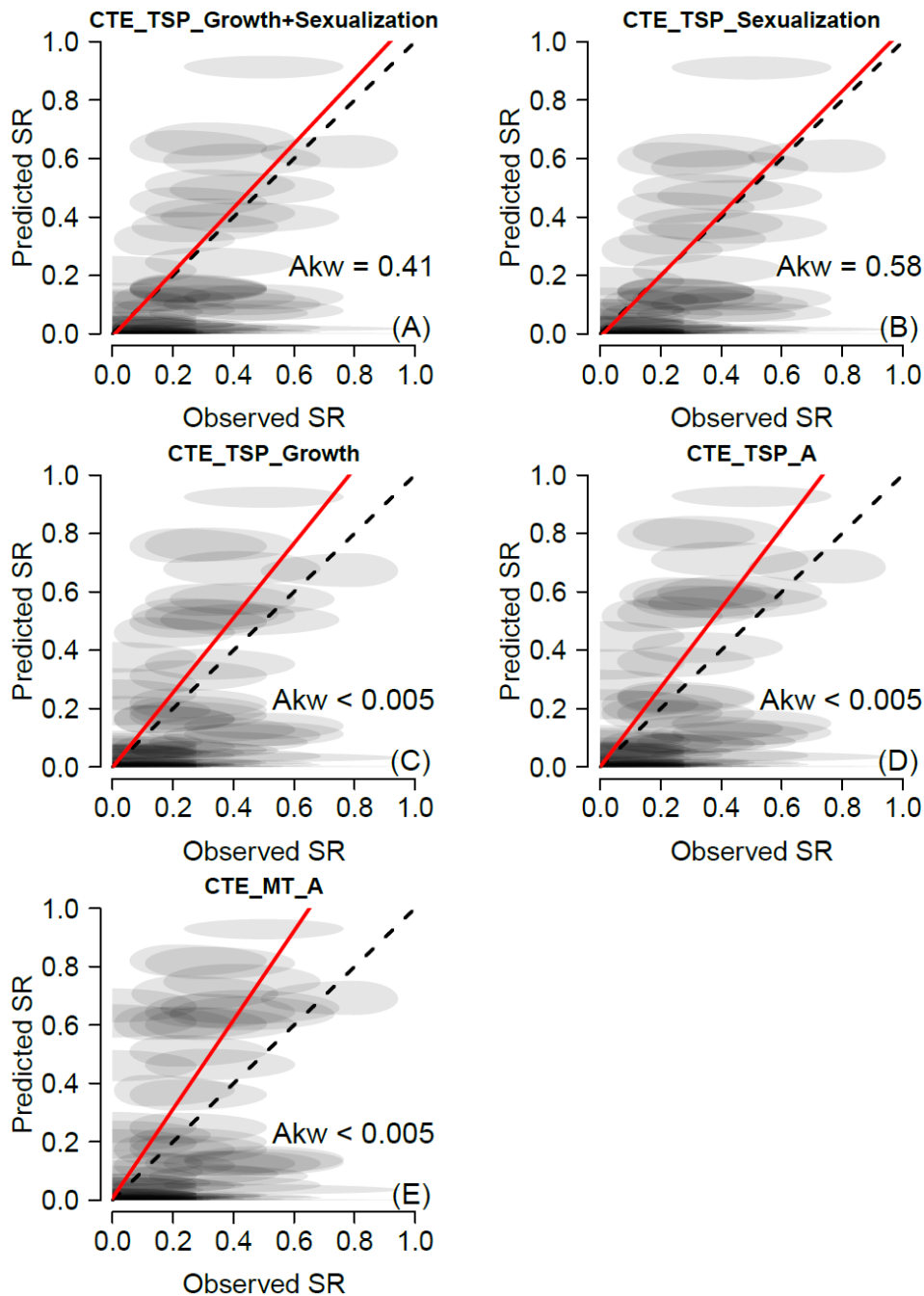


Figure 5: Predicted vs. observed sex ratio (SR) and respective Akaike weights (Akw) for each method. Estimates are based on the thermal reaction norm for sexualization inferred via the $CTE_{Sexual+Growth}$ (A) or the $CTE_{Sexualization}$ (B) during the TSP. Estimates are based on the CTE_{Growth} (C). Estimates are based on the simple mean temperature during the TSP (D) and during the middle third of incubation (E). Lines of equality are indicated by black dotted lines and least rectangles lines are indicated by red lines. Confidence interval of sex ratio observations and upper quantiles (97.5%) and lower quantiles (2.5%) of sex ratio predictions are indicated by grey shaded ellipses.

4. Discussion

Sex ratio is an important demographic parameter that must be investigated to forecasting population dynamics of species with TSD in a changing thermal environment and thus assessing species' vulnerability under current and future climate change constraints (Janzen 1994, Girondot et al. 2004, Boyle et al. 2014). Indirect methods to predict sex ratio of hatchlings based on temperature-based proxies have the advantage to produce large datasets and are particularly convenient when the species of interest lack external sexual characters before adult stage. However, these methods remain questionable (Wyneken and Lolavar 2015). Indeed, our results suggest that previous methods do not provide relevant estimates of sex ratio for the loggerhead sea turtle but also for other reptiles with TSD as similar mechanisms are at play. According to our dataset, previous methods (mean temperature during either the middle third of incubation or the TSP as well as the CTE weighted by embryo growth) tend to overestimate the proportion of males as actual proportion of males increases. This might lead to the erroneous conclusion that a nesting population is resilient when the species is known to usually experience highly female-biased primary sex ratio (sex ratio of hatchlings produced at a nesting beach), such as sea turtles. Our method based on the thermal reaction norm for sexualization overcomes such problem by providing accurate estimates of sex ratio. First, the TSP does not match the middle third of incubation when temperatures varies (Valenzuela 2001, Georges et al. 2004, Georges et al. 2005, The Chu et al. 2008, Neuwald and Valenzuela 2011, Woolgar et al. 2013, Stubbs et al. 2014, Fuentes et al. 2017, Girondot et al. In press) which invalidates the use of the middle third of incubation as an approximation of the actual TSP. The bias introduced by this approximation is expected to be higher as the magnitude of thermal fluctuations increases (Georges et al. 2004) which is the case for species that dig shallow nests (virtually all reptiles except sea turtles). Secondly, we found that mean temperature during the TSP and even the CTE weighted by embryo growth during the TSP, an equivalent of the CTE proposed by Georges et al. (1994), may also yield erroneous sex ratio estimates. This is due to the fact that the effect of temperature on sexual fate is mediated by both gonadal growth and the cellular contents of sex determinants (Girondot et al. 2010, Diaz-Hernandez et al. 2015).

Here we show that sex ratio of hatchlings of the loggerhead sea turtle is appropriately estimated when using the $CTE_{Sexualization}$ (i.e. the average temperature weighted by the differential sexualization within the TSP). The thermal reaction norm for sexualization integrates simultaneously the effect of temperature on all temperature-sensitive elements underlying sex determination: Aromatase activity and aromatase expression (Girondot et al. 2010) through

temperature-dependent expression of at least one master gene (Rhen and Schroeder 2010, Bachtrog et al. 2014) even if potentially mediated by the effect of temperature on DNA methylation (Matsumoto et al. 2013, Venegas et al. 2016). The sexualization for loggerhead populations nesting in Florida is quasi-null at 22.5°C and starts increasing exponentially up to this temperature. This result is consistent with a previous model of aromatase activity based on the effect of temperature on both the enzyme activity and its transcriptional regulation for the European pond turtle (*Emys orbicularis*) (Girondot et al. 2010).

Obviously, all methods are equivalent in predicting highly female-biased (or male-biased) sex ratio when temperature is very high (or very low) but this holds true only for species with one pivotal temperature (TSDIa and TSDIb) and only when incubation temperature fluctuates out of the transitional range of temperature. Importantly, a study shown that sex ratio of a lizard with 2 pivotal temperatures (*Amphibolorus muricatus*) was not related with the CTE (*sensu* Georges et al. 1994) but rather with the magnitude of thermal fluctuations (Warner and Shine 2010). The new CTE described in the present study (CTE_{Sexualization}) integrates the effect of thermal fluctuations on sex determination and thus could be applied to predict sex ratio of species with multiple pivotal temperatures.

Investigating the thermal reaction norm for sexualization among reptiles with TSD will (i) enable providing ecologically relevant estimates of sex ratio of hatchlings in order to properly assess the viability of populations in the context of climate change and more broadly (ii) enhance our understanding of TSD in natural conditions.

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Supplementary files

Table S1: Summary table of incubation and sex ratio data

Nest ID	Season	Beach	Laying	Hatching	Males	Females	Ambiguous
1	2002	BR	15/05/2002	18/07/2002	3	4	0
2	2002	BR	17/05/2002	13/07/2002	2	8	0
3	2002	BR	18/05/2002	17/07/2002	3	7	0
4	2002	BR	10/06/2002	05/08/2002	0	10	0
5	2002	BR	10/06/2002	05/08/2002	0	10	0
6	2002	BR	10/06/2002	04/08/2002	2	8	0
7	2002	BR	12/06/2002	08/08/2002	0	10	0
8	2002	BR	22/07/2002	05/09/2002	0	10	0
9	2002	BR	22/07/2002	06/09/2002	1	9	0
10	2002	BR	22/07/2002	09/09/2002	0	10	0
11	2002	JU	15/05/2002	12/07/2002	1	9	0
12	2002	JU	15/06/2002	11/08/2002	1	9	0
13	2002	JU	15/06/2002	09/08/2002	0	10	0
14	2002	JU	02/08/2002	19/09/2002	0	10	0
15	2002	JU	02/08/2002	19/09/2002	0	10	0
16	2002	HI	16/05/2002	13/07/2002	3	7	0
17	2002	HI	17/05/2002	16/07/2002	2	8	0
18	2002	HI	30/05/2002	23/07/2002	3	7	0
19	2002	HI	17/06/2002	08/08/2002	2	8	0
20	2002	HI	22/06/2002	26/08/2002	2	8	0
21	2002	HI	26/07/2002	12/09/2002	0	10	0
22	2002	HI	01/08/2002	02/10/2002	0	10	0
23	2002	HI	02/08/2002	27/09/2002	0	10	0
24	2002	ME	24/05/2002	19/07/2002	2	8	0
25	2002	ME	11/06/2002	12/08/2002	2	7	0
26	2002	ME	12/06/2002	10/08/2002	1	9	0
27	2002	ME	12/06/2002	08/08/2002	0	10	0

28	2002	SA	10/06/2002	11/08/2002	4	6	0
29	2002	SA	18/06/2002	16/08/2002	3	7	0
30	2002	SA	18/07/2002	12/09/2002	5	5	0
31	2002	SA	24/07/2002	10/09/2002	0	9	0
32	2002	SAR	20/05/2002	12/07/2002	4	6	0
33	2002	SAR	21/05/2002	21/07/2002	5	5	0
34	2002	SAR	23/05/2002	21/07/2002	8	2	0
35	2002	SAR	29/05/2002	26/07/2002	3	7	0
36	2002	SAR	19/06/2002	10/08/2002	1	9	0
37	2002	SAR	20/06/2002	18/08/2002	2	8	0
38	2002	SAR	21/06/2002	18/08/2002	0	10	0
39	2002	SAR	21/06/2002	10/08/2002	1	9	0
40	2002	SAR	20/07/2002	03/09/2002	0	10	0
41	2002	SAR	24/07/2002	12/09/2002	1	9	0
42	2002	SAR	26/07/2002	12/09/2002	1	9	0
43	2002	SAR	27/07/2002	12/09/2003	4	6	0
44	2003	BR	01/07/2003	17/08/2003	0	8	0
45	2003	BR	30/06/2003	17/08/2003	0	10	0
46	2003	BR	30/06/2003	18/08/2003	0	10	0
47	2003	ME	28/06/2003	18/08/2003	0	10	0
48	2003	ME	27/06/2003	22/08/2003	0	10	0
49	2003	ME	18/08/2003	09/10/2003	0	9	0
50	2003	ME	17/08/2003	10/10/2003	3	6	0
51	2003	ME	16/08/2003	11/10/2003	4	5	0
52	2004	BR	16/07/2004	04/09/2004	NA	NA	NA
53	2004	BR	20/08/2004	25/09/2004	NA	NA	NA
54	2004	BR	20/08/2004	29/09/2004	NA	NA	NA
55	2004	HI	25/06/2004	12/08/2004	2	8	0
56	2010	SA	15/06/2010	06/08/2010	2	7	0
57	2010	SA	22/06/2010	08/08/2010	0	10	0
58	2010	SA	28/06/2010	16/08/2010	0	10	0
59	2010	BR	16/06/2010	02/08/2010	0	9	1
60	2010	BR	28/06/2010	15/08/2010	0	10	0
61	2011	BR	16/05/2011	04/07/2011	0	10	0
62	2011	BR	18/05/2011	06/07/2011	0	10	0
63	2011	BR	21/05/2011	10/07/2011	0	33	0
64	2011	BR	21/06/2011	12/08/2011	NA	NA	NA
65	2011	BR	25/06/2011	14/08/2011	0	28	0
66	2011	BR	26/06/2011	17/08/2011	0	8	0
67	2011	SA	24/05/2011	20/07/2011	0	8	0
68	2011	SA	03/06/2011	27/07/2011	0	8	0
69	2011	SA	13/06/2011	31/07/2011	0	9	0
70	2011	SA	24/06/2011	15/08/2011	0	9	0
71	2011	SA	23/06/2011	15/08/2011	0	9	0
72	2012	BR	31/07/2012	21/09/2012	0	9	0
73	2013	BR	11/07/2013	01/09/2013	0	4	0
74	2013	BR	25/07/2013	12/09/2013	0	10	0
75	2013	BR	07/08/2013	24/09/2013	NA	NA	NA

76	2014	BR	06/05/2014	28/06/2014	4	6	0
77	2014	BR	10/05/2014	01/07/2014	0	10	0
78	2014	BR	16/05/2014	08/07/2014	NA	NA	NA
79	2014	BR	20/05/2014	09/07/2014	0	5	0
80	2014	BR	14/06/2014	07/08/2014	0	1	0
81	2014	BR	18/06/2014	06/08/2014	0	7	0
82	2014	BR	09/07/2014	27/08/2014	1	8	0
83	2014	BR	02/08/2014	20/09/2014	0	9	0
84	2014	BR	09/08/2014	27/09/2014	0	7	0
85	2015	BR	18/05/2015	07/07/2015	0	5	0
86	2015	BR	05/06/2015	22/07/2015	0	5	0
87	2015	BR	12/06/2015	01/08/2015	0	9	0
88	2015	BR	30/06/2015	18/08/2015	0	6	0
89	2015	BR	06/07/2015	23/08/2015	0	7	0
90	2015	BR	18/07/2015	04/09/2015	0	9	0
91	2015	BR	28/07/2015	16/09/2015	0	9	0
92	2016	BR	09/05/2016	27/06/2016	0	10	0
93	2016	BR	09/06/2016	29/07/2016	0	10	0
94	2016	BR	19/06/2016	05/08/2016	0	10	0
95	2016	BR	02/07/2016	17/08/2016	0	10	0
96	2016	BR	08/07/2016	26/08/2016	NA	NA	NA
97	2016	BR	10/07/2016	26/08/2016	0	10	0
98	2016	BR	15/07/2016	01/09/2016	0	8	0
99	2016	BR	21/07/2016	10/09/2016	0	10	0
100	2016	BR	01/08/2016	18/09/2016	0	1	0
101	2016	BR	01/08/2016	18/09/2016	0	10	0
102	2016	BR	06/08/2016	23/09/2016	0	7	0
103	2016	BR*	21/05/2016	09/07/2016	0	4	0
104	2016	BR*	21/05/2016	11/07/2016	0	10	0
105	2016	BR*	24/05/2016	10/07/2016	0	10	0
106	2016	BR*	24/05/2016	13/07/2016	NA	NA	NA
107	2016	BR*	28/05/2016	14/07/2016	0	9	0
108	2016	BR*	28/05/2016	14/07/2016	0	9	0
109	2016	BR*	04/06/2016	27/07/2016	0	6	0
110	2016	BR*	04/06/2016	28/07/2016	0	4	0
111	2016	BR*	09/06/2016	27/07/2016	0	9	0
112	2016	BR*	09/06/2016	30/07/2016	0	10	0
113	2016	BR*	26/06/2016	15/08/2016	0	8	0
114	2016	BR*	26/06/2016	15/08/2016	NA	NA	NA
115	2016	BR*	14/07/2016	31/08/2016	0	3	0
116	2016	BR*	14/07/2016	31/08/2016	0	10	0
117	2016	BR*	15/07/2016	02/09/2016	0	2	0
118	2016	BR*	15/07/2016	02/09/2016	0	4	0
119	2016	BR*	15/07/2016	02/09/2016	0	8	0
120	2016	BR*	15/07/2016	02/09/2016	0	10	0
121	2016	BR*	16/07/2016	04/09/2016	0	3	0
122	2016	BR*	16/07/2016	04/09/2016	0	10	0
123	2016	BR*	17/07/2016	05/09/2016	0	8	0

124	2016	BR*	17/07/2016	03/09/2016	0	10	0
125	2016	BR*	18/07/2016	11/09/2016	0	10	0
126	2016	BR*	18/07/2016	07/09/2016	0	2	0
127	2016	BR*	18/07/2016	06/09/2016	0	10	0
128	2016	BR*	18/07/2016	06/09/2016	0	10	0
129	2016	BR*	20/07/2016	11/09/2016	0	8	0
130	2016	BR*	20/07/2016	07/09/2016	0	10	0
131	2016	BR*	19/08/2016	05/10/2016	0	10	0
132	2016	BR*	19/08/2016	10/10/2016	0	10	0
133	2016	BR*	16/08/2016	05/10/2016	5	5	0

Table S1: Summary table of laying dates, hatching dates and the number of males and females in clutches for which incubation temperature was recorded. Data are from six beaches in Florida: Boca Raton (BR), Juno Beach (JU), Hutchinson Island (HI), Melbourne (MEL), Sanibel (SA), and Sarasota (SAR). *Clutches which were split for another experimental design not presented here.

Table S2: Summary table of proxies' values

Nest ID	CTE_MT_A	CTE_TSP_A	CTE_TSP_Growth	CTE_TSP_Sexualization	CTE_TSP_Growth+Sexualization
1	28.59	28.84	28.97	29.31	29.22
2	29.62	29.64	29.72	30.02	29.96
3	28.74	28.83	28.93	29.27	29.19
4	29.07	29.51	29.60	30.37	30.20
5	28.71	29.31	29.48	30.36	30.16
6	28.73	29.30	29.42	30.12	29.96
7	29.61	30.22	30.39	31.54	31.31
8	31.92	32.03	32.06	32.27	32.22
9	31.61	31.68	31.68	31.76	31.75
10	31.63	31.67	31.68	31.76	31.75
11	29.88	29.85	29.93	30.22	30.16
12	29.27	29.73	29.91	30.50	30.37
13	29.80	30.28	30.46	31.17	31.02
14	32.03	32.07	32.08	32.19	32.17
15	31.97	31.95	31.95	32.05	32.04
16	30.10	30.08	30.10	30.13	30.12
17	28.95	28.91	28.99	29.14	29.10
18	28.63	28.77	28.81	28.99	28.95
19	29.88	30.40	30.59	31.33	31.18
20	29.78	29.85	29.87	30.01	29.98
21	31.16	31.34	31.41	31.66	31.61
22	30.81	30.85	30.85	30.89	30.88
23	30.68	30.69	30.69	30.73	30.72
24	28.09	28.19	28.32	28.75	28.65
25	28.46	28.76	28.92	29.40	29.28
26	28.73	28.93	29.06	29.50	29.40
27	28.58	29.09	29.25	29.84	29.70
28	28.34	28.45	28.53	28.79	28.73
29	29.06	29.17	29.31	29.69	29.60
30	30.08	30.14	30.17	30.47	30.41

31	31.77	31.89	31.89	32.07	32.04
32	30.10	30.18	30.20	30.25	30.24
33	27.46	27.48	27.50	27.61	27.58
34	28.51	28.53	28.56	28.73	28.69
35	28.13	28.17	28.31	28.68	28.58
36	30.14	30.38	30.47	31.13	31.00
37	29.31	29.65	29.79	30.00	29.95
38	31.21	31.44	31.59	32.50	32.33
39	31.40	31.55	31.65	32.41	32.27
40	31.46	31.66	31.67	31.79	31.76
41	30.70	30.84	30.85	31.06	31.01
42	31.78	31.90	31.94	32.37	32.28
43	31.78	31.76	31.81	31.99	31.95
44	32.71	32.88	32.87	33.06	33.02
45	33.00	33.06	33.05	33.21	33.18
46	33.28	33.31	33.30	33.47	33.44
47	30.58	30.63	30.65	30.83	30.79
48	30.70	30.72	30.73	30.91	30.87
49	30.27	30.34	30.34	30.46	30.44
50	30.38	30.34	30.36	30.51	30.47
51	28.65	28.74	28.80	29.03	28.98
52	30.24	30.47	30.54	30.97	30.88
53	31.60	31.09	31.24	32.39	32.19
54	30.07	29.85	30.02	32.22	31.75
55	31.33	31.45	31.45	31.54	31.52
56	30.32	30.41	30.61	31.36	31.21
57	31.91	32.16	32.20	32.39	32.35
58	31.86	32.08	32.11	32.52	32.43
59	32.15	32.22	32.26	32.66	32.59
60	33.06	33.13	33.11	33.28	33.25
61	31.19	31.43	31.44	31.60	31.56
62	32.20	32.27	32.27	32.42	32.39
63	32.09	32.12	32.12	32.31	32.28
64	33.84	33.56	33.61	34.17	34.07
65	33.80	33.82	33.82	34.09	34.04
66	33.39	33.38	33.39	33.64	33.60
67	30.59	30.66	30.73	30.90	30.85
68	29.70	29.72	29.80	30.16	30.08
69	30.10	30.58	30.71	32.04	31.75
70	32.06	32.07	32.10	32.25	32.22
71	31.68	31.66	31.70	31.90	31.86
72	30.89	31.03	31.16	31.71	31.61
73	32.56	32.60	32.59	32.95	32.88
74	33.17	33.15	33.17	33.34	33.31
75	32.25	32.29	32.30	32.64	32.58
76	30.67	30.85	30.88	31.32	31.23
77	31.33	31.30	31.34	31.62	31.56
78	30.46	30.48	30.58	30.85	30.78

79	30.68	30.64	30.68	30.99	30.92
80	30.90	30.92	30.97	31.43	31.34
81	31.41	31.67	31.73	32.28	32.17
82	32.14	32.21	32.21	32.33	32.31
83	34.15	34.09	34.10	34.21	34.19
84	32.46	32.52	32.53	32.75	32.71
85	32.00	32.05	32.07	32.83	32.67
86	33.94	34.05	34.04	34.26	34.23
87	33.97	33.93	33.95	34.30	34.24
88	31.71	31.71	31.78	32.11	32.04
89	31.75	31.95	31.95	32.56	32.45
90	33.05	33.13	33.17	33.40	33.36
91	34.24	34.21	34.24	34.41	34.37
92	31.61	31.69	31.77	32.06	32.00
93	34.11	34.13	34.10	34.40	34.35
94	33.35	33.40	33.42	33.47	33.46
95	33.76	33.85	33.85	34.11	34.06
96	33.41	33.39	33.41	33.46	33.45
97	32.77	32.72	32.74	32.79	32.78
98	32.99	33.03	33.02	33.09	33.08
99	33.21	33.15	33.12	33.34	33.30
100	32.85	32.80	32.84	32.93	32.91
101	33.37	33.32	33.37	33.54	33.51
102	31.95	31.93	31.96	32.35	32.28
103	31.71	31.85	31.90	32.32	32.24
104	31.14	31.21	31.26	31.57	31.51
105	31.69	32.01	32.09	32.90	32.73
106	31.36	31.42	31.49	31.99	31.89
107	32.36	32.59	32.62	33.21	33.10
108	31.52	31.70	31.72	32.13	32.06
109	33.35	33.14	33.18	33.40	33.35
110	31.39	31.22	31.25	31.37	31.34
111	33.49	33.57	33.55	33.76	33.72
112	32.38	32.40	32.38	32.58	32.55
113	33.93	33.94	33.94	33.99	33.98
114	32.92	32.93	32.94	33.07	33.04
115	32.90	32.93	32.94	33.09	33.06
116	31.99	32.03	32.03	32.25	32.21
117	32.57	32.67	32.67	32.87	32.83
118	31.45	31.58	31.58	31.88	31.83
119	33.06	33.12	33.12	33.24	33.22
120	31.78	31.87	31.86	32.05	32.02
121	32.22	32.31	32.30	32.46	32.43
122	31.21	31.31	31.31	31.52	31.48
123	32.64	32.70	32.69	32.88	32.85
124	31.89	31.96	31.95	32.11	32.08
125	31.26	31.14	31.15	31.45	31.40
126	31.51	31.42	31.42	31.75	31.69

127	33.22	33.21	33.20	33.31	33.29
128	31.85	31.83	31.82	32.09	32.04
129	32.85	32.76	32.73	33.15	33.08
130	31.61	31.65	31.65	31.97	31.91
131	31.84	31.95	31.96	32.16	32.12
132	30.39	30.50	30.53	30.63	30.60
133	30.03	29.99	30.03	30.13	30.10

Table S2: Summary table of proxies' value used to predict sex ratio using different methods: simple mean temperature during the middle third of incubation (CTE_MT_A) and during the thermosensitive period for sex determination (CTE_TSP_A). Proxies for sex ratio can also be calculated by the temperature weighted by the differential growth (CTE_TSP_Growth), or weighted by the differential sexualization (CTE_TSP_Sexualization) or weighted by both (CTE_TSP_Growth+Sexualization) during the thermosensitive period for sex determination.

Encadré 1 : Résultats clés présentés dans ce manuscrit

1. Les populations de tortue Caouanne (*Caretta caretta*) nidifiant en Turquie et en Libye présentent des normes de réaction thermiques du taux de croissance similaires tandis que l'environnement thermique d'incubation des œufs est différent.
2. Les différents estimateurs utilisés jusqu'à présent pour prédire le sex ratio d'une ponte en milieu naturel produisent des résultats différents.
3. Un avancement de la saison de ponte ne permettra pas à toutes les populations de tortue Caouanne de contrecarrer un effet potentiellement néfaste du réchauffement du climat sur le développement des embryons.
4. Chez une population de tortue Caouanne nidifiant au Brésil, la date du début de la saison de ponte a lieu plus tôt lorsque la température augmente au niveau de deux sites de nourrissage et la durée de la saison de ponte diminue lorsque la production primaire nette augmente au niveau de deux autres sites de nourrissage.
5. La norme de réaction thermique de la sexualisation permet de prédire le sex ratio d'une ponte en milieu naturel avec une grande précision tandis que les autres méthodes utilisées jusqu'à présent tendent à surestimer la proportion réelle de mâle.

Encadré 2 : Détection d'un signal phylogénétique des normes de réaction thermiques du taux de croissance embryonnaire

J'ai tenté d'élargir la comparaison des normes de réaction thermiques (Géorgie, Floride, Brésil, Afrique du Sud) pour détecter d'éventuelles contraintes évolutives en testant la présence d'un signal phylogénétique. Pour ce faire, j'ai récupéré les distances génétiques (F_{st}) entre les différentes populations de tortue Caouanne obtenues grâce à une étude phylogéographique (Shamblin et al. 2014). J'ai ensuite réalisé un dendrogramme des distances génétiques entre les populations pour lesquelles je dispose d'une information sur les normes de réaction thermiques du taux de croissance embryonnaire. J'ai d'abord exclu les distances génétiques non significatives (Chypre) et calculé les distances cophénétiques basées sur le dendrogramme regroupant l'ensemble des populations étudiées dans l'étude originale. Les normes de réaction thermiques étant des caractères continus, j'ai calculé la matrice des distances selon la méthode d'analyse Procrustéenne généralisée (Gower 1975). J'ai ensuite calculé le signal phylogénétique (K) en suivant la procédure de Blomberg (Blomberg et al. 2003). Cette méthode permet de tester si un caractère est distribué sur un arbre phylogénétique selon un mouvement purement Brownien ($K = 1$) ou si deux taxons évolutivement proches ont plus tendance à présenter des similitudes (K proche de 0) en permutant aléatoirement les états de caractères (ici 100000 permutations). J'ai obtenu un signal phylogénétique de 0.41 ce qui laisserait à penser que ces normes de réaction thermiques sont, en partie, évolutivement contraintes. Cependant, le test n'étant pas significatif ($p = 0.23$), des données provenant d'autres populations sont nécessaires pour conclure quant aux contraintes évolutives imposées sur ce caractère.

Discussion

Estimer la vulnérabilité des reptiles ovipares sous contrainte du changement du climat reste un défi majeur à l'heure actuelle. Les tortues marines sont des espèces migratrices dont l'aire de répartition s'étend à l'échelle mondiale. Ces espèces présentent un degré important de fidélité aux sites de ponte ce qui pourrait contraindre leur capacité à suivre l'enveloppe climatique favorable à l'incubation des œufs. Néanmoins, la phénologie des pontes semble répondre aux variations de température, ce qui offrirait une stratégie efficace pour atténuer l'effet potentiellement néfaste du réchauffement du climat sur le développement des embryons. Dans ce manuscrit, j'ai montré qu'une telle plasticité dans le comportement de nidification ne permettra pas à toutes les populations de tortue Caouanne (*Caretta caretta*) de contrecarrer l'effet du réchauffement du climat sur le sex ratio et la réussite d'incubation (Encadré 1). Cependant, notre capacité à estimer le potentiel adaptatif des tortues marines reste améliorable. Notamment, nous ne savons pas dans quelles mesures les normes de réaction thermiques du taux de croissance embryonnaire peuvent répondre à la sélection naturelle. J'ai montré que deux populations de tortue Caouanne nidifiant en Turquie et en Libye présentent des normes de réaction thermiques similaires tandis que l'environnement thermique d'incubation des œufs est différent (Encadré 1). Cela suggère que ces normes de réaction sont évolutivement contraintes mais une analyse plus détaillée est requise. Concernant la phénologie des pontes, j'ai montré que la date de début de la saison dépend de la température tandis que la durée de la saison dépend de la disponibilité en ressources chez une population de tortue Caouanne nidifiant au Brésil (Encadré 1). Ce paramètre doit désormais être pris en compte pour prédire la période pendant laquelle l'incubation des œufs a lieu et, *in fine*, prédire le sex ratio primaire. J'ai aussi montré que les différents estimateurs du sex ratio produisent des résultats différents et qu'il est nécessaire d'estimer la norme de réaction de la sexualisation pour correctement prédire le sex ratio des pontes en milieu naturel (Encadré 1). Cette nouvelle méthode doit désormais être appliquée pour prédire le sex ratio des nouveaux nés chez les espèces à détermination du sexe sensible à la température.

J'ai montré que les populations de tortue Caouanne (*Caretta caretta*) nidifiant en Turquie et en Libye présentent des normes de réaction thermiques similaires pour le taux de croissance embryonnaire tandis que les conditions thermiques d'incubation sont différentes (Encadré 1). Une limite possible à ce résultat est que les conditions thermiques ne sont peut-être pas si différentes à l'échelle de plusieurs générations. Il est cependant difficile d'obtenir cette information chez les tortues marines puisque ce sont des organismes à temps de génération

long. Ceci complique aussi l'estimation de l'héritabilité de ce caractère. J'ai tenté de détecter un éventuel signal phylogénétique des normes de réaction thermique du taux de croissance embryonnaire afin de comprendre les contraintes évolutives sous-jacentes (Encadré 2). Cependant l'analyse n'est pas concluante et nécessite d'intégrer des données provenant d'autres populations. Il est aussi important de noter que les normes de réaction thermiques estimées dans ce manuscrit sont une moyenne à l'échelle d'une population. Nous n'avons donc pas d'information quant à la variabilité de ces normes de réaction à l'échelle des individus, ce qui contraint notre capacité à évaluer dans quelles mesures ce caractère peut répondre à la sélection.

Le sex ratio primaire est un paramètre démographique important pour comprendre la dynamique des populations de tortues marines (Girondot et al. 2004) et ainsi estimer leur vulnérabilité face au changement du climat (Fuentes et al. 2011, Fuentes et al. 2013, Hamann et al. 2013). J'ai montré que les différents estimateurs du sex ratio d'une ponte en milieu naturel produisent des résultats différents qui tendent à surestimer la proportion réelle de mâles (Encadré 1). La nouvelle méthode basée sur la norme de réaction de la sexualisation se révèle efficace pour obtenir des estimations précises du sex ratio en milieu naturel. Par conséquent, cette méthode doit désormais remplacer les précédentes afin de pouvoir discuter et comparer le sex ratio primaire chez les tortues marines. Il est regrettable que de récentes études proposent des mesures de conservation (dei Marcovaldi et al. 2014, Laloë et al. 2014, dei Marcovaldi et al. 2016, Esteban et al. 2016, Laloë et al. 2016) ou réalisent des méta-analyses (Hays et al. 2017) basées sur des estimations fausses et non comparables.

Il a été proposé que la plasticité phénotypique dans la phénologie des pontes soit la stratégie la plus efficace chez les tortues marines pour atténuer ou éviter les effets néfastes liés à un réchauffement du climat (Horne et al. 2014, Butt et al. 2016). J'ai montré que ce type de plasticité ne permettra pas à toutes les populations de contrecarrer les augmentations de température prédite d'ici 2100 au niveau des sites de pontes (Encadré 1). Ce résultat souligne l'importance de considérer les traits d'histoire de vie dépendant de la température spécifiquement pour chaque population afin de correctement identifier les sites de ponte potentiellement vulnérables. J'ai montré que la plasticité dans la phénologie des pontes semble dépendre d'un autre paramètre écologique important, la disponibilité en ressources (Encadré 1). Cette étude mérite d'être réalisée chez d'autres populations pour améliorer notre compréhension des paramètres biotiques et abiotiques qui contrôlent la phénologie des pontes des tortues marines.

Chez les tortues marines, la survie des embryons est un des facteurs principaux contraignant la persistance d'un site de ponte dans un contexte de réchauffement climatique (Poloczanska et al. 2009, Pike 2013, 2014). La survie des embryons dépend en partie de la température d'incubation (Howard et al. 2014) notamment médiée par la disponibilité en oxygène (Ackerman 1980). Jusqu'à présent, les études ayant exploré l'effet de la température sur la survie des embryons en milieu naturel n'ont considéré que la température moyenne pendant l'incubation (Santidrian Tomillo et al. 2012, Horne et al. 2014, Santidrian Tomillo et al. 2015). Cependant la température moyenne est un mauvais indicateur de la survie des embryons puisque cette mesure n'intègre pas l'effet des variations de température sur la réponse des embryons à un stress thermique. Une autre perspective d'amélioration serait donc de développer un modèle mécaniste prenant en compte l'effet de l'amplitude des fluctuations de température sur la survie des embryons. Deux études récentes permettront d'apporter quelques pistes quant aux processus moléculaires à l'œuvre au niveau cellulaire lorsque des embryons sont soumis à un stress thermique (Tedeschi et al. 2015) ainsi qu'au potentiel adaptatif du seuil critique de tolérance thermique (Tedeschi et al. 2016). Il est important de noter que la survie des embryons nécessite aussi de prendre en compte l'effet des précipitations (Santidrian Tomillo et al. 2015) qui peuvent, selon les caractéristiques physiques du substrat d'incubation, compromettre la disponibilité en oxygène (Ackerman et al. 1985).

Diagnostiquer la vulnérabilité d'un site de ponte face au changement du climat nécessite aussi de prendre en compte l'élévation du niveau de la mer, réduisant directement la surface disponible pour pondre, et la fréquence des événements météorologiques extrêmes, détruisant directement les nids (Fuentes et al. 2011, Fuentes et al. 2013, Hamann et al. 2013). Ce n'est qu'en intégrant des données génétiques, physiologiques, démographiques, écologiques et climatiques que nous serons en mesure d'évaluer la vulnérabilité des tortues marines face à l'évolution actuel du climat pour finalement établir des mesures de conservation adaptées.

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ANNEXE 1 : Modelling the sex ratio of natural clutches of the European Pond Turtle (*Emys orbicularis*) from air temperature

Modelling the sex ratio of natural clutches of the European Pond Turtle (*Emys orbicularis*) from air temperature

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Abstract: Temperature-dependent sex determination, or TSD, is a kind of sex determination in which gonadal sex is dependent on the incubation temperature of the eggs. Within reptiles, TSD occurs in many turtles, all crocodylians, the tuataras and some lizards. The European pond turtle, *Emys orbicularis*, has been the most studied species for TSD, and the biochemistry of TSD is well-understood in this species. However, predicting the sex ratio when incubation temperature is not constant remains challenging. Here we propose a new methodology to predict the sex ratio for natural nests, and we apply this methodology to eggs of *E. orbicularis* incubated under natural conditions. Finally, we give recommendations on how to apply this method to other natural populations or species with TSD.

Keywords: Air temperature, Soil temperature, Nest temperature, Thermosensitive period of sex determination, embryonic development, Temperature-dependent sex determination

Introduction

Two sex determination mechanisms are found in reptiles: genotypic sex determination (GSD) where the sex is predetermined during egg fertilization by the genotype, and temperature-dependent sex determination (TSD) where the temperature of the embryo during incubation defines the sex (Pieau 1996). In reptiles, TSD was first demonstrated for the lizard *Agama agama* (Charnier 1966), and a few years later for the turtles *Testudo graeca* and *Emys orbicularis* (Pieau 1971, 1972). The latter has been the model species for the study of TSD by Claude Pieau for 35 years and is so far the most studied reptile for TSD (Pieau and Dorizzi 2004). However, only individuals from the center of France have been studied for TSD, though the species ranges from Portugal (Sillero et al. 2014) to Kazakhstan (Mazanaeva and Orlova 2004).

Animals with temperature dependent sexual determination are susceptible to experience a sex ratio bias depending on incubation conditions. The sex ratio bias could be still enhanced due to climate change. Sex ratio bias can have profound consequences in term of population dynamics and then for conservation because it can promote failure in fertilization rate if one

sex is depleted, over-exploitation of resources if males and females do not use the same resources in the environment, and loss of genetic variation impacting the viability of populations due to lower effective size of population. This lowering of genetic variation could jeopardize the ability of turtle populations to adapt to climate change and then drive the population to an extinction vortex.

The European Pond Turtle, *E. orbicularis*, shows the Males-Females (MF) pattern of TSD typical for many turtles: incubation temperatures lower than 27°C are masculinizing, while temperatures higher than 30°C are feminizing (Girondot 1999). The range of temperatures (28.51±1.15°C for *E. orbicularis*; Girondot 1999) producing both sexes is the transitional range of temperatures (TRT); the temperature producing both sexes in equal proportion is the pivotal temperature (PT) (Mrosovsky and Pieau 1991). Usually, TRT and PT are identified for incubations at constant temperatures. In nature, where the temperature often changes during development, these statistics do not predict the sex ratio correctly (reviewed in Hulin et al. 2008).

However, the embryo temperature is important for the sex determination only during the thermosensitive period (TSP), or the period of the development during which temperature exerts its influence on the sexual phenotype of the gonad (Mrosovsky and Pieau 1991). The TSP in *E. orbicularis* is between stages 16 (when the gonads appear) and 21, when embryos have mass 160–180 mg and 650–750 mg, respectively (Pieau and Dorizzi 1981). When incubation temperature is constant, this range of masses is observed during the middle third of incubation. However, this correspondence is not true when temperature changes during incubation according to daily fluctuations and seasonal trends. In natural nests, embryos experience a fluctuating regime of temperatures. TSD has been mostly studied under laboratory conditions at constant incubation temperatures, and consequently the effects of fluctuating temperatures during incubation are understood much less. Thus in order to correctly predict the timing of temperature effect for sex determination during incubation, it is necessary to stage the embryo. Staging the embryo requires to open the egg which cannot be done for threatened species. An alternative is to model the embryo development to determine the boundaries of the TSP during incubation and then predict with better accuracy the sex ratio.

In this paper we present the successive steps for predicting sex ratio under natural conditions using the European Pond Turtle, *E. orbicularis*, as a target species but the methodology can be applied to any species with TSD. We start with obtaining air or soil temperatures recorded

by meteorological stations in the vicinity of nesting sites, and we describe how to predict nest temperatures. Then, we show how nest temperatures may be used for determining the TSP, and how the TSP duration or temperatures, in turn, can be used to estimate nest sex ratio.

Step 1: Obtaining air and soil temperatures

Besides planting automated dataloggers (e.g. iButtons, Hobo...) in the field, often data from meteorological stations are (freely) available and can provide a convenient starting point. Guidelines for meteorological stations deployments are used worldwide to ensure that comparable measurements are obtained (World Meteorological Organization 2008). Measurements can represent highest, lowest, and mean air temperatures, and ground temperature or soil temperatures at various depths. General recommendation for meteorological stations indicate that measurement equipment must be located in the middle of a flat area of 25 m × 25 m without any trees or building in proximity that could cast shade. The ground should be covered only by short grass or the local typical soil cover. The observed air temperature should be representative of the free air conditions surrounding the station. Temperature must be recorded at a height of 1.2–2.0 m above ground level. To ensure that the thermometer is at true air temperature, protect it from radiation by a screen. For all measurements, each thermometer must be compared with a reference thermometer, or thermometers of the same type can be compared with each other. This calibration should also be done when data loggers are used during fieldwork to ensure that differences between sites do not reflect differences between thermometer calibrations. If data are collected using personal dataloggers, similar considerations should be taken into account to have reproducible and comparable temperature measurements.

The daily air temperature typically shows an irregular sinusoidal pattern with a 24-hours period (the pattern is irregular because the time for maximum is not at the exact intermediate between the two times for the minima). For a particular day this pattern can be degraded by local meteorological events, but here we only discuss the average pattern, which is representative of the local climate. To illustrate the pattern of daily temperatures, we used air temperature recorded by the laboratory Ecology, Systématique, Evolution each 30 minutes from 2006 to 2014 in Barbeau, ~32 km south-east of Paris in France (N43°03'07.801'' E22°56'34.148''). Time was converted to Universal Time Coordinated (UTC) to prevent hour shift between summer- and wintertime. The time of sunrise and sunset were obtained for each day at this location based on classical formula (Teets 2003). It is important to use the local time for sunrise or sunset because time can be different by more than one hour as compared to

sun time between the two extreme longitudes of a time zone. For each day, the UTC times of the daily minimum and maximum temperatures were calculated based on the time series of temperatures. The mode for the minimum and maximum temperature time has been estimated according to UTC time of sunrise grouped by 30 minutes using the method of Asselin de Beauville (1978). Minimum temperature is approximately 30 minutes after sunrise. Maximum temperature is generally between 13:00 and 15:00 h depending on the latitude and the time of sunrise, thus the season (Fig. 1). There is little chance that the true minimum and maximum are exactly represented in time series when temperatures are recorded every couple of hours, so the true daily minimum and maximum must be estimated using fit of irregular sinusoidal pattern (function `minmax.periodic()` in the `HelpersMG` R package; see Girondot 2016b).

The standard depths for soil temperature measurements in meteorological station are 5, 10, 20, 50 and 100 cm below the surface. As a standard procedure the soil type, soil cover and the degree and direction of the ground's slope should be recorded. Whenever possible, the physical soil constants, such as bulk density, thermal conductivity, the level of the water table (if within 5 m of the surface) and the moisture content at field capacity should also be indicated. The soil temperature follows a similar daily pattern as the air temperature, but with some time shift depending on depth, amplitude change, and soil heat conductivity. Soil heat conductivity is itself dependent on the granular constitution of the soil, its composition and its humidity (Abu-Hamdeh and Reederb 2000). The physics of heat transfer into heterogeneous media such as soil is complicated and correlative studies are often the only way to estimate heat conductivity (Farouki 1981).

Step 2: From air or soil temperatures to nest temperatures

Like soil temperatures, temperature within the nest depends on the air temperature, sun irradiation but also soil heat conductivity. At the depth of a typical nest of *E. orbicularis* (around 10 cm), the time shift with air temperature is very small, around 20 minutes (Guyot 2013) and the nest temperature is correlated with the soil temperature but their averages and amplitudes can be different. As an example, temperatures have been recorded in nine *E. orbicularis* nests in Brenne, 200 km south of Paris, in July and August 2009 every 3 hours; corresponding temperatures for air at 2 m and soil between 7 to 26 cm have been obtained from ECMWF project every 6 hours at a nearby station (Fig. 2).

Two methods can be used to predict the nest temperature from the soil or air temperatures obtained by meteorological stations. A physical model of heat transfer can be defined

(Farouki 1981), but many physical constants must be determined and such data are often not available. A more practical solution is to calibrate a model relating nest temperature obtained locally with data loggers to air or soil temperatures obtained from meteorological stations or from data loggers close to the nesting area. Rather than fitting average temperature and amplitude, we find it more suitable to fit minimum and maximum daily temperatures separately. A generalized linear mixed model (Gaussian error and identity link) relating the nest temperature to the air or soil temperatures for the minimum and maximum temperatures must be built separately for calibration. The maximum daily temperature is from 0:00 h of day i to 0:00 h of day $i+1$ and the minimum daily temperature is from 12:00 h of day i to 12:00 h of day $i+1$. This shift permits the fit of some cases when the minimum temperature could be before midnight due to local meteorological conditions (Eccel 2010). Thus, we obtain the following models, where i indicates the day, ε_i is the Gaussian error term for the day i , and μ_L is the random effect dependent on the logger L :

$$T_{\max} \text{ Nest } (0:00 \text{ h} \rightarrow 0:00 \text{ h})_i \sim T_{\max} \text{ Air or Soil } (0 \text{ h} \rightarrow 0 \text{ h})_i + \mu_L + \varepsilon_i$$

$$T_{\min} \text{ Nest } (12:00 \text{ h} \rightarrow 12:00 \text{ h})_i \sim T_{\min} \text{ Air or Soil } (12 \text{ h} \rightarrow 12 \text{ h})_i + \mu_L + \varepsilon_i$$

The construction of these linear models must include autocorrelation and moving average within the error structure (ARIMA model) and the data loggers must be used as a random factor to reflect their different localization. The air or soil temperatures are treated as fixed effects. The output for fixed factors provides estimates for mean-differences or slopes. Random factors, on the other hand, are defined by a distribution and not by differences. The output for a random factor is an estimate of this variance and not a set of differences from a mean (Pinheiro and Bates 2000). Distribution of the random factor can be used as a measure of heterogeneity of nest temperatures as compared to air or soil temperature. For the nine nests monitored in Brenne (Fig. 2), the distributions of logger random factors for minimum and maximum temperatures are shown in Figure 3: heterogeneity of temperatures is higher for maximal temperatures than for minimal temperatures. Sometimes, the temperature difference can be easily explained by the amount of vegetation cover or tree shade, but sometimes the locations look similar and the difference is probably the consequence of difference in soil conductivity due to difference of soil composition and humidity. Using these statistical tools, it is possible to calibrate nest temperatures based on air or soil temperatures recorded from meteorological stations, integrating thermal heterogeneity. This calibration can then be used

to predict nest temperatures in a nesting area from a local pattern of air or soil temperature variation.

Step 3: From nest temperature to thermosensitive-period of development for sex determination

A model of embryo growth can be used to estimate the exact location of the TSP during development for a nest incubated in natural conditions. Determining the thermal reaction norm for *E. orbicularis*' embryonic growth rate was necessary to build such a model. A reaction norm is the relationship between a phenotypic character and an environmental factor. Here the environmental factor is the incubation temperature and the phenotypic character is the embryonic growth rate. When the thermal reaction norm for embryo growth is known, it is possible to model the embryo mass or straight carapace length (SCL) change for any time series of nest temperatures. Such a model has been published recently for the marine turtle *Caretta caretta* (Girondot and Kaska 2014) and has been converted here for *E. orbicularis*. Information of quantitative development for eggs incubated at constant temperatures (Fig. 4) and from two nests incubated at non-constant temperatures (Fig. 5) has been used to model the thermal reaction norm for embryo growth (Fig. 6). We choose to model the change in straight carapace length (SCL) rather than mass because SCL is strictly increasing during development whereas mass can decrease due to loss of water in dry substrate or at high incubation temperature. The thermosensitive period during embryo growth was determined using a table for correspondence between mass or SCL and embryological stage (Pieau and Dorizzi 1981). On the graphs in figure 5, it is clear that the TSP is not located at the middle third of incubation when temperature is not constant.

Step 4: Constant temperature incubations and sex ratio

A total of 1,259 eggs of *E. orbicularis* have been incubated at constant temperatures and sexed for Claude Pieau' researches from 1970 to 1998 (reviewed by Girondot 1999). The relationship between sex ratio and incubation temperature can be fitted using different sigmoidal functions (Girondot 1999, Godfrey et al. 2003, Hulin et al. 2009). We introduce here a new one that we name double-Richards. It is similar to the Richards equation (see Godfrey et al. 2003) with two K (parameter for asymmetry) values (K1 and K2) below and above the PT. Among these models only the logistic model is symmetrical. An asymmetrical model (Hulin) is selected based on Akaike information criterion and Akaike weight (Table 1). Thus, we identified $P=28.48^{\circ}\text{C}$ as the PT [CI 95% = (28.481, 28.482)] (Fig. 7), with the sex

ratios at $P-0.3^{\circ}\text{C}$ and $P+0.3^{\circ}\text{C}$ being $0.95=0.5+0.45$ and $0.21=0.5-0.19$, respectively. In other words, the feminizing effect of temperature is not symmetrical around the PT, which means that it is necessary to add more thermal energy to feminize when the temperature increases below PT rather than above.

Step 5: From thermosensitive-period of development for sex determination to sex ratio

A common mistake is to use the average temperature recorded during incubation to predict sex ratio. This has been elegantly demonstrated for the marine turtle *C. caretta* when eggs were incubated at the same average temperature but with different daily amplitude: the larger the amplitudes, the more feminized the embryos (Georges 1989, Georges et al. 1994). To understand the effect of changing temperatures on sex ratio, imagine that you could freeze embryos and that they will survive. Freezing temperatures should be masculinizing for a MF TSD pattern. But the embryos do not develop at all and, as a direct consequence, this low temperature does not impact their metabolism and will have no effect for sex determination. Thus, the effect of a temperature must be weighted by the growth of embryo at this temperature (Pieau 1984, Booth and Freeman 2006). However, this effect is not sufficient to explain the sex ratio bias observed in this experiment (MARC GIRONDOT, unpublished observation).

The incubation duration has been proposed as a proxy for the sex ratio as it integrates the influence of temperature during all the development (Godfrey et al. 1999, Mrosovsky et al. 2009). However, this solution is not entirely satisfactory because the temperature influences incubation duration during all of incubation, whereas temperature influences sex only during the TSP. Thus, one can imagine a situation with long (respectively short) incubation duration because the beginning or the end is cold (respectively warm) but the temperature within the TSP is warm (respectively cold). In such a situation, the sex ratio will be estimated wrongly.

An alternative solution is to use the TSP duration as a proxy for sex ratio. TSP duration can be evaluated at constant incubation temperature for calibration using the embryo growth model and thermal reaction norm of embryonic growth rate (see previous section) (Fig. 8). The relationship between TSP duration and sex ratio for these nests incubated at constant temperature has been fitted using the same method as the relationship between temperature and sex ratio (Table 2, Fig. 9). Using this relationship, TSP duration for natural nests can be transformed into sex ratio. The confidence interval for the sex ratio can be obtained based on the confidence interval from the relation used for calibration (Fig. 9). To test the procedure,

the length of the TSP for the two nests shown in Figure 5 was determined and their sex ratio predicted using the calibration curve (100% males, Fig. 9). This prediction is in agreement with the sex ratios actually observed (reviewed by Girondot 1999).

However, the thermal reaction norms for any biological reaction, and of course for embryonic growth rate, are bell shaped with a maximum at an intermediate optimal temperature (Schoolfield et al. 1981). Thus, the same incubation duration is expected below and above this optimal temperature where 100% males or 100% females can be obtained respectively. This situation is unlikely to be observed for *E. orbicularis* thermal reaction norm for embryonic growth because the optimum (temperature at which the embryonic growth rate is maximum) is higher than 35°C (Fig. 6), a temperature that is rarely attained within natural nests (see Fig. 2).

We have shown that incubation duration could lead to wrong conclusions about sex ratio due to the non-monotonous trend of temperatures effect on growth rate. The solution that we advocate here using TSP duration is also not fully satisfactory because there is no evidence that the thermal reaction norm for embryo growth is the same as the thermal reaction norm for sexualisation which describe how the temperature affects more or less the sexualisation of the gonad. The best solution would be to convert temperatures within TSP into sex ratio taking into account this new thermal reaction norm for sexualisation. The shape of this reaction norm cannot be deduced from the thermal reaction norm of sex ratio due to the threshold effect of the proportions (a proportion cannot be lower than 0 and higher than 1). For example, 35°C is a more feminizing temperature than 30°C (Pieau 1982), but 100% females are observed at both temperatures. We can predict that the sexualisation thermal reaction norm is non-linear according to temperature as for all thermal reaction norms (Schoolfield et al. 1981).

Incubations at changing temperatures with sexed embryos are necessary to estimate the thermal reaction norm for sexualisation. These data are not still available for *E. orbicularis* and only the TSP duration can be used as a proxy for sex ratio at current time.

General conclusions

The different steps presented in this paper permit one to convert air, soil, nest or TSP duration or temperatures into sex ratio. We used two nests incubated in nature to test the method, but these two nests were 100% masculinized thus the test has low statistical power. It would be better to have more nests and some of them with mixed sex ratio.

The method that we preconize to estimate sex ratio from nest temperatures requires that the thermal reaction norm for embryo growth and the thermal reaction norm for sexualisation are known. The thermal reaction norms are genetically determined and can be different for different populations based on genetic drift or selection (Angilletta et al. 2003). Selection can be of primary importance because the thermal niche for different populations can be very different depending on the latitude and the climate. As a note of caution, the heterogeneity of nest temperatures is probably lower than the heterogeneity of soil temperatures at the scale of entire ecosystems because turtles do not choose nesting site randomly: they are generally located in sandy soil, in a small hill exposed to the west. Of course, females have plastic behavior and they can nest in other places when this situation is not available. Thus we encourage the teams working in the field to evaluate these two reaction norms for the populations of their working area rather than using the one described here for *Emys orbicularis* from Brenne in the center of France.

To be in the best situation to estimate sex ratio of natural nests, first it is necessary to get calibration values for the thermal reaction norms for embryo growth and for sexualisation. A set of nests must be monitored using temperature data loggers. The higher the diversity of temperatures both within and between nests, the better the estimates thus a lower number of nests are necessary. For example, even only two nests with large range of temperatures permit one to estimate the thermal reaction norm for embryo growth but the confidence interval was large (Fig. 6). Thus it is better to monitor nests in different soil media, with different exposure and at different times to sample a large range of incubation temperatures. Incubation duration, being defined as the time from egg laying until piping (when the embryo egg tooth breaks the shell) or emergence, must be recorded and juveniles must be sexed for each nest. The higher the number of sexed juveniles, the better. Until recently the only method for sexing hatchlings was lethal because it requires examining gonads histologically. Laparoscopy could be used only for large individuals but is also lethal for *Emys orbicularis* hatchlings (PIEAU and GIRONDOT, unpublished). Two recent studies have reported that steroid hormones can be detected in amniotic fluid obtained from eggshells after hatching and differential levels permit to distinguished males and females for eggs incubated at constant incubation temperatures in marine turtles (*Chelonia mydas* and *Caretta caretta*) (Xia et al. 2011, Kobayashi et al. 2015). This method must be still evaluated in natural conditions. These methods cannot be used at large spatial and temporal scales but could be used to calibrate sexualisation thermal reaction norm and then sex ratio could be determined from time series of nest temperatures.

If the sex ratio cannot be easily determined for a natural nest, the thermal reaction norm for embryo growth rate can be still determined and then TSP duration could be used as a proxy of sex ratio.

All the statistical methods described in this paper are available in two R packages: *HelpersMG* (Girondot 2016b) for meteorological statistical tools and *embryogrowth* (Girondot 2016a) for statistical tools related to thermal reaction norm for embryonic growth and temperature-dependent sex determination. Both packages are available in the Comprehensive R Archive Network (CRAN) website: www.cran.org.

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Tables

Table 1: Fit of sex ratio depending on constant incubation temperatures for 1,259 eggs (reviewed in Girondot 1999). Hulin model is selected based on AIC and Akaike weight and is shown graphically in Figure 6. AIC is the Akaike Information Criterion which is a measure of the fit of the model corrected for over-parametrization, Δ AIC is the difference with the lowest AIC, and Akaike weight is the probability that a model is really the best (Burnham and Anderson 2002).

	Parameters	AIC	ΔAIC	Akaike weight
Logistic	2	93.44	9.22	0.008
Hill	2	93.14	8.92	0.01
Richards	3	88.40	4.18	0.10
Hulin	4	84.22	0.00	0.83
Double-Richards	4	90.09	5.87	0.04

Table 2: Fit of sex ratio upon duration of the thermosensitive period of development for sex determination. Hulin model is selected based on AIC and Akaike weight and is shown graphically in Figure 8. See table 1 for the legend of columns.

	Parameters	AIC	ΔAIC	Akaike weight
Logistic	2	92.60	13.99	0.00
Hill	2	93.33	14.71	0.00
Richards	3	90.19	11.57	0.00
Hulin	4	78.61	0.00	0.99
Double-Richards	4	91.49	12.88	0.00

Figures

Figure 1: Time for minimum and maximum daily air temperature from 2006 to 2014 at Barbeau in France (N43°03'07.801" E22°56'34.148") according to the time of sunrise.

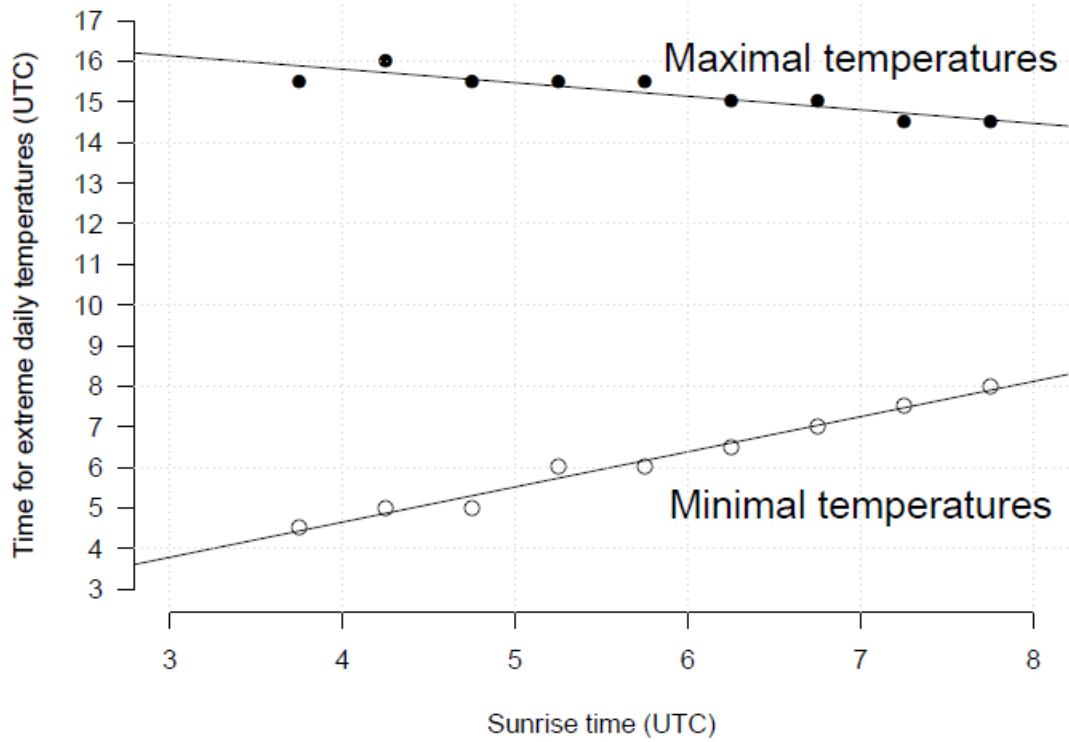


Figure 2: *Emys orbicularis* nest temperature recorded in nine nests in 2005 in Migné, France (N46°42'36'' E01°18'36''), air temperature at 2 m high and soil temperature between 7 to 26 cm depths from ECMWF project (N46°36'00'' E01°15'00'').

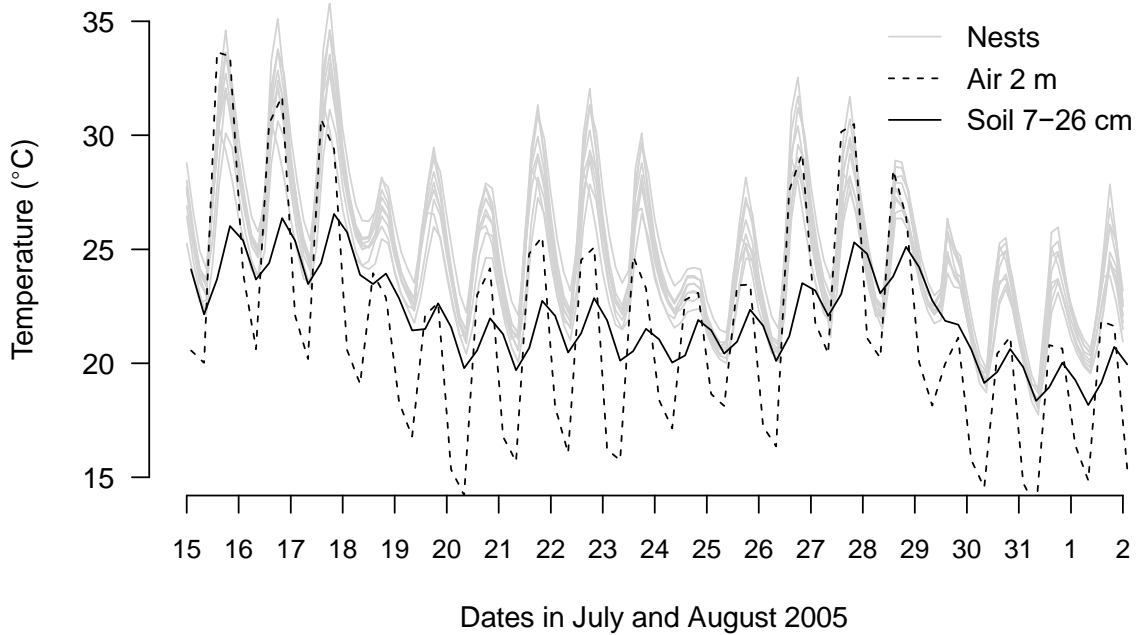


Figure 3: Distribution of random factor in generalized linear mixed model (GLMM) measuring the heterogeneity of different location for daily minimum and maximum temperatures, estimated from nine nests of *Emys orbicularis* monitored in 2005.

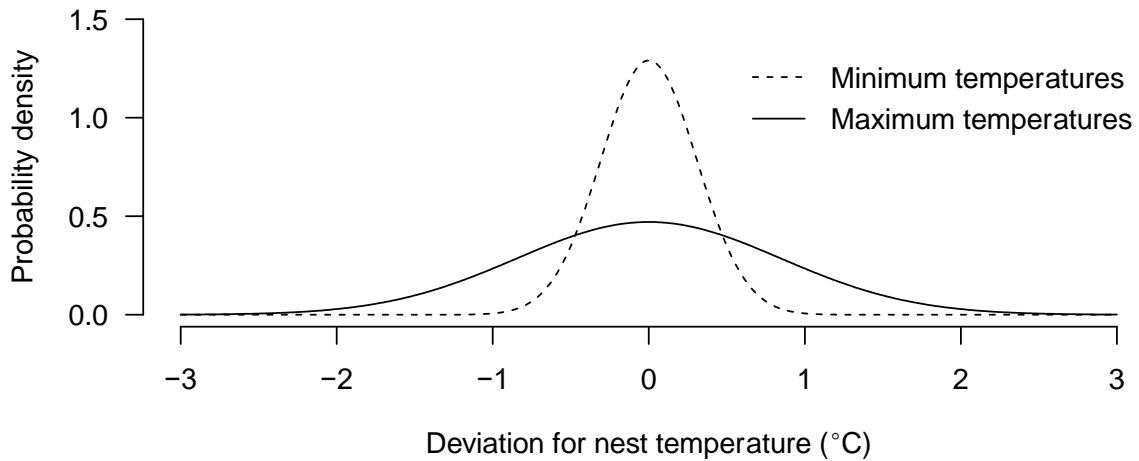


Figure 4: (A) Embryo mass and (B) straight carapace length (SCL) during development at two constant incubation temperatures (points and error bars being $2 \times SD$) and fit of Gompertz growth model at both temperatures with a common size at day 0 (X_0 parameter) and K parameter.

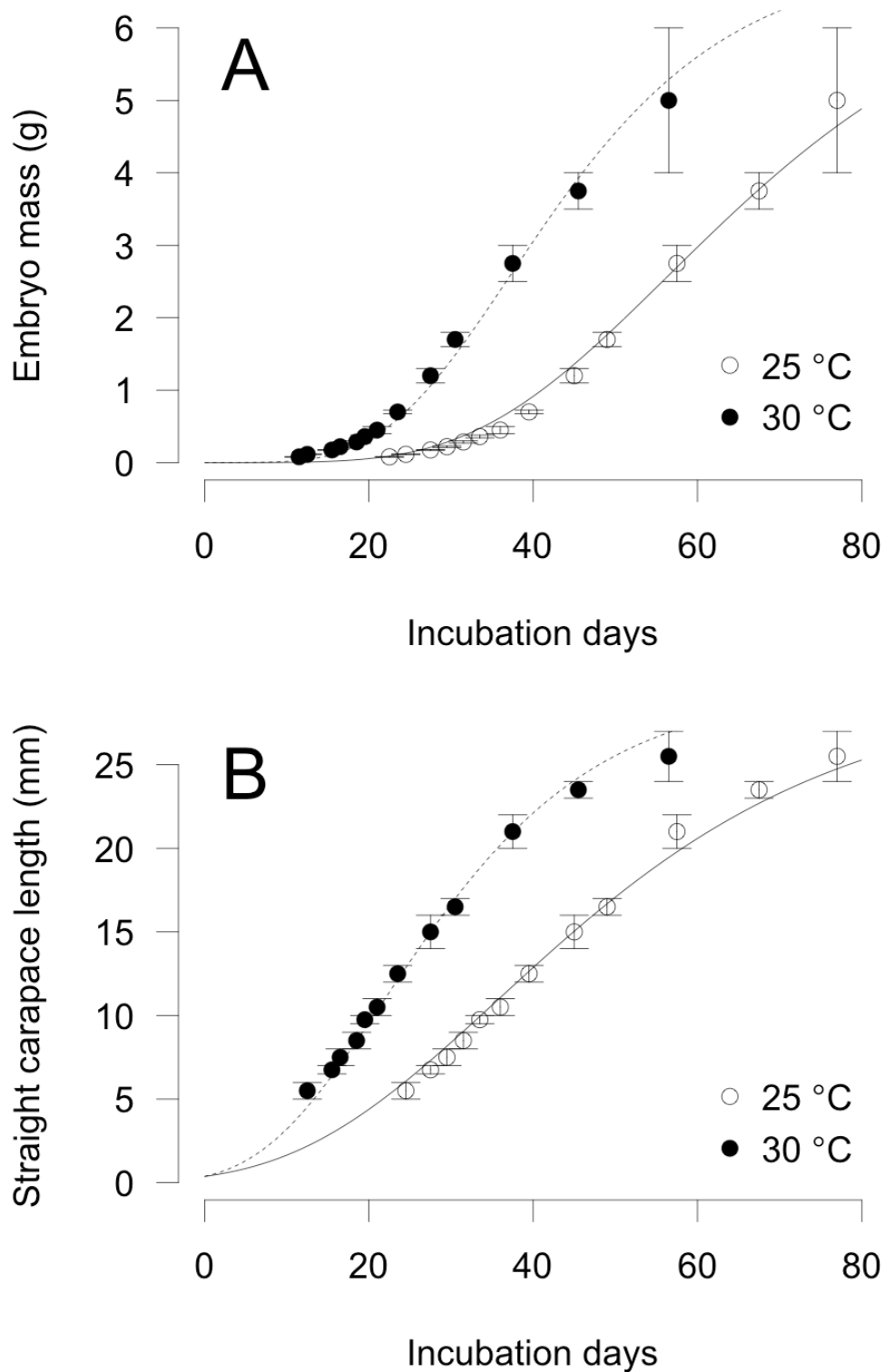


Figure 5: Modeled *Emys orbicularis* embryonic straight carapace length (SCL) growth incubated *in situ* in (A) 1996 and (B) 2004. Grey lines are the temperatures within the nest and the black lines represent the model for the growth of the embryo. Horizontal lines indicate the observed Straight Carapace Length (SCL) of *Emys orbicularis* hatchlings and its 95%

confidence interval. Grey rectangle is the position of thermosensitive-period for sex determination according to embryo SCL on SCL-axis (Pieau and Dorizzi 1981) and its projection on time-axis. Vertical dot lines and the corresponding arrows at the top delineate the second-third of incubation.

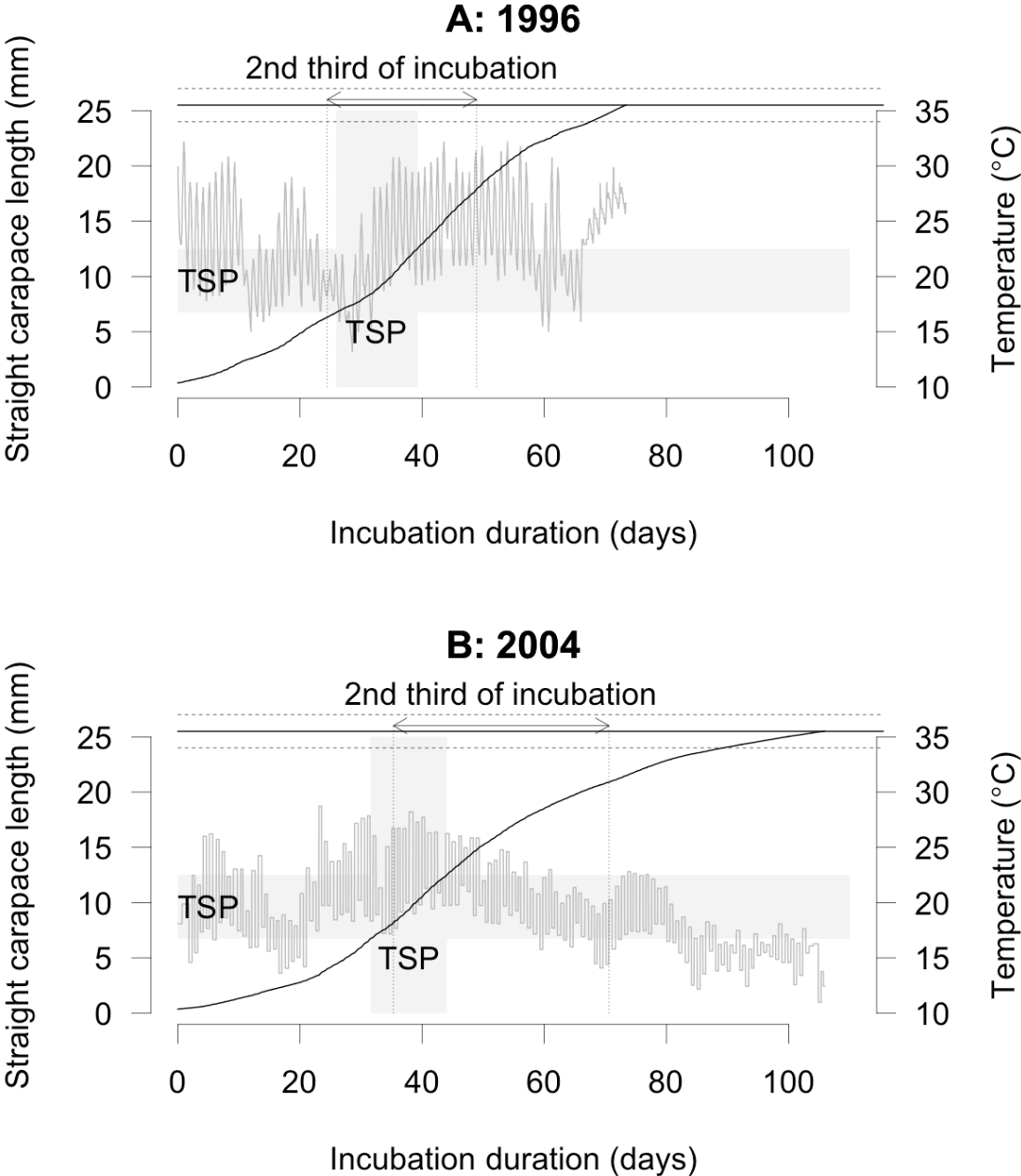


Figure 6: Embryo growth thermal reaction norm (line) and the histogram of temperatures in two nests used for fitting.

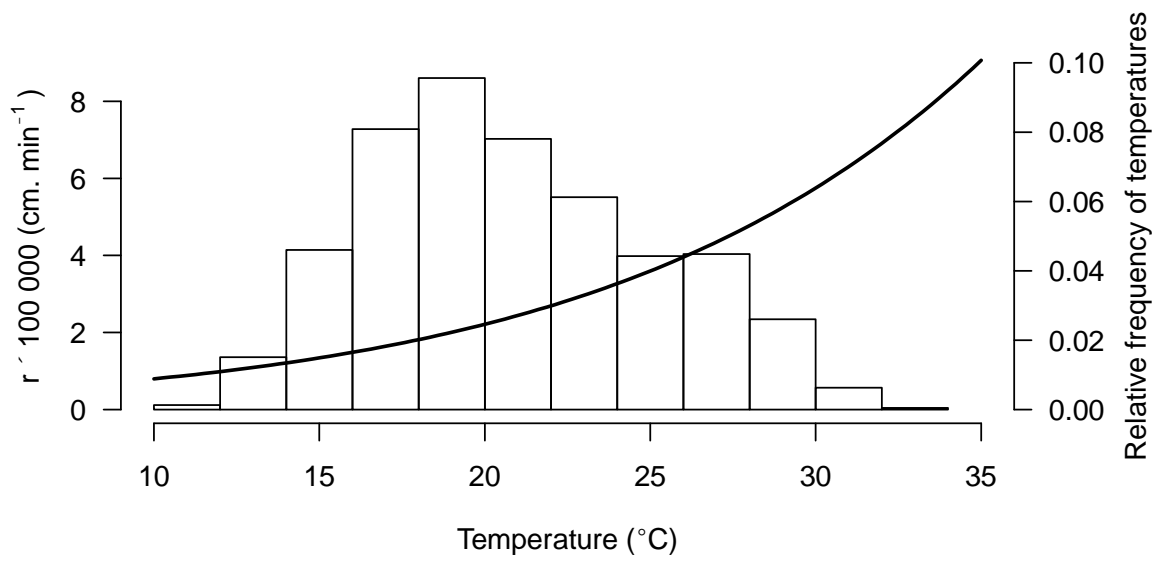


Figure 7: Sex ratio (proportion of males) of *Emys orbicularis* embryos from Brenne (France) incubated at constant temperatures. The error bars represent twice the standard deviation for each point estimate. The solid line shows the selected model based on table 1. The pivotal temperature is 28.48°C (standard error <0.001) and the transitional range of temperatures (shaded area) defined as the range of temperatures producing sex ratio between l and $1-l$ ($l=5\%$) is 1.05°C (standard error <0.001) [28.16°C–29.21 °C].

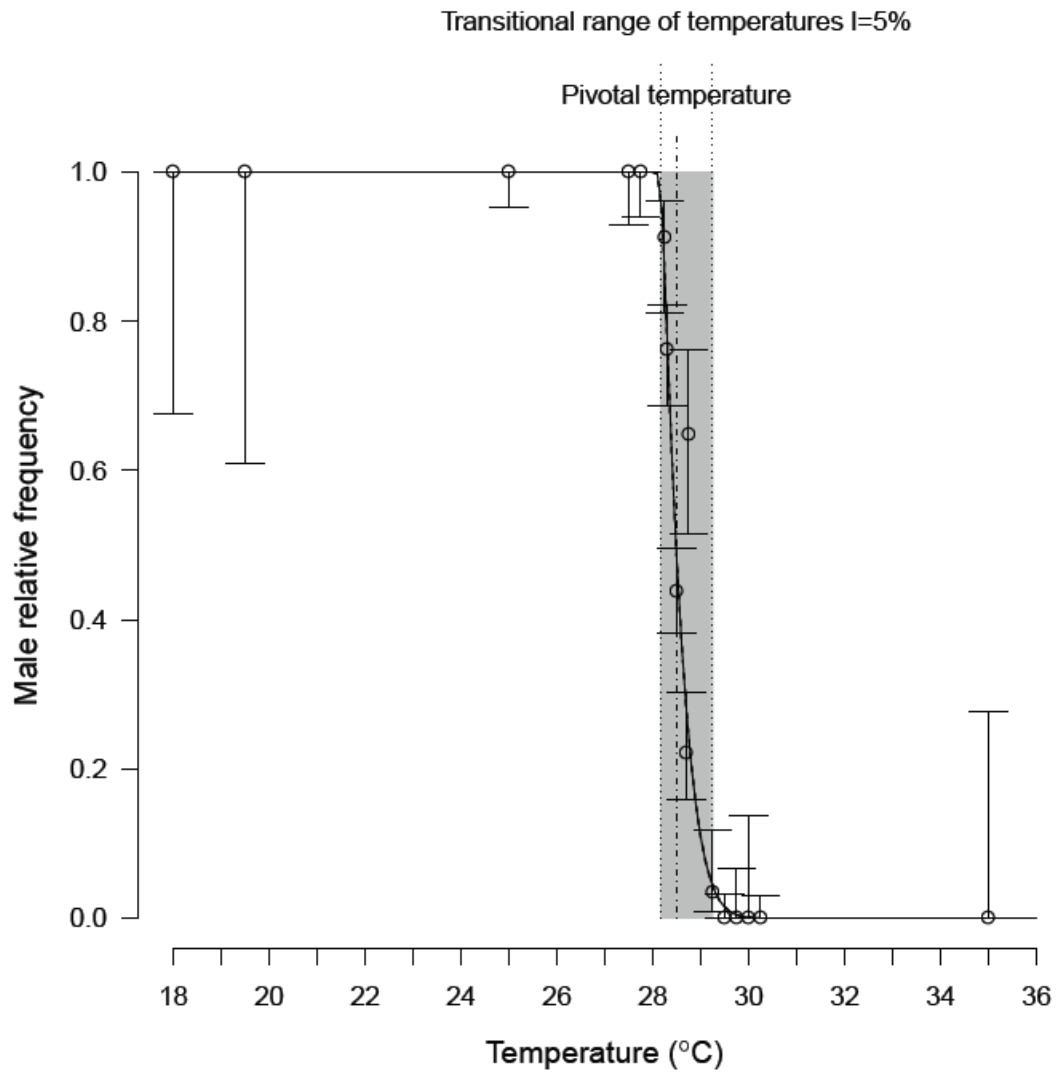


Figure 8: Relationship between constant incubation temperature and duration of the thermosensitive period for sex determination in days based on the modeled thermal reaction norm for embryo growth (see Figure 6). Temperatures for pivotal temperature (PT) and transitional range of temperatures (TRT) are shown (see Figure 7).

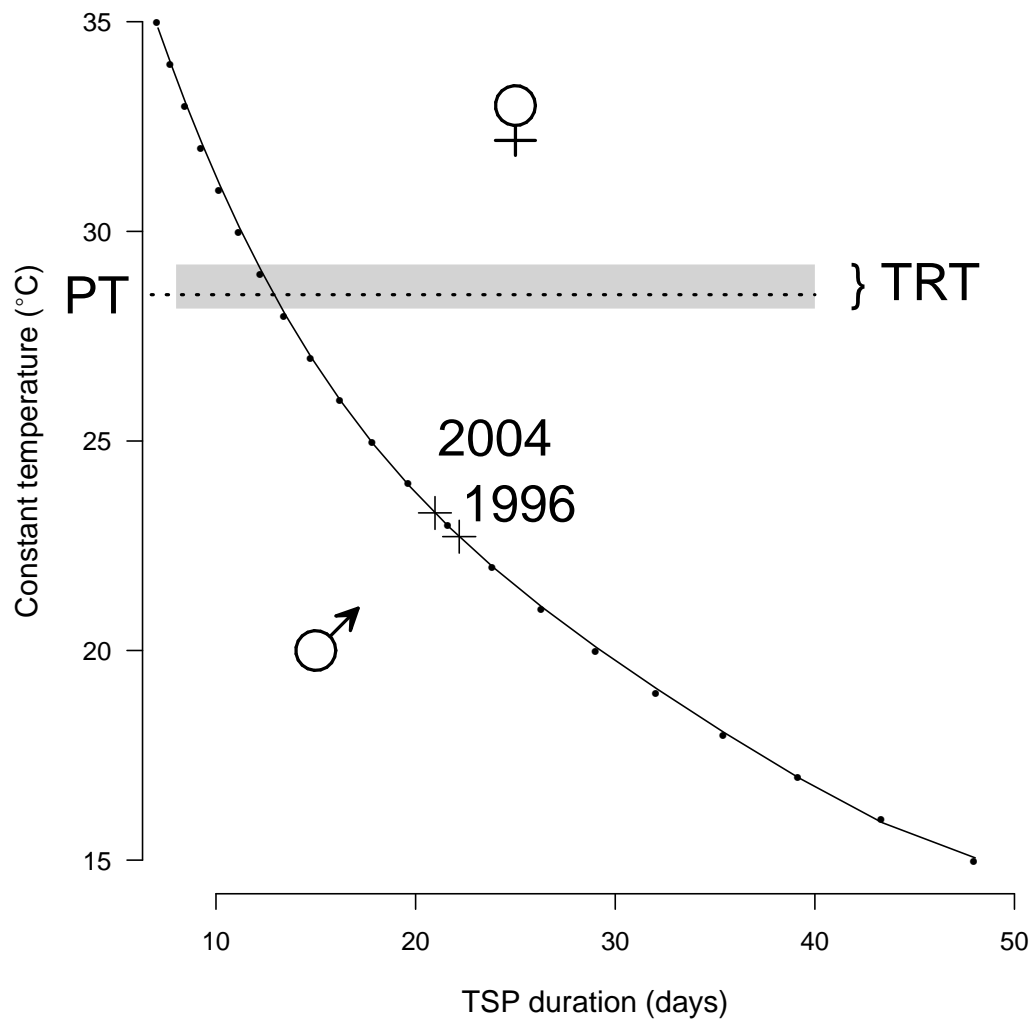
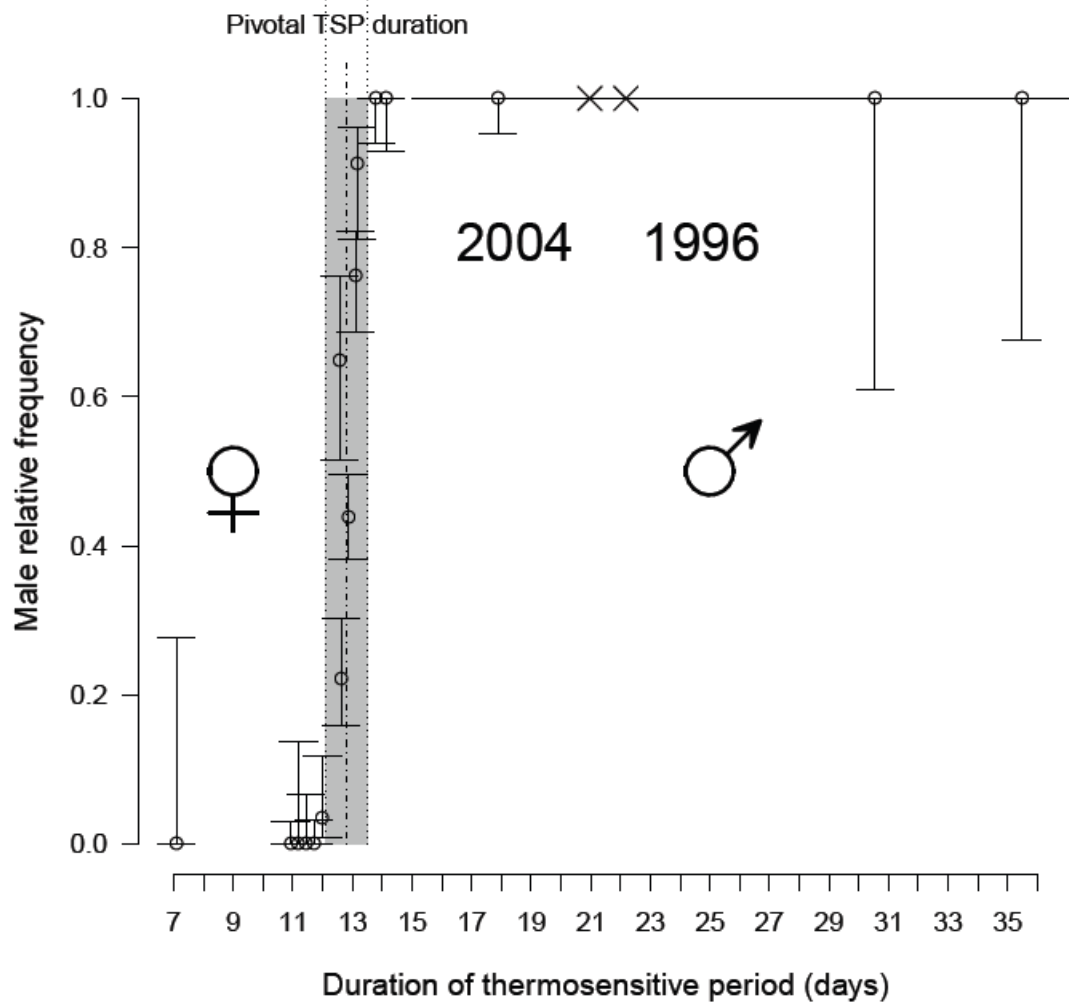


Figure 9: Calibration curve of sex ratio as a function of TSP duration, using incubations at constant temperatures. The error bars represent twice the standard deviation for each point estimate. The line shows the selected model based on table 2. Arrows indicate the estimated TSP duration for two nests incubated in natural condition from 1996 (Figure 5A) and 2004 (Figure 5B) and their corresponding sex ratios.

Transitional range of TSP durations $I=5\%$



ANNEXE 2 : Reaction norm of embryo
growth rate dependent on incubation
temperature in the marine turtle Olive
Ridley, *Lepidochelys olivacea*, from
Pacific Central America

Reaction norm of embryo growth rate dependent on incubation temperature in the marine turtle Olive Ridley, *Lepidochelys olivacea*, from Pacific Central America

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Abstract: Olive Ridleys (*Lepidochelys olivacea*) and loggerheads (*Caretta caretta*) are two closely phylogenetically related marine turtles that nest in very different thermal habitat. Olive Ridleys nest in pan-tropical beaches whereas loggerheads nest in more temperature beaches. In the context of climate change, the temperature in temperature beaches will increase. We have determined the thermal reaction norm for embryonic growth in both species. We show that the optimum temperature for the growth of embryos is much lower for *Caretta caretta* than for *Lepidochelys olivacea*. It makes Loggerhead turtles particularly sensitive to increase of beach temperature as it is expected due to climate change. On the other hand, Olive Ridleys are more resilient.

Keywords: Growth, Embryo, temperature, Gompertz, Turtle, *Lepidochelys olivacea*, Olive Ridley, Loggerhead, *Caretta caretta*

Introduction

Climate warming presents major challenges to organisms (Parmesan 2006, Intergovernmental Panel on Climate Change 2007). There is a pressing need in conservation biology and climate change research for cross-species, quantitative, objective criteria for assessing the susceptibility of species to climate change-induced extinction (Bernardo et al. 2007). Overall, current estimates of biodiversity loss due to climate change are very variable, depending on the method, taxonomic group, biodiversity loss metrics, spatial scales and time periods considered (Bellard et al. 2012). Yet, the majority of models indicate alarming consequences

for biodiversity, with the worst-case scenarios leading to extinction rates that would qualify as the sixth mass extinction in the history of the earth (Pereira et al. 2011).

One attempt to address this shortcoming is phenomenological, using climatic descriptions of contemporary ranges coupled with expected temperatures to predict future ranges (Bellard et al. 2013). These bioclimatic models are best case scenarios because, while they reflect existing interspecific differences in projected ranges under global warming, they also implicitly assume that all species have similar potential to access and exploit predicted climate space. The insidious threat from climate change is that it will attenuate or preclude ecological responses by species that are physiologically constrained; yet, quantitative, objective criteria for assessing relative susceptibility of diverse taxa to warming-induced stress are wanting (Bernardo et al. 2007). Consequently, biologists are endeavouring to develop robust ways to evaluate the differential vulnerability of organisms to climate change (Williams et al. 2008) and then to evaluate and implement management strategies tailored for species judged most at risk (Lee and Jetz 2008).

The extent of adaptive variation in heat-tolerance is likely to have important consequences for the resilience of many ectothermic species in a rapidly warming world (Baker et al. 2004, Jensen et al. 2008, Baskett et al. 2009, Somero 2010, Eliason et al. 2011). This has led to calls for an ‘adaptive evolutionary’ approach to conservation, which seeks to conserve functional diversity (rather than simply genetic marker diversity) at whatever scale it occurs (Fraser and Bernatchez 2001).

The two marine turtles *Lepidochelys olivacea*, Olive Ridley, and *Caretta caretta*, loggerhead, are two phylogenetically closely related species (Guillon et al. 2012) that nest in very different thermal habitats. Olive Ridley turtles nest in intertropical zone while loggerheads nest in more temperature beaches (Wallace et al. 2010). Virtually all biological rates are affected by temperature including development times (Gillooly et al. 2002) and growth rates (Gillooly et al. 2001) of ectothermic animals. Egg incubation temperatures affect the duration of embryogenesis (Miller 1985), the probability of embryo survival (Van Damme et al. 1992), the sex determination for species with temperature-dependent sex determination such as *Caretta caretta* (Pieau 1996) and the morphology, and body size at hatching (Du and Ji 2003). In addition, long-term effects of incubation temperature on the physiology and behaviour of hatchlings has been observed (Burger 1989, Sibly and Atkinson 1994). Thus, the fitness of developing embryos is strongly dependent on the temperature within the nest during incubation.

Recently, a general way to model sigmoidal embryo growth with variable incubation temperature during development obtained from field has been proposed and tested with data from *Caretta caretta* from Dalyan beach, Turkey (Girondot and Kaska 2014a). We apply this model to new data of incubation temperatures gathered in 2011-2012 in Guatemala for *Lepidochelys olivacea* nesting in Pacific coast to compare the reaction norm for the embryo growth to temperature with the anticipated potential impact of climate change for these species.

Materials and methods

FIELD DATA

Olive Ridley marine turtles nest along all the Pacific Coast of Guatemala. Egg collection for human consumption is authorized as long as 20% of the eggs of each collected nest is given to hatcheries located along all the littoral. A total of 80 nests have been used in this experiment. Among them, 40 have been incubated in hatchery (hatchery nests) and 40 have been used in 4 different experiments (experimental nests). Half of these 40 nests were buried in sand at 40 cm depth and half at 60 cm depth. Among each group of these 40 experimental nests, half (10) have been incubated in open beach under shade and half (10) at sun.

ANALYSIS OF INCUBATION DATA

For each of the 80 monitored nests, we obtained: incubation duration, longitudinal temperature data and 10 hatchling straight carapace length at the nearest 1/10 mm. Effect of experimental treatment on mean incubation temperature, incubation duration and hatchling size has been analysed using generalized linear model (mean incubation temperature and incubation duration) or generalized linear mixed model (hatchling size) with nest identity as random factor. In all cases, fixed factors were depth of nests (40 or 60 cm) and with or without shade and their interaction. Mean incubation temperature and its first order interaction with other factors were added for analysis of mean incubation temperature and hatchling size. Gaussian distribution with identity link has been used. Variability of daily temperature among nest from similar treatment has been established.

THERMAL GROWTH RATE REACTION NORM

The model of embryo growth integrates in a single framework both the growth rate dependency on temperature and the embryo growth based on growth rate (Girondot and Kaska 2014a). The growth rate dependency on temperature and the embryo growth model are

fitted using maximum likelihood to best describe the observed hatchling size according to time series of temperature within nests. It is summarized here briefly but a complete description can be found in the original publication (Girondot and Kaska 2014a).

Biological temperature-dependent rate models based on Arrhenius' and Eyring's equations have been formulated by Sharpe and DeMichele (1977). The original formulation of Sharpe and DeMichele was modified by Schoolfield et al. (1981) to remove the very high correlations of parameter estimators. Two kinds of equations using 4 or 6 parameters produced a curve with a maximum at an intermediate temperature and decreased below and above this temperature. The level as well as the position of the maximum can be manipulated using the parameters values.

The early growth of embryos is modeled using a modification of the Gompertz model (Ricklefs 2010) proposed by Laird (1964):

$$X(t) = X_H r_K \exp\left(\ln\left(\frac{X(0)}{K}\right) \exp(-r(T) t)\right) \text{ [Equation 1]}$$

Where $X(0)$ is the size or mass at nesting time (time=0), $r(T)$ is the growth rate at the beginning of the curve, and K is the carrying capacity with $\lim_{t \rightarrow +\infty} X(t) = K$. X_H is the hatchling size and $r_K=2.09$ is a constant used to slowdown growth at the end of incubation (Girondot and Kaska 2014a) to ensure that embryological stages are well positioned during incubation.

The dynamic of $X(t)$ is governed by the Gompertz differential equation (eqn 2):

$$X'(t) = r(T) \ln\left(\frac{K}{X(t)}\right) X(t) \text{ [Equation 2]}$$

The gastrula is approximately a disk of 1.7 mm diameter and this size will be used as $X(0)$ (Kaska and Downie 1999).

PARAMETER FITTING

$r(T)$ can be calculated with the 4 or 6 parameters of the Schoolfield et al. (1981) model and an incubation temperature T . With $X(0)$ and K , and a time-series of $r(T)$, the pattern of change of size for this nest is evaluated using Runge-Kutta method of order 4 for the approximation of solutions of ordinary differential equations.

Estimation of parameters was performed using maximum likelihood with an identity link and a Gaussian distribution of SCL. The standard error of parameters was estimated using the square-root of the inverse of the Hessian matrix which is an asymptotic approximation of the variance-covariance matrix (Abt and Welch 1998). The model is implemented as an R package (Girondot 2016).

COMPARISON BETWEEN SETS OF DATA

$r(T)$ has been fitted for hatchery and experimental *Lepidochelys olivacea* nests first separated and second grouped in a single dataset. We used AIC and Akaike weight to select between 4 and 6-parameters model. AIC is a measure of the relative quality of fit, which penalized for too many parameters in the model (Akaike 1974) and Akaike weight gives the relative statistical support of several models tested on the same dataset (Burnham and Anderson 2002). Likelihood ratio test has been used to test whether a single model for hatchery and experimental nests was sufficient or not to describe data. The statistics $LRT = -2 \frac{\ln L_{complex\ model}}{\ln L_{simple\ model}}$ is distributed as a χ^2 with the degree of freedom being the difference of number of parameters between the most complete model and the simplest one.

Results

ANALYSIS OF INCUBATION DATA

Incubation temperatures in the 80 nests from *Lepidochelys olivacea* incubated in the beach of Monterrico, Guatemala are shown in figure 2 as well as the temperatures recorded in *Caretta caretta* nests from Turkey (Girondot and Kaska 2014b). Average incubation temperatures for each of the 80 nests ranges from 29.11°C to 33.56°C (mean=31.00°C sd=1.53°C). Only shading status for experimental nests is significant (p=10 15) to explain the difference between nests. Depth and interaction between shading status and depth are not significant (p>0.45). Shaded nests are cooler than those exposed to the sun. Nests from hatchery are also significant cooler than the experimental ones (paired t-tests with Bonferonni correction, both p<10 9) (Fig. 3A).

Incubation duration ranges from 43 to 55 days (mean=49.86 days sd=3.63). Significant effect of mean incubation temperature for experimental data is observed (p<0.01) but not of depth or shading as well as all interactions (all p>0.1). Incubation duration is longer for cooler temperatures (Fig. 3B).

Significant effect for hatchlings straight carapace length (mean 40.86 mm, sd=1.82) is noticed for experimental data for depth factor ($p=0.01$) but not for any other factors (all $p>0.05$). Embryos incubated at 40 cm are smaller (40.22 mm) than those incubated at 60 cm (41.07 cm) (Fig. 3C).

The variability of daily temperatures among nests has been measured as the average of the daily standard deviation of temperatures recorded in nests from each treatment (shading status and depth). Based on a t-test with unequal variances, both an effect of shading at 60 cm and 40 cm (both $p<0.01$) but also an effect of depth for shade and sun-exposed nests (both $p=0.04$) was noticed.

GROWTH RATE DEPENDENT ON TEMPERATURE

Parameters maximizing likelihood of observed hatchling size for each nest have been fitted first using the total set of 80 nests using the 4 and the 6-parameters equation describing instantaneous growth rate dependency to temperature. AIC for 4-parameters model is 380.97 whereas it is 390.05 for the 6-parameters model. Akaike weight gives a very strong support to retain the 4-parameters model ($p=0.99$). In a second step, parameters have been fitted separately for hatchery nests ($\ln L=-97.83$), for experimental nests ($\ln L=-79.79$) and for all nests together ($\ln L=-186.55$) with LRT being 1.90 ($df=4$, $p=0.75$). Thus a single model for the two categories of nests was sufficient.

The fitted pattern of embryo growth for the 80 nests is shown in figure 5. It should be noted that all fitted embryo sizes at the end of the incubation are comprised within the 95% confidence interval of observed hatchling sizes.

The instantaneous growth rate according to temperature is shown in figure 6 for *Lepidochelys olivacea* from Guatemala as well as the curve fitted for Mediterranean *Caretta caretta* (Girondot and Kaska 2014b, Monsinjon et al. In press).

Discussion

Temperature during incubation of ectothermic animals can have profound consequences on the fitness of individuals and then we expect that selection acts to adapt response of embryos to temperature.

The effect of shading on incubation temperature was anticipated based on several previous reports for turtles (Janzen 1994, Schmid et al. 2008, Refsnider et al. 2013) but also lizards (Doody et al. 2006). Indeed, we found that the shaded experimental nests have a lower mean

incubation temperature than the experimental nests exposed to the sun. Hatchery nests are also shaded and have still a lower temperature (Fig 3A). However we do not detect a significant effect of depth probably because the differential between both depths (40 and 60 cm) was not sufficient to induce enough change in temperature. As a consequence, shaded nests take longer to emerge as compared to the nests directly exposed to sun and incubation temperature mediates this effect (Fig 3B).

Hatchlings from eggs incubated at 40 cm were significantly smaller than those from eggs incubated at 60 cm (Fig. 3C) but a direct effect of temperature or shading was not observed. We do not have definitive explanation for this effect. Mass of hatchling has been shown to be dependent on incubation temperature in many reptiles (Brana and Ji 2000, Ji and Du 2001a, b, Ji et al. 2002) but also on dryness of substrate (Delmas et al. 2008). On the other hand, effect on size is not consistent among studies nor within the same study. For example, no effect has been observed in the turtle *Pelodiscus sinensis* (Du and Ji 2003) but a slight decrease of carapace length has been measured in the marine turtle *Caretta caretta* as the moisture increased (McGehee 1990). But, in the same experiment and for the same turtles, carapace width and plastron length do not show any consistent pattern (McGehee 1990).

Here we do not detect an effect of temperature on size but an effect of depth of the nest. This effect could be mediated by moisture difference between two depths: it is possible that eggs incubated at 40 cm lost more water than those located at 60 cm, closer to the water table (Nielsen 1990). The dry-incubated eggs accumulated less water during incubation (Finkler 1999). Water within the eggs is known to play a key role in the mobilization of yolk reserves by reptile embryos and consequently influences hatchling mass (Packard and Packard 2001).

The pattern of growth rate depending on temperature show an increase from 20°C to 35°C. However, it should be noted that the part of the curve from 20°C to 27°C is extrapolated based on equation 2 because no lower temperature than 27°C was experienced by *Lepidochelys olivacea* nests (Fig. 2). On the other hand, temperature as low as 22°C was recorded in *Caretta caretta* nests in Turkey (Girondot and Kaska 2014b) and the pattern of growth rate for these both species at low temperatures is very similar (Fig. 6). However, the pattern differs completely for incubation at high temperatures. The growth rate continues to increase up to 35°C for *Lepidochelys olivacea* whereas it goes down for *Caretta caretta*. This is consistent with the observation that incubation duration increases at 32°C as compared to 31°C (Monsinjon et al. In press). The distribution of nesting sites for both of these species is very different. *Caretta caretta* nests mainly out of the intertropical region whereas

Lepidochelys olivacea has a distribution more centered on the equator (Wallace et al. 2010). The northernmost nest for a marine turtle has been deposited by a *Caretta caretta* in South of France at the latitude 43°16.05'N (Sénégal et al. 2008). As a consequence, the nest temperatures experienced by *Lepidochelys olivacea* (Fig. 2A and B) are generally much higher than temperatures experienced by *Caretta caretta* (Fig. 2C). However, we lack data to generalize this observation.

The variability of temperatures among different nests incubated in same conditions was measured. We detect an effect of depth but much more an effect of shading (Fig. 4). Such a measure is particularly important because it permits to explain why, in the same beach and at the same time, heterogeneity of incubation conditions is observed. The thermal microhabitats have been overlooked for marine turtle and generally for reptiles. This is particularly important in the context of prediction of the impact of climate change. The climate models available give prediction at the regional scale (Christensen et al. 2007) very far away from the beach scale and even intra-nesting beach level as shown here. This downscaling is challenging to be able to produce realistic models of incubation of reptile eggs.

Acknowledgments

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Figures

Figure 1: Map of Central America with greyed Guatemala. Monterrico nesting beach is indicated.

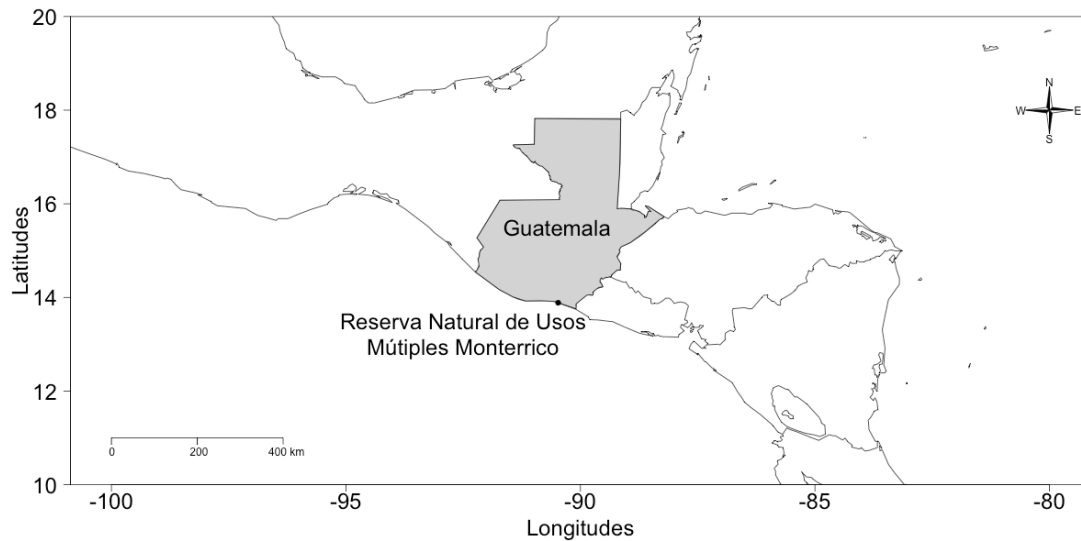


Figure 2: Temperatures recorded in hatchery (A) and experimental (B) *Lepidochelys olivacea* nests from Monterrico, Guatemala. As a comparison, temperatures recorded in 21 *Caretta caretta* nests from Turkey are shown (Girondot and Kaska 2014b) (C).

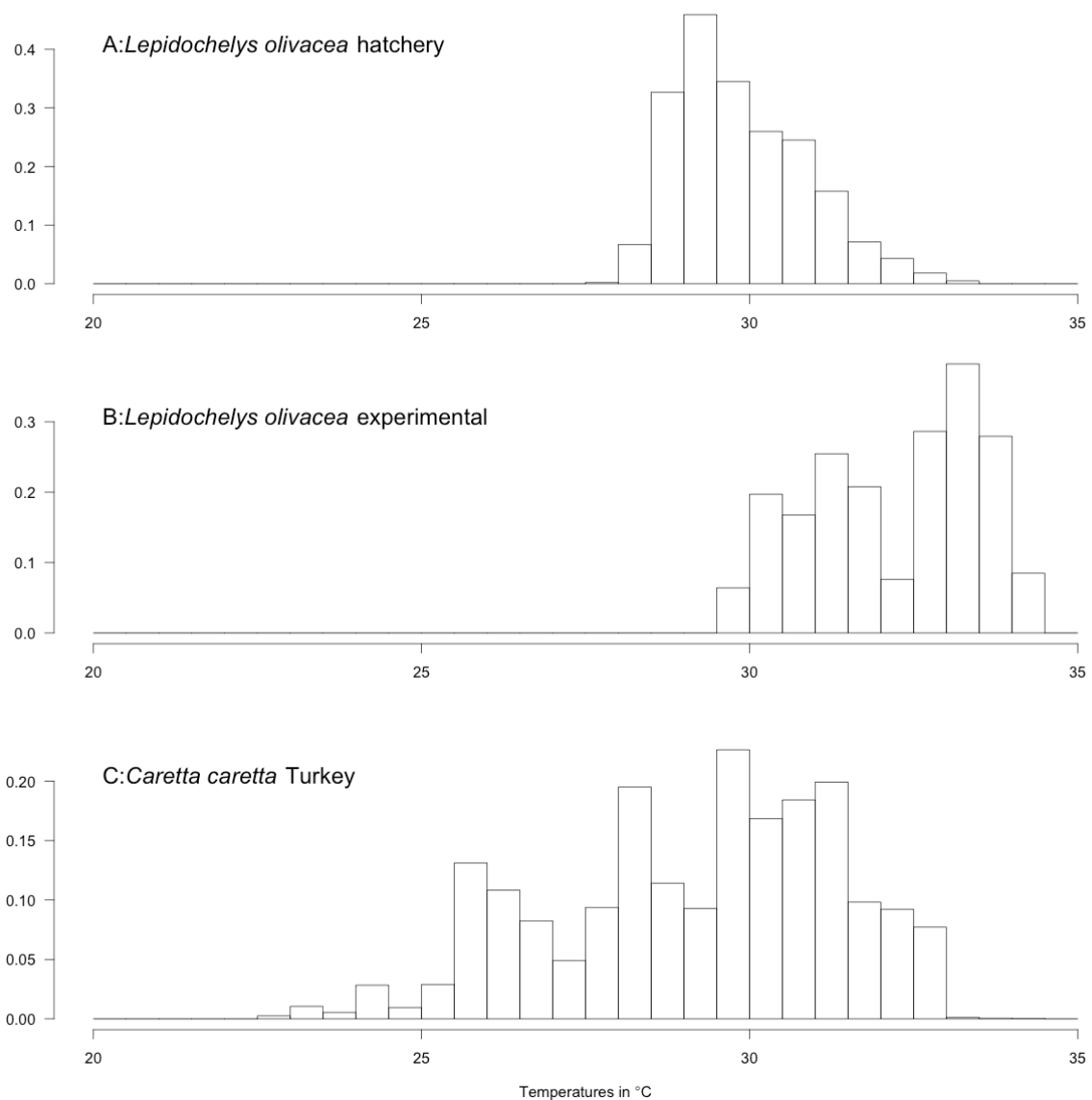


Figure 3: Descriptive statistics of incubation data for *Lepidochelys olivacea* nests from Monterrico, Guatemala plotted according to the significant factors explaining the differences between treatments. (A) Mean incubation temperature, (B) incubation duration, and (C) hatchling size.

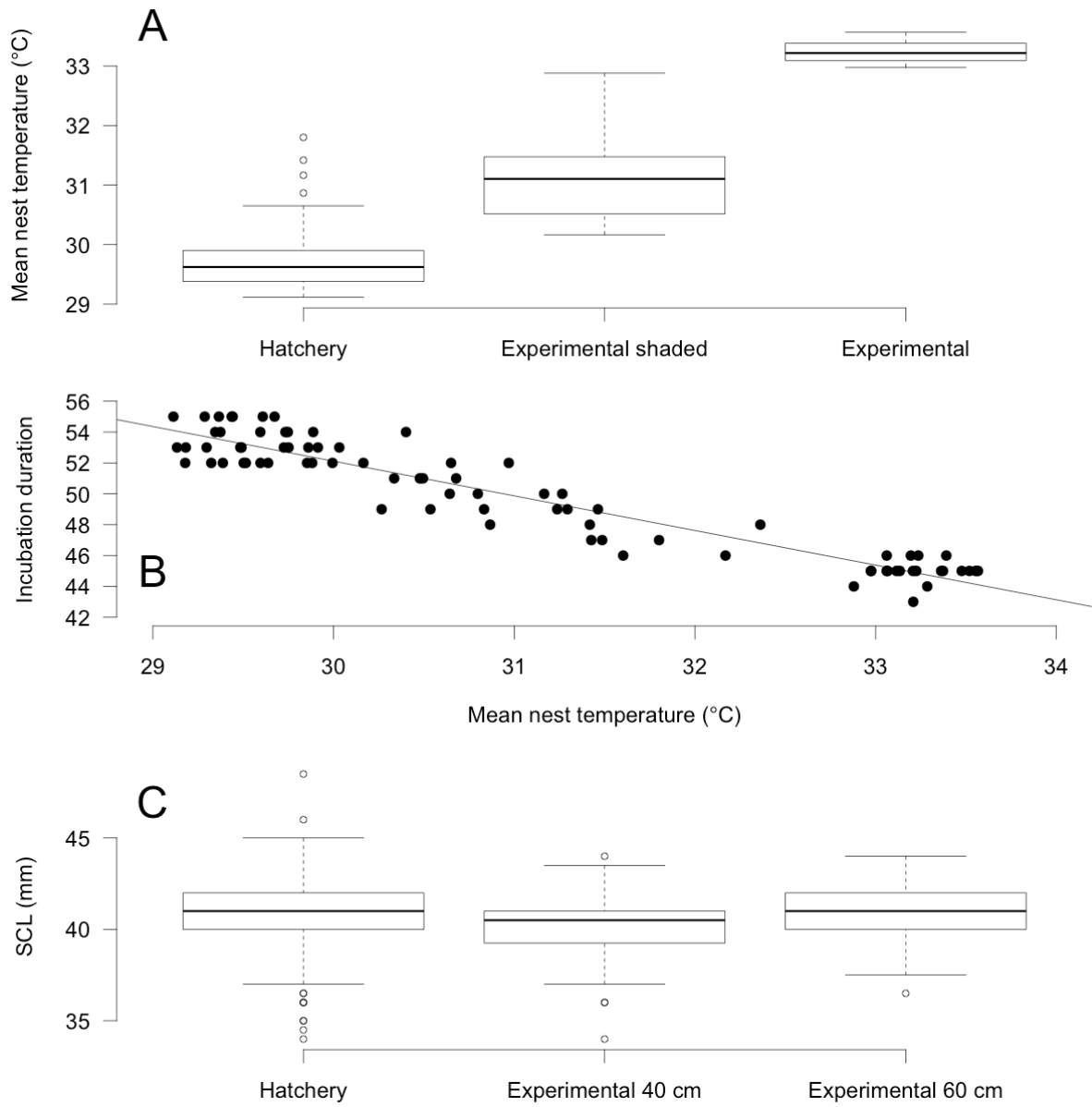


Figure 4: Average of the daily standard deviation of temperatures recorded in nests from each treatment (shading status and depth).

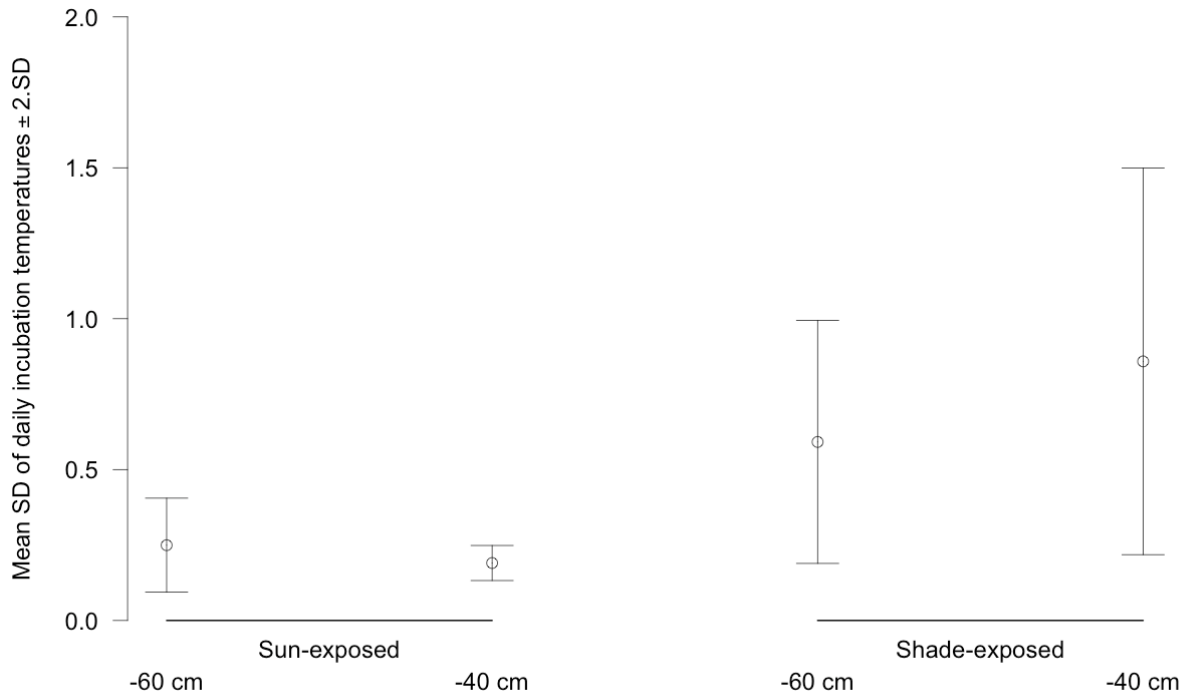


Figure 5: Fitted straight carapace length (SCL) depending on incubation time for the 80 monitored nests of *Lepidochelys olivacea*. The horizontal lines are the observed hatching size and twice the standard deviation.

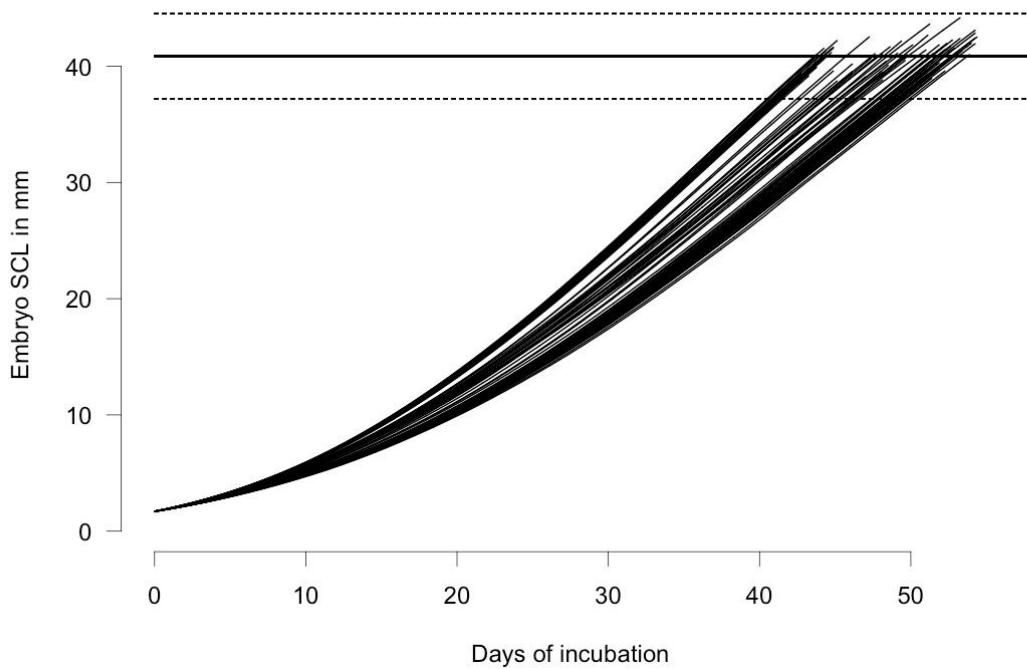
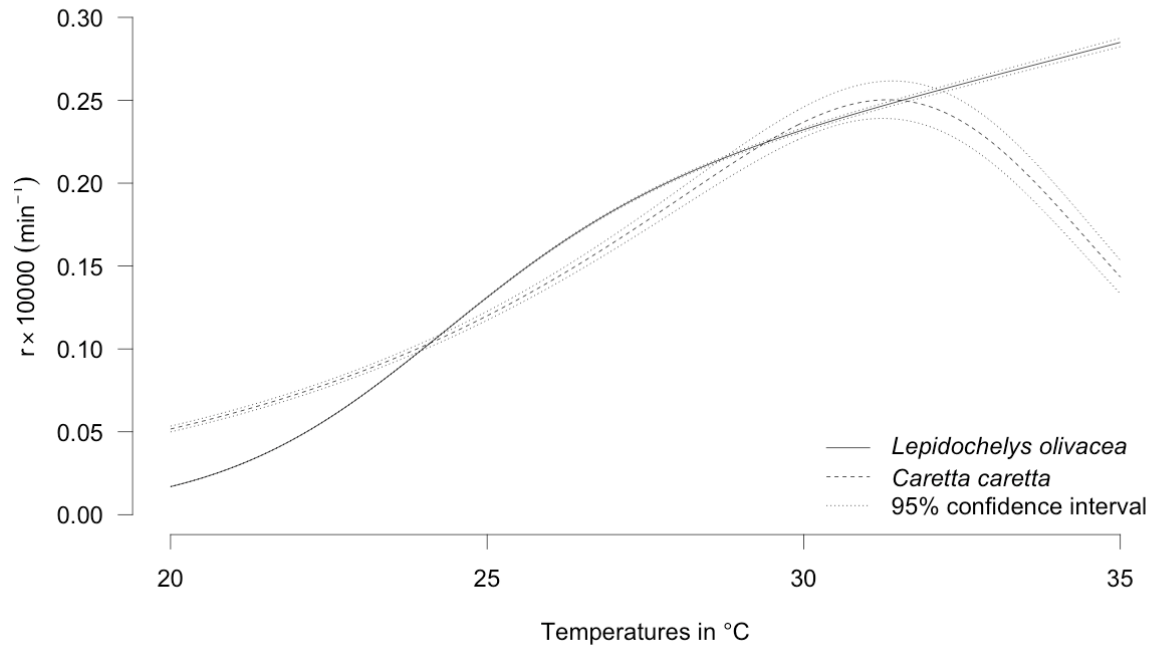


Figure 6: Fitted growth rate $r(T)$ of straight carapace length (SCL) depending on incubation temperature T for *Lepidochelys olivacea* and *Caretta caretta* (Monsinjon et al. In press). The envelope in dashed lines is twice the standard errors.



ANNEXE 3 : Delimitation of the embryonic thermosensitive period for sex determination using an embryo growth model reveals a potential bias for sex ratio prediction in turtles

Delimitation of the embryonic thermosensitive period for sex determination using an embryo growth model reveals a potential bias for sex ratio prediction in turtles

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Abstract: The sexual phenotype of the gonad is dependent on incubation temperature in many turtles, all crocodylians, and some lepidosaurians. At hatching, identification of sexual phenotype is impossible without sacrificing the neonates. For this reason, a general method to infer sexual phenotype from incubation temperature is needed. Temperature influences sex determination during approximately one third of the embryonic development, starting when the gonad begins to form. However, due to the technical difficulties associated with determining the timing of the TSP in natural nests, current methodology uses the middle third of incubation duration as a proxy for the middle third of development. While valid for constant incubation temperatures, this approximation has never been tested for fluctuating regimes of temperature, like those found in natural nests. A new method based on modelling the embryo growth is proposed, allowing the precise identification of the thermosensitive period for sex determination from a natural regime of temperatures. Results from natural nests and simulations show that the approximation of the thermosensitive period for sex determination to the middle third of incubation may create a quasi-systematic bias to lower temperatures when computing the average incubation temperature during this period and thus a male-bias for sex ratio estimate.

Keywords: Temperature, incubation, reptile, turtle, norm of reaction, temperature-dependent sex determination, thermosensitive period, sex ratio

1. Introduction

Sex determination is the process whereby an embryo has the ability to become male or female. Temperature-dependent sex determination (TSD), a special case of environmental sex determination, is frequent in reptiles: all crocodylians have TSD, as do many turtles and some lepidosaurians. In such a system, the sexual phenotype of the embryo depends on incubation temperature during part of development. Sex determination is not a point process but rather spans several days during development (Raynaud and Pieau 1985). The time during development when temperature influences the sexual phenotype of the gonad is referred to as

the thermosensitive period (TSP) for sex determination. This article focuses on how to evaluate the TSP when temperature changes during embryological development.

Mrosovsky and Pieau (1991) defined the TSP as the interval of time when the variation of temperature (any kind of temperature variation) has been shown to have some significant effect (any kind of effect) on sex ratio. From an operational perspective, development is a continuous process that is discretized into embryological stages. To specify when the TSP occurs and to discover the embryological stages with which it is associated, eggs have been shifted once or twice at different stages of embryonic development, from a male- to a female-producing temperature (or vice versa) (Bull and Vogt 1981, Pieau and Dorizzi 1981, Yntema and Mrosovsky 1982, Bull 1987, Burke and Calichio 2014). Using these methods, it has been demonstrated that the TSP begins with the formation of the genital ridge, which is the onset of the gonad formation and coincides approximately with the middle third of incubation at constant temperature in all species that have been studied to date (Bull and Vogt 1981, Pieau and Dorizzi 1981, Yntema and Mrosovsky 1982, Bull 1987, Wibbels et al. 1991, Merchant-Larios et al. 1997, Young et al. 2004). The embryonic stages 21 and 26 according to Miller (1985), mark the limits of the TSP for all studied sea turtles (Pieau and Dorizzi 1981, Yntema and Mrosovsky 1982, Bull 1987, Desvages et al. 1993), including *Caretta caretta*. Other systems for delimitation of developmental stages are used in some other species (e.g. Yntema 1968, Rhen and Lang 1994, Werneburg 2009) and the limits of the TSP must be defined accordingly within these other systems.

Most of the data on the relationship between temperature and sex determination in reptiles with TSD have been obtained from artificial incubation at constant temperatures. However, estimating the TSP for fluctuating regimes of temperature is useful in making predictions on the sex ratio of hatchlings from natural nests. At hatching, identification of sexual phenotype is impossible without sacrificing the neonates and a general method to infer sexual phenotype from incubation temperature is needed. Given the difficulty of estimating the TSP for natural regimes of temperature, many authors use the middle third of incubation as a proxy for the TSP (e.g. Wibbels et al. 1991, Hanson et al. 1998, Godley et al. 2002, Kamel and Mrosovsky 2006, Merchant-Larios et al. 2010, LeBlanc et al. 2012, Sarı and Kaska 2015). Here, we use a model of embryo growth for *Caretta caretta* (Girondot and Kaska 2014) to determine the time at which the embryos reach the sizes that correspond to the beginning and the end of the TSP under fluctuating temperatures. The TSP is estimated for natural nests and simulated regimes of temperatures that may be encountered in natural nests. Our results show that the temporal

duration and the location of the TSP may shift considerably depending on the thermal regime experienced and that the estimated average temperature during the middle third of incubation is a quasi-systematically male-biased estimate of the average temperature during the thermosensitive period of incubation.

2. Materials and methods

2.1. The model of embryo development

First, we briefly explain the embryonic growth model proposed by Girondot and Kaska (2014). The model integrates within a single framework:

- (i) embryo growth modelled using the Gompertz equation (Gompertz 1825). This model uses 3 parameters, X_0 for the size at time 0, K for the asymptotic metric and $a(T)$ being the growth rate which is dependent on temperature T .
- (ii) the dependency of the growth rate on temperature—also called the growth rate thermal reaction norm named $a(T)$ with T being a temperature. The biological temperature-dependent rate models based on the Arrhenius and Eyring equations were formulated by Schoolfield et al. (1981). These equations allow the modelling of any biological temperature-dependent process when rates are low at both low and high temperatures, thus showing an optimum at intermediate temperatures. Two models of thermal reaction norms were described by Schoolfield et al. (1981), one with 6 parameters and one being a simplified version using only 4 parameters. A Weibull model (Weibull 1951) was also tested as being a simpler model using only 3 parameters.

The models for growth rate dependency on temperature and embryo growth were fitted using maximum likelihood to best describe the observed hatchling size according to a time series of temperature within the nests. The 3-, 4- and the 6-parameters models for thermal reaction norm were compared using the Akaike Information Criterion (Akaike 1974) corrected for small sample size (AICc) (Burnham and Anderson 2002). The AICc measures the quality of fit, penalizing for too high a number of parameters in the model. The confidence interval for the $a(T)$ function was calculated using the delta-method (Davison 2003).

2.2. Developmental table and TSP limits

Developmental stages, days of incubation and straight carapace length (SCL) were described for *Caretta caretta* from the Eastern Mediterranean (Kaska and Downie 1999). The relationship between SCL and days of incubation was fitted using a Gompertz equation

(Gompertz 1825). The fit was done using bivariate normal distribution as uncertainties on both incubation days and SCL size were reported. Confidence interval was built based on the square-root of the diagonal of the inverse of the Hessian matrix.

The mean SCL size at the beginning of the TSP (stage 21 defined by Miller 1985) and the end of the TSP (stage 26) are 0.84 mm (n=21) and 2.35 mm (n=125) respectively (Kaska and Downie 1999). The mean SCL size at hatching time is 39.33 mm (Girondot and Kaska 2014). Then the ratio of the SCL at beginning and end of TSP over SCL at emergence are 0.21 and 0.71 for *Caretta caretta*. SCL at emergence varies slightly among nests but the ratios were assumed here to be constant. When X (SCL at emergence) is known, the sizes at the beginning and end of TSP are then $X \times 0.21$ and $X \times 0.71$ respectively. Knowing these sizes, it is possible to locate precisely the TSP during incubation as being the time during incubation when embryo SCL crosses these limits.

2.3. Field nests

A total of 26 time-series of temperatures and corresponding incubation durations from the Eastern Mediterranean *Caretta caretta* population were obtained from a literature survey. The temperatures of 21 loggerhead turtle nests were recorded during the nesting season on Dalyan Beach (Turkey) (Sarı 2011), two on Fethiye beach (Turkey) (Kaska et al. 2006), two in Alagadi (Northern Cyprus) (Godley et al. 2001a) and one on Patara beach (Turkey) (Öz et al. 2004) using Tiny Talk temperature recorders (resolution 0.37°C, Orion Components Ltd., UK). These were placed in the middle of the nests (ca. 45 cm depth) during oviposition, on the same night or the following morning in the case of relocated nests (Godley et al. 2001a, Öz et al. 2004, Kaska et al. 2006, Girondot and Kaska 2014). Development of the embryo was modelled for each of these nests and the limits of the thermosensitive period for sex determination were established based on embryo size as described above.

2.4. Simulated nests

Eight time series of temperatures are simulated to represent theoretical situations matching the most common field observations. The temperature is maintained constant (A) to mimic incubation in laboratory at constant temperature. An increasing trend in temperature (B) can result from the phenology of the nesting season when nesting begins in a cooler period (Binckley et al. 1998, Baptistotte et al. 1999, Casale et al. 2000, Doody et al. 2004), but it can also be a result of metabolic heating during development (Broderick et al. 2001, Godley et al. 2001b, Zbinden et al. 2006). A decreasing trend in temperature (C) mimics the incubation

when nesting begins in a warmer period (Leslie et al. 1996, Godley et al. 2001a, Kamel and Mrosovsky 2006). Nesting may occur just before a drop in temperature (D) (Mrosovsky 1994) or just before a peak of seasonal temperature (E) (Mrosovsky and Provancha 1992, Godley et al. 2002). Three regimes of temperature are used to mimic the cooling effect of heavy rainfall (Houghton et al. 2007) or nest wash over by the sea (Mrosovsky et al. 1984) at the beginning (F), middle (G) and end (H) of incubation. Development of the embryo was modelled for each of these simulated temperature regimes and the limits of the thermosensitive period for sex determination were established using the 0.21 and 0.71 SCL ratios (see above).

2.5. Statistics describing incubation

Different statistics were calculated for each nest:

- Timing and duration of the middle third of incubation;
- Timing of the thermosensitive period for sex determination (TSP);
- Average temperature during: (i) the total incubation, (ii) the middle third of incubation, and (iii) the TSP;
- Average temperature weighted by the growth rate of embryo (The Chu et al. 2008) during: (i) the total incubation, (ii) the middle third of incubation, and (iii) the TSP. The formula used is $\sum(T_i \Delta SCL_i) / \sum \Delta SCL_i$, where T_i and ΔSCL_i are the temperature and change of SCL during the time interval i , respectively.

The difference between TSP and middle third of incubation average temperatures, or temperatures weighted for growth of embryo, permits us to quantify the error made when the middle third of incubation is used as a proxy of TSP.

2.6. Sex ratio estimates

The relationship between constant incubation temperatures and sex ratios for *Caretta caretta* nesting in Greece (Mrosovsky et al. 2002) was fitted using maximum likelihood under binomial distribution and logit link (Girondot 1999). The sex ratio and its 95% confidence interval can be predicted using this relationship from any constant temperature equivalent (CTE). Four CTEs were used: average temperature and average temperature weighted by embryo growth rate, each being calculated during the middle-third of incubation time or during the TSP.

3. Results

3.1. Developmental table

Developmental data fitted to the incubation days show a tendency of SCL to slow down growth just before hatching (Figure 1). The fitted values are $X_0=0.34$ mm (SE=0.19 mm) and $K=47$ mm (SE=3.59 mm) and then $rK=K/(SCL \text{ at emergence})=1.209$ ($r^2=0.97$). These values are very similar to the values fitted for the development of European freshwater turtle, *Emys orbicularis*, $X_0=0.37$ mm and $rK=1.209$ (Monsinjon et al. In press)

3.2. Embryo growth thermal reaction norm

Three models of thermal reaction norms have been fitted using the 26 nests from Turkey and Cyprus: one with 6 parameters, one being a simplified version using only 4 parameters (Schoolfield et al. 1981) and the last one being the 3-parameters Weibull model. The simple model using 3 parameters shows the lower AICc and higher Akaike weight (3-parameters, AICc=92.01; 4-parameters, AICc=92.74; 6-parameters, AICc=95.25; Akaike weight for 3-parameters model=0.52) and has been retained thereafter (Goodness of fit using Pearson chi-square=1.36, $p>0.999$). Figure 2 shows the embryo growth rate thermal reaction norm and its 95% confidence interval. The histogram of temperatures available for these 26 nests is shown at the bottom of the thermal reaction norm.

3.3. Statistics about field nests incubation

The temperature profiles from 26 *Caretta caretta* nests in the Eastern Mediterranean show a general pattern of increasing temperatures with time, which occurs during the advance of summer in this region. Metabolic heating is also observed in some nests (Godley et al. 2001a). The TSP has been determined for each of these nests (Figure 3). The constant temperature equivalent (CTE), calculated as average temperature during the TSP or growth-weighted average temperature during the TSP, may be higher or lower than the one calculated for the total incubation period (Figure 4, first row). The range of differences is from 0.49 to 1.08 °C (using average temperature) or 0.56 to 0.20 °C (using growth-weighted average) respectively. In contrast, the average temperature during the TSP is generally higher than the one estimated during the middle third of incubation (Figure 4, second row). The range of differences is from 0.07 to 0.89 °C (using average temperature) or 0.06 to 0.66 °C (using growth-weighted average) respectively.

The relationship between sex ratio and constant incubation temperatures for *Caretta caretta* in Greece is shown in figure 5A. The sex ratios predicted for the different nests when CTE is the

average temperature or the average temperature weighted by growth rate are shown in figure 5B and 5C respectively. As expected, predicted sex ratios are unchanged when temperatures are very low or very high (Figure 5B and C). However, for a few nests, sex ratios calculated using temperatures recorded during the middle-third of incubation (y-axis) are significantly masculinized as compared with the sex ratios calculated using temperatures recorded during the TSP (x-axis). For example, the sex ratio of the top-right nest from Figure 3 was estimated to be 17% (male relative frequency) by Öz et al. (2004), using the average temperature during middle-third incubation as proxy. In contrast, we predict a sex ratio of 0% (95% confidence interval 0 to 3%) for this nest, using both the average and the growth-weighted average temperatures during the TSP. Similar comparison using literature could not be made for the other nests from our dataset.

3.4. Simulated nests

To extend the study to other incubation profiles possibly found in nature, the following eight temperature regimes were generated: (A) no temperature trend, (B) increasing and (C) decreasing temperature trends, (D) U-shaped and (E) inverted U-shaped trends, and a cooling pulse at the (F) beginning, (G) middle, or (H) end of incubation. First, our results confirm the standard observation that at a constant incubation temperature the limits of the TSP are very close to the limits of the middle third of incubation (Figure 6A, Table 1). When the temperature increases during incubation, the TSP is shifted to a later period of time than the middle third of incubation, and when the temperature decreases during incubation, the TSP is shifted to an earlier period of time (Figure 6B & 6C, Table 1). As expected, when lower temperatures occur in the middle of incubation, the TSP is longer than the second third of incubation and when higher temperatures occur in the middle of incubation, the TSP is shorter than the second third of incubation (Figure 6D & 6E, Table 1). For cooling events, the TSP is shifted later or earlier than the middle third of incubation when the cooling occurs before or after the TSP respectively (Figure 6F & 6H, Table 1). The TSP is longer than the middle third of incubation when temperature cooling occurs during the TSP (Figure 6G, Table 1).

When temperature is not constant during the TSP, the average temperature during the TSP is different from the average temperature during the second third of incubation. An interesting result is that this difference is always positive or null (Table 1): the mean temperature during the TSP is higher than the mean temperature calculated for the second third of incubation. A bias toward lower values during the middle third of incubation, as compared to the TSP, is

also found when growth-weighted average temperature is used instead of average temperature (Table 1).

4. Discussion

The embryonic thermosensitive period for sex determination is a useful concept in species with temperature-dependent sex determination. It permits to delimit the range of development when the temperature influences the sexual phenotype. When eggs are incubated at constant temperature, the TSP is located in the middle third of incubation (Yntema 1979, Bull and Vogt 1981, Yntema and Mrosovsky 1982). From this information, many studies also used the “middle third rule” for nests incubated in natural conditions when temperature changes during incubation. A recent survey of literature about temperature-dependent sex determination in marine turtles (Rees1 et al. 2017) retrieved 18 papers published between 2011 and 2016 studying sex ratio and nest temperature. Among these, 15 out of 18 used middle third incubation average temperature and 5 out of 18 used total incubation average temperature to infer nest sex ratio based on recorded temperature. However, whereas the “middle third rule” can apply at constant temperature, the validity of its use at changing incubation temperatures had never been evaluated.

In the present study, we characterize the TSP using an embryo growth model. We fit the thermal reaction norm of embryo growth rate for the population of *Caretta caretta* in the Eastern Mediterranean (Figure 1). The reaction norm obtained differs slightly from the one described in Girondot and Kaska (2014) because new nests have been added with higher incubation temperatures that were absent in the previous study. The inclusion of nests from other places in western Turkey and Cyprus is justified by the high rate of genetic exchange between these nesting sites (Carreras et al. 2007).

Using this reaction norm, the growth of embryos for natural and simulated regimes of temperature was modelled. We show that the exact timing of the thermosensitive period depends on the regime of temperature experienced during incubation and that the TSP generally differs from the middle third of incubation. This result is obtained for temperature regimes experienced in natural nests (Figure 3) as well as simulated temperature regimes (Figure 6; Table 1). As embryo growth rate depends on temperature, a variation in temperature during incubation translates into a variation of embryo growth rate. A higher variation in temperatures or steeper reaction norms lead to higher change in embryo growth rate. The dependency of embryo growth rate on temperature thus results in the temporal shift

between TSP and middle third of incubation observed both for the 26 natural nests and the 8 simulated nests. A temporal shift between the TSP and the middle third of incubation is the normal situation in the wild, including other *Caretta* populations or other turtle species, because the regime of temperature in natural nests usually shows variation.

The shift of the thermosensitive period, as compared to the middle third of incubation, has an effect on the average temperature experienced during the time when sex is determined. In the studied conditions, the average temperatures or the growth-weighted average temperatures computed during the middle third of incubation are generally lower than the ones estimated during the TSP. The use of the middle third of incubation as a proxy for the TSP is thus likely to produce an underestimation of the temperature experienced during sex determination. Thus, the sex ratio predicted from the average temperature during the middle third of incubation is expected to be too masculinised as compared to the true sex ratio. This result was obtained for 24 out of the 26 studied natural nests (Figure 4) and for simulated regimes of temperatures when temperature varies during TSP (Table 1). Finally, we observe that the average temperature during total incubation reflects very poorly the average temperature during the TSP (Figure 4, Table 1). Total incubation temperature as a proxy of TSP temperature to estimate sex ratio would produce too much error to be of any use.

The quasi-systematic lower temperature during the middle third incubation as compared to the temperature during the TSP can be explained by linking the change of growth rate to the change of temperature. For example, when temperature increases during incubation, a progressive shortening of embryological stage duration is observed as long as incubation progresses. Consequently, the time from the beginning of incubation to embryological stages corresponding to the beginning of the TSP is longer than one third of incubation. Similarly, the time to get from embryological stages corresponding to the end of TSP to the end of incubation is shorter than one third of incubation. As a result, the TSP is shifted later than the middle third of incubation and the temperatures within the TSP are enriched in higher temperatures as compared to the middle third incubation period.

A general observation is that the TSP is shifted as compared to middle third of incubation at a time when growth rate is higher (Figure 6). Because growth rate is an increasing function of temperature for most of the temperature range experienced in natural conditions (Figure 1), a period of higher growth rate corresponds to higher temperatures. This explains why the average temperature during the TSP is higher than during the middle third of incubation. We expect this difference to be higher when the embryo growth reaction norm is steeper.

However, for even higher incubation temperatures, growth rate is expected to become a decreasing function of temperature due to sub-lethal effect (Figure 2 in Pieau 1978). In this case, the opposite result is observed (not shown): average temperatures are lower for the TSP than for the middle third. However, the incubation at sub-lethal temperatures must be strongly counter-selected and this effect should be rarely seen in natural conditions.

Our results have some bearing in view of the many studies that use the middle third of incubation as a proxy for the TSP. There are two main types of studies that use the middle third of incubation for sex ratio prediction. Firstly, authors may draw a correlation curve between observed nest sex ratios and the average temperatures recorded in the nest during the middle third of incubation. This correlation is then used to infer sex ratios from new values of average temperature (Kaska et al. 1998, Kaska et al. 2006). The validity of this method depends on the close correlation between sex ratio and the average temperature. The average temperature during the TSP, as determined by a model of embryo growth as shown here, should thus improve the correlation, and therefore the accuracy of the method. Secondly, authors may try to directly predict nest sex ratios by comparing the average temperature measured during the middle third of incubation with the sex ratios obtained under incubation at constant temperatures (Binckley et al. 1998, Hanson et al. 1998, Broderick et al. 2001). In the presence of variation of incubation temperature, it has already been shown that this method performs poorly (De Souza and Vogt 1994, Valenzuela 2001). The first reason is that the embryo does not spend the same amount of development at high versus low incubation temperatures (Pieau 1982). The use of growth-weighted average temperatures has gone a long way in solving this problem (Georges 1989, Georges et al. 1994) and in this context a model of embryo growth such as the one described in this study should be helpful. However, our results suggest that the temporal shift in the TSP, as compared to the middle third of incubation, could still be responsible for a bias in sex ratio estimation (Figure 4, Table 1).

In the close nesting area of Zakynthos (Greece), the TRT was estimated to be 1.53 °C (Hulin et al. 2009). This means that an error in average temperature of 0.89 °C as observed in the present study when using middle third temperature rather than TSP temperature could result in a large overestimation of male proportion in nest sex ratio. Indeed, our results show that the production of males is overestimated if nest sex ratios are predicted from the average temperature measured during the middle third of incubation (Figure 5). The exact level of bias depends in a complex way on the nature of the temperature dynamics. Since the TRT is often

lower than 4 °C in turtles (Hulin et al. 2009), a significant overestimation of nest sex ratio towards males could happen when the same method is applied to other species or populations.

As highlighted before, the sex ratio deduced from average temperatures during the middle third of incubation is too masculinised when compared to the real sex ratio (Figure 5). This difference may have important implications for conservation of sea turtles. Indeed, the sex ratio in nesting beaches is sometimes highly biased towards female production (Mrosovsky and Provanha 1992, Marcovaldi et al. 1997). This observation has given rise to concerns about the viability of some populations. In cases where the middle third is used instead of the TSP for sex ratio prediction, the female bias could be more important than estimated. The necessity of a conservation program should thus be re-evaluated in light of our results. The protection of nesting beaches yielding both males and females or the choice of beaches for relocation could also be affected. In addition, when a female bias is underestimated, the impact of global warming on the sex ratio of nesting beaches would be even more pronounced than previously thought (Hawkes et al. 2007).

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Tables

Table 1: Shift for the beginning and end of the expected position of the TSP as compared to the middle third of incubation (MT), and difference in the average temperature (CTEa) or growth-weighted average temperature (CTEw-a) within the TSP as compared to during the middle third of incubation (MT) or total incubation (TI).

	Difference between TSP and middle third of incubation (MT) or total incubation (TI) for:					
	Begin TSP-MT (Days)	End TSP-MT (Days)	CTEa TSP-MT (°C)	CTEw-a TSP-MT (°C)	CTEa TSP-TI (°C)	CTEw-a TSP-TI (°C)
A: Constant	-0.77	0.51	0.00	0.00	0.00	0.00
B: Linear increase	4.47	4.31	0.60	0.53	0.60	-0.16
C: Linear decrease	-3.86	-3.81	0.50	0.45	0.50	0.36
D: U-shaped	-4.66	3.61	0.13	0.13	-1.31	-1.07
E: Inverse U-shaped	1.49	-1.28	0.12	0.10	2.52	1.34
F: Cooling at the beginning	2.30	2.05	0.00	0.00	0.91	0.20
G: Cooling at the middle	-2.31	2.05	0.51	0.27	-1.32	-0.50
H: Cooling at the end	-2.31	-2.56	0.00	0.00	0.91	0.29

Figures

Figure 1: Straight carapace length (SCL) in mm according to the duration of incubation in days for *Caretta caretta* in the Eastern Mediterranean. The ellipses show twice the standard deviations for both SCL and days of incubation. The curve shows the fitted Gompertz model and its confidence interval at 95%.

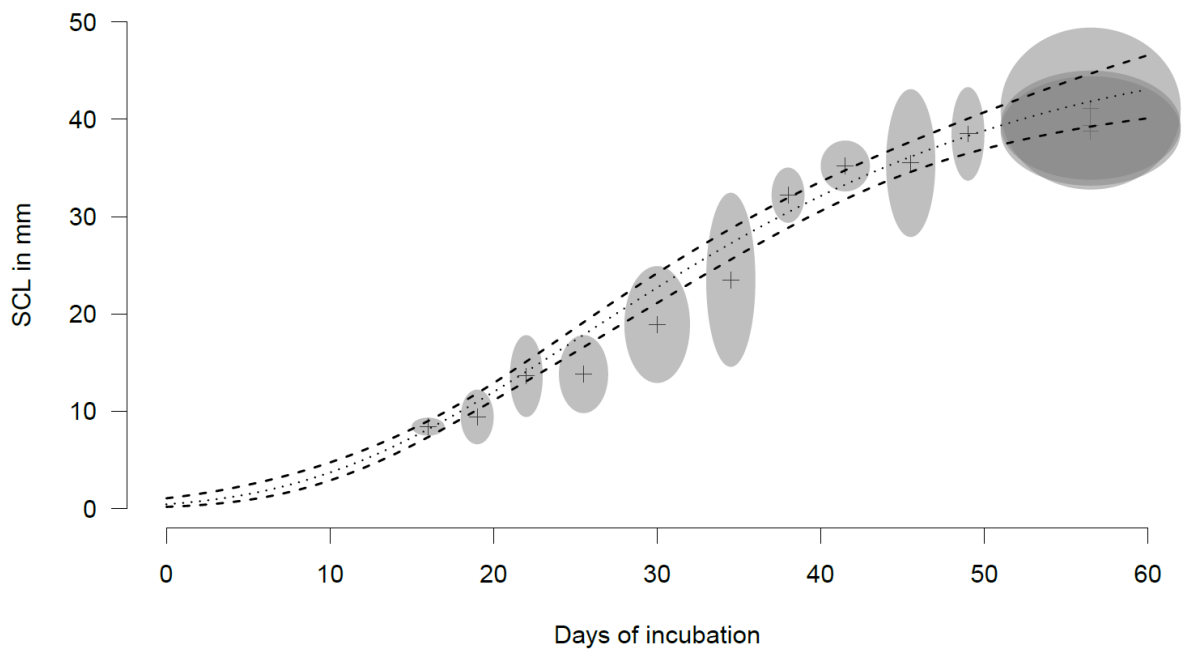


Figure 2: Thermal reaction norm of embryo growth in the marine turtle *Caretta caretta* from the Eastern Mediterranean, also showing the histogram of density of temperatures in nests included in the study. $a(T)$ is the instantaneous growth rate at temperature T (bold line) and dashed lines are the confidence interval at 95%.

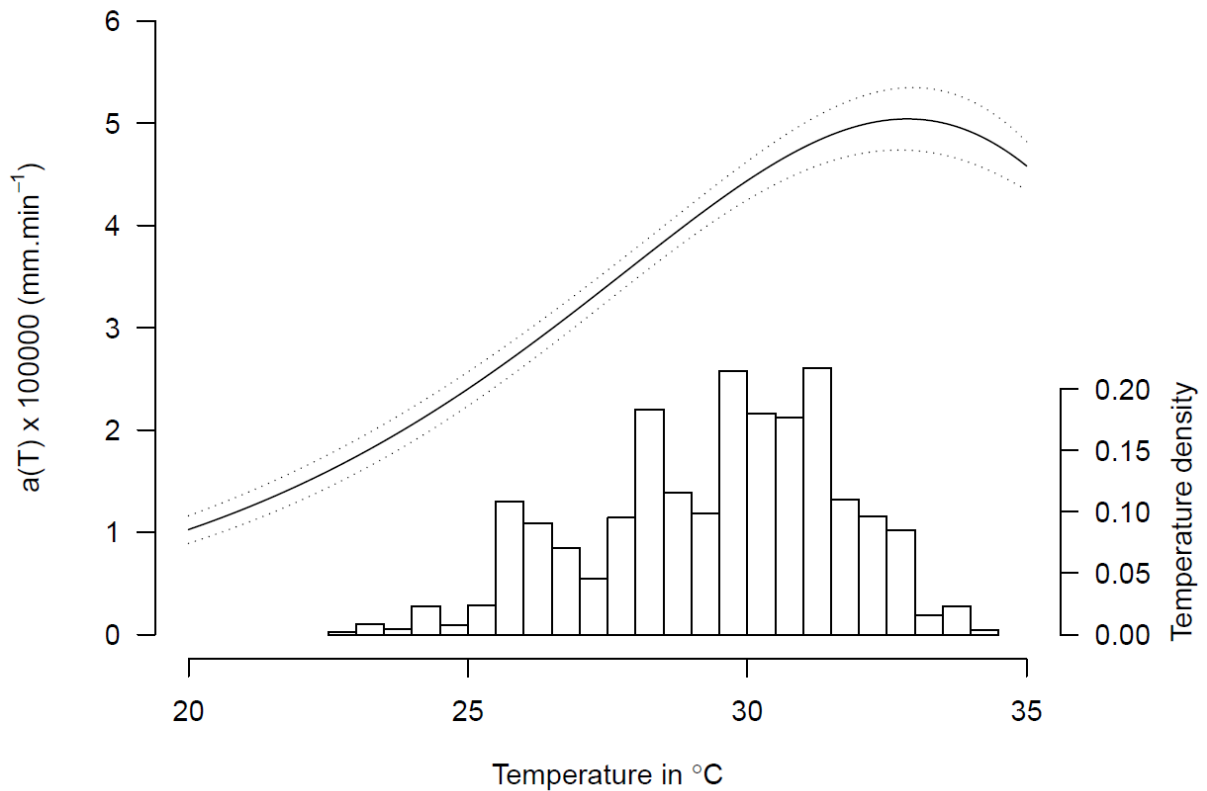


Figure 3: Embryo growth for 4 nests from the Eastern Mediterranean. Distribution of straight carapace length (SCL) at emergence (Girondot and Kaska 2014) is shown as horizontal plain and dashed lines for 95% confidence interval. Temperatures are shown as a grey line, while straight carapace length and its confidence interval (95%) are shown as continuous and dashed lines, respectively. The thermosensitive period (TSP) is shown in grey and its limits are defined based on embryo sizes that are projected onto the time axis to define the temporal position of TSP. Vertical dashed lines bind the middle third of incubation.

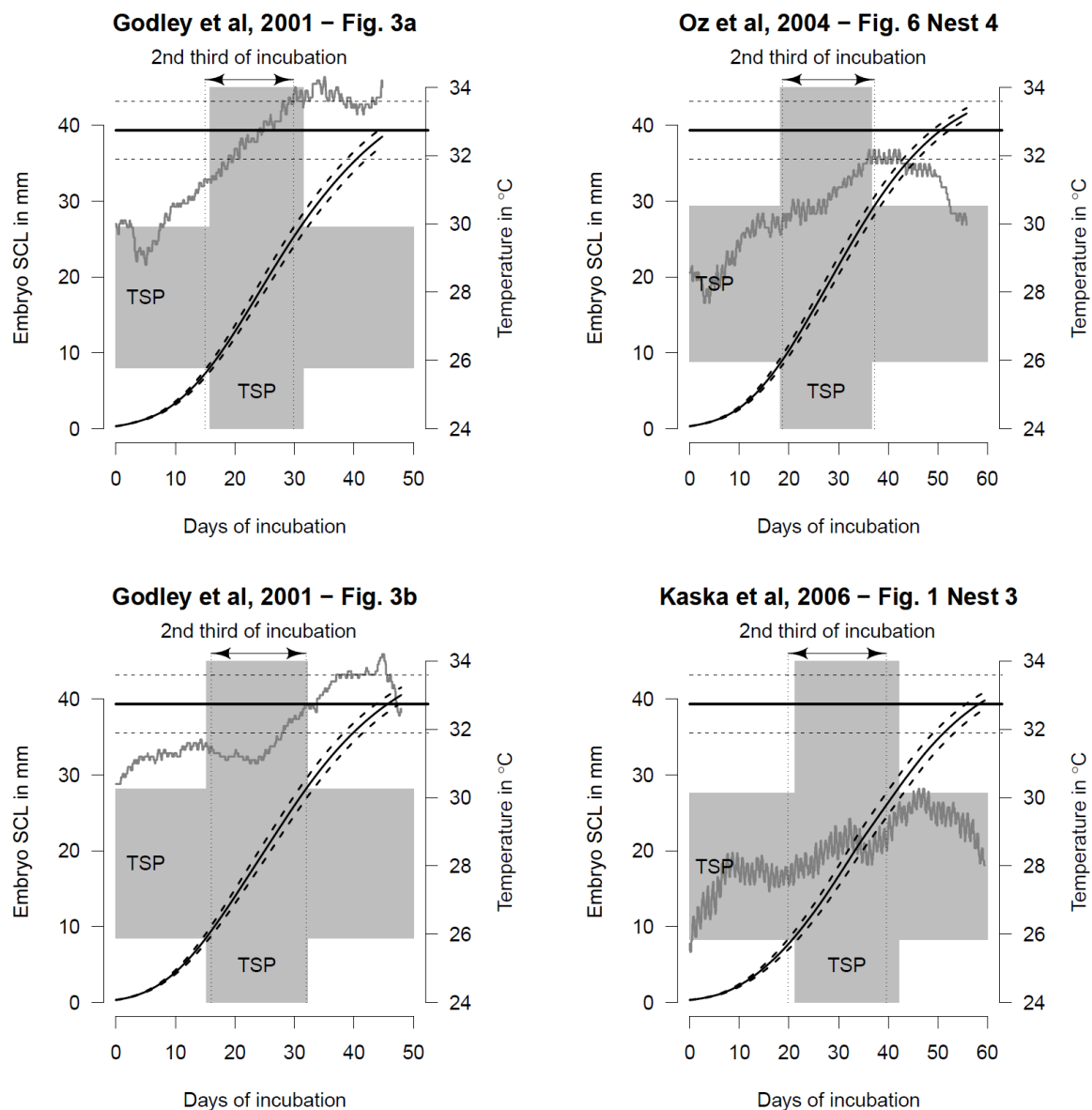


Figure 4: Distribution of difference for average temperature (1st column) or growth-weighted average temperature (2nd column) between the TSP and total incubation (1st row) or between the TSP and middle third of incubation (2nd row).

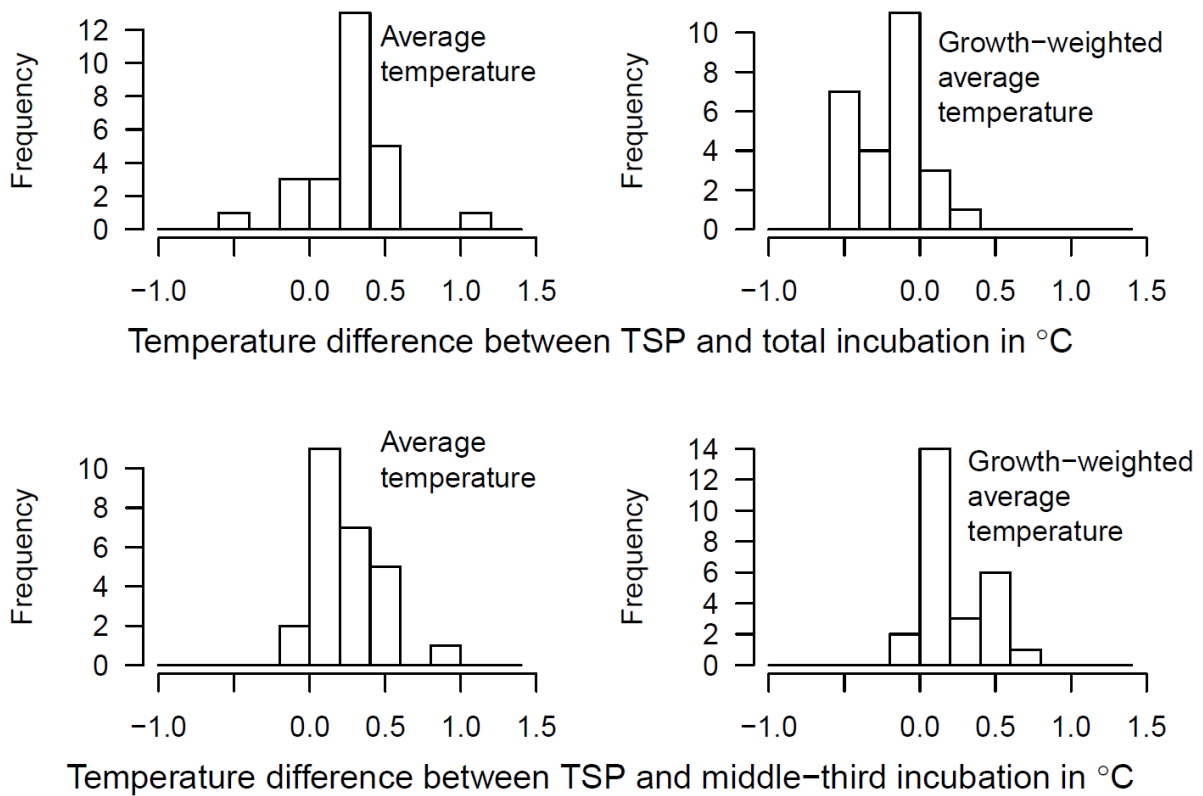


Figure 5: (A) Thermal reaction norm for sex ratio of *Caretta caretta* from Zakynthos population, Greece (95% confidence interval between dashed lines); (B) prediction of sex ratio based on average temperature and (C) average of temperatures weighted by embryo growth rate, calculated within the TSP (x-axis) or the middle-third of incubation (y-axis). The dashed line represents the equality between TSP- and middle-third-based sex ratio predictions. Bars represent 95% confidence intervals.

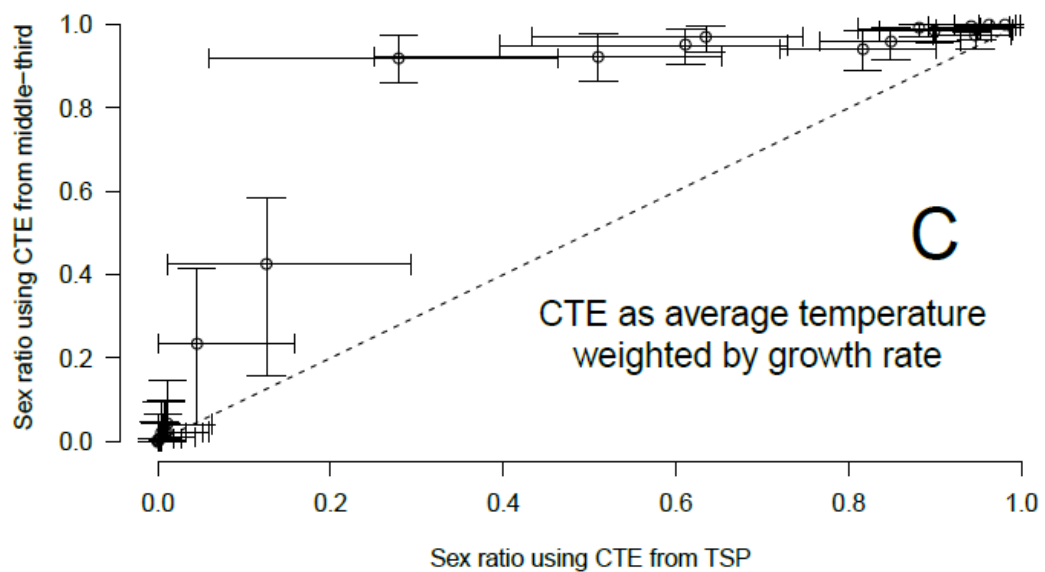
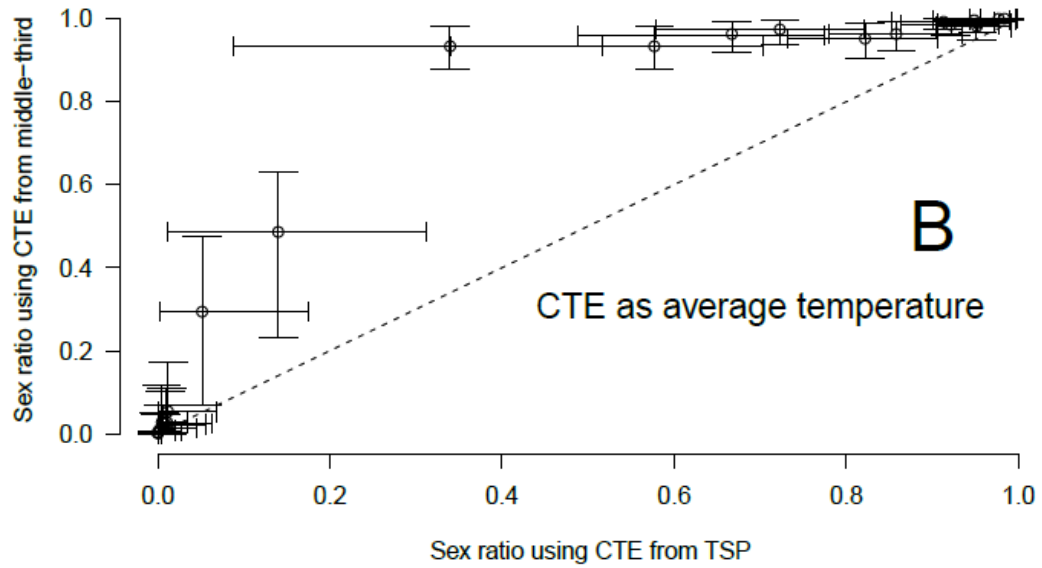
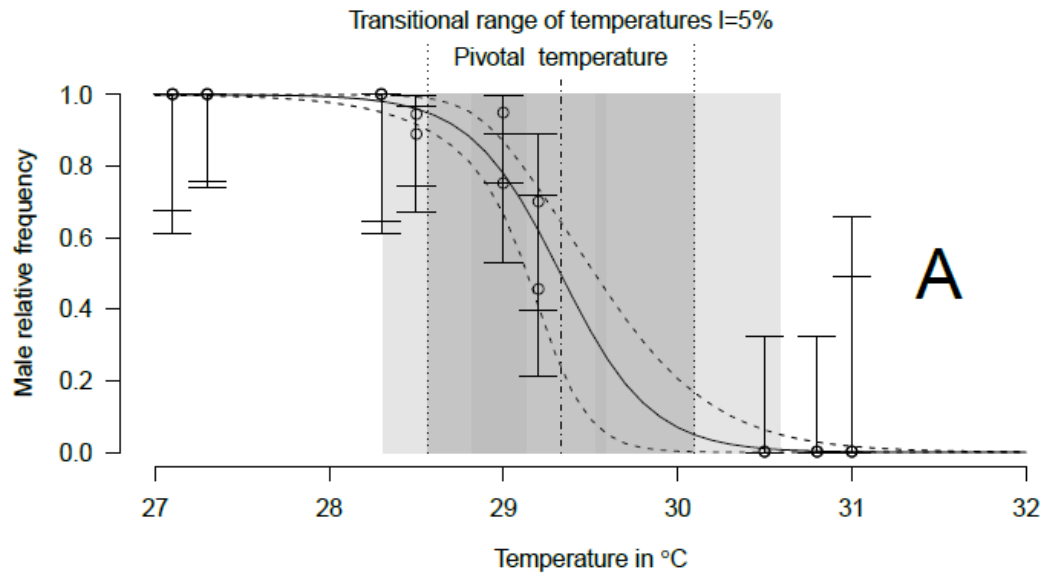
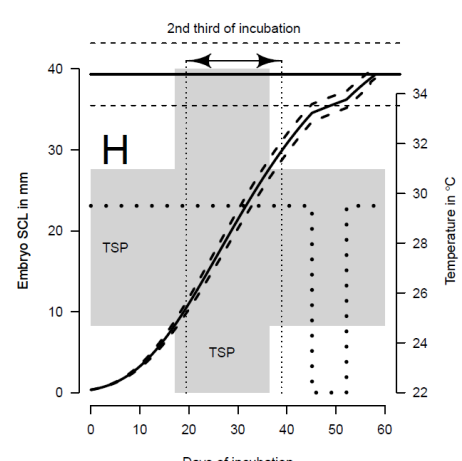
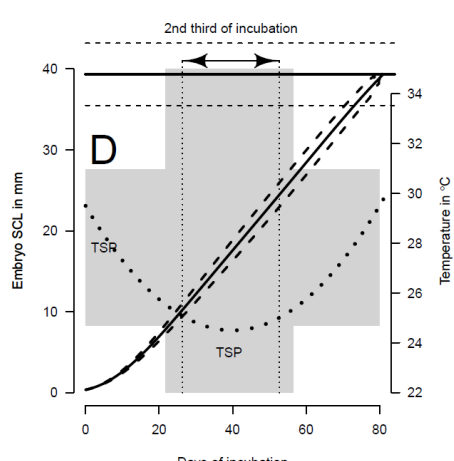
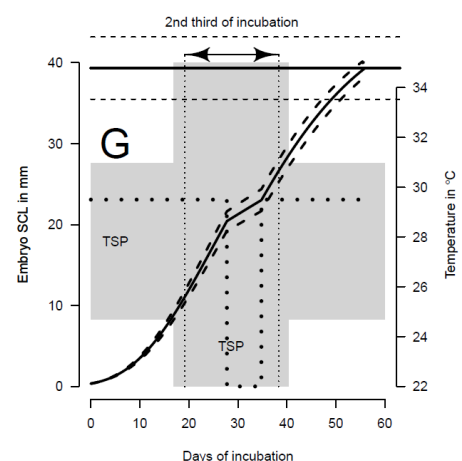
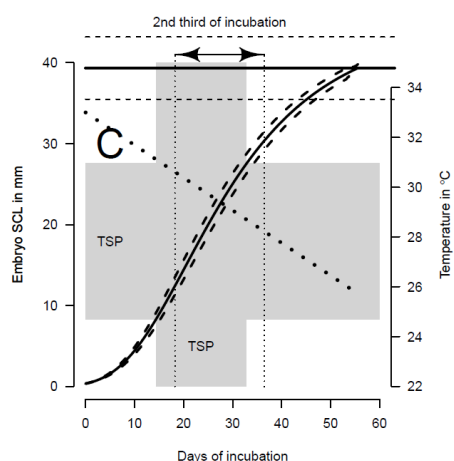
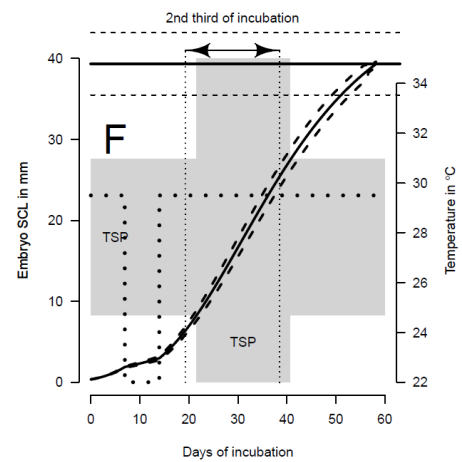
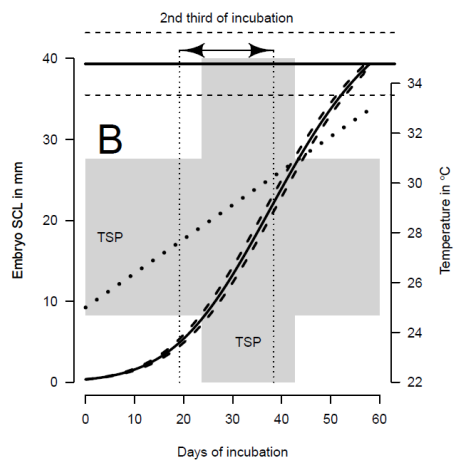
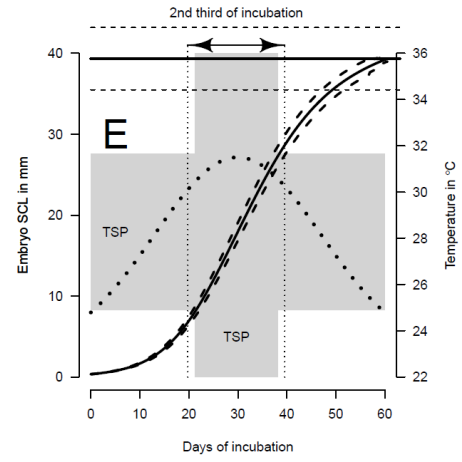
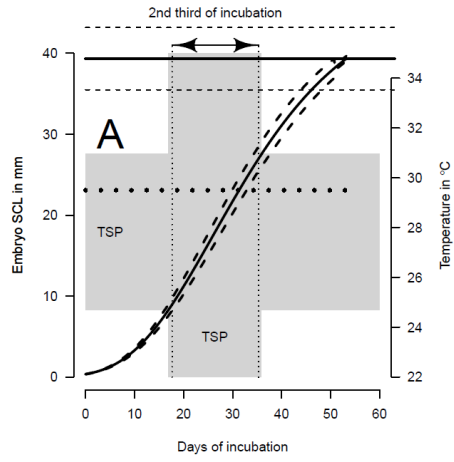


Figure 6: Patterns of embryo growth for different trends of temperature change during incubation. Distribution of straight carapace length (SCL) at emergence is shown as horizontal plain and dashed lines for 95% confidence interval. Temperatures are shown as a thick dotted line, while straight carapace length (SCL) and its confidence interval are shown as continuous and dashed lines, respectively. The thermosensitive period (TSP) is shown in grey and its limits are defined based on embryo sizes that are projected onto the time axis to define the temporal position of TSP. Trends in temperature are (A) Constant, (B) Linear increase, (C) Linear decrease, (D) U-shaped, (E) Inverse U-shaped, (F) Cooling at the beginning, (G) Cooling at the middle, and (H) Cooling at the end.



ANNEXE 4 : Les engins et techniques de
pêche utilisés dans la baie de Loango,
République du Congo, et leurs incidences
sur les prises accessoires

Les engins et techniques de pêche utilisés dans la baie de Loango, République du Congo, et leurs incidences sur les prises accessoires

by

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By-catch

Résumé. – Une étude a été menée pour évaluer les risques de captures accessoires par les différents engins et techniques de pêche utilisés dans la baie de Loango au Congo-Brazzaville. La liste des espèces cibles est donnée de même que les espèces non-ciblées. Parmi celles-ci, seules des tortues marines sont observées. L'étude montre que les filets dormants à grandes mailles sont plus nocifs pour les tortues marines que les filets à petites mailles. Les filets à petites mailles sont toutefois peu sélectifs et peuvent compromettre le renouvellement de la ressource. La réglementation congolaise n'autorise que des mailles dont la taille est supérieure à 10 cm. Cette situation correspond à un conflit d'usage entre la ressource à préserver et la biodiversité à maintenir. Cinq des sept espèces de tortues marines fréquentent le littoral congolais. La pêche artisanale constitue une importante menace pour ces espèces au Congo. Ces résultats seront mis à profit pour expérimenter un filet conforme à la réglementation locale et regroupant des caractéristiques identifiées comme pouvant réduire la fréquence des prises accidentelles de tortues marines tout en maintenant le taux de capture des espèces commerciales (poissons et crustacés).

Abstract. – Fishing gears and techniques used in the Bay of Loango, Republic of Congo, and their by-catch risks.

A study was undertaken to assess the by-catch risk in the different type of fishing gears and techniques commonly used in the Republic of Congo (Congo-Brazzaville). Targeted and non-targeted species were listed. Among the non-targeted species, only sea turtles were observed. The study shows that dormant-gillnets with large meshes are more noxious for sea turtles than small meshes dormant gillnets. Small meshes are nevertheless poorly selective and thus can compromise the renewal of the resources. The Congolese law does not allow meshes smaller than 10 cm stretched. According to the literature, other factors can influence the risk of sea turtle by-catch. The nets badly tightened or little vertical increase the risk, the implementation of delusions or phosphorescent or bright elements may reduce the risk. Five of the seven species of sea turtles are present along the coast of the Republic of Congo. Artisanal fishing is one of the main threats for these species. The results of this study will be used to create and experiment a fishing net prototype fulfilling the Congolese regulation requirements and putting together factors and specifications identified as able to reduce the frequency of sea turtle incidental captures while maintaining the commercial species catch rate (fishes and crustaceans).

Selon des statistiques de 2011, près de 30% des stocks de poissons mondiaux sont surexploités, 57% sont exploités à la limite supérieure de leur productivité et seulement 13% ne sont pas pleinement exploités (FAO, 2011). La plupart des zones de pêche subissent donc une très forte pression et de nombreuses espèces cibles sont surexploitées (Worm *et al.*, 2006). Les prises accessoires, définies comme les espèces non ciblées par l'activité de pêche subissent elles aussi de fortes pressions. Certaines de ces prises accessoires sont des espèces patrimoniales menacées et/ou protégées localement ou au niveau international. Citons les cétacés, les requins et les tortues marines (Moore *et al.*, 2009). Autant il est possible d'avoir de l'information, même si elle est biaisée et par-

tielle, de la part des pêcheries industrielles qui sont suivies, autant il y a un manque criant d'information sur l'impact de la pêche artisanale. Or celle-ci peut être prépondérante dans certaines régions du monde (Wallace *et al.*, 2010b). Par exemple, à notre connaissance, les seules données disponibles sur ce sujet au Congo datent de plus de 25 ans (Gobert, 1986).

Située à cheval sur l'équateur, la République du Congo est un pays d'Afrique centrale pourvu d'une façade maritime étroite de 170 km environ donnant sur l'Atlantique. Le Congo dispose d'une ZEE (Zone Economique Exclusive) qui s'étend sur environ 60 000 km² (FAO, 2006). Le plateau continental sur lequel s'effectue l'activité de pêche s'étend

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sur une largeur de 50 km et une surface estimée à environ 10 700 km² (FAO, 2006). Le littoral maritime borde la partie Ouest des départements du Kouilou et de Pointe-Noire au sud du pays (Fig. 1). La côte présente des baies (Pointe Noire, Loango) et des pointes (Pointe-Indienne, Pointe-Noire). La baie de Loango se situe sur ce littoral au Nord de la capitale économique Pointe-Noire. Cette baie est limitée au sud par le cap de Loango, appelé Pointe-Indienne, et l'embouchure du fleuve Kouilou au nord. Une bande rocheuse sous marine présente à la Pointe-Indienne (Giresse, 1980) constitue un lieu favorable au développement de la faune marine. Une intense activité de pêche artisanale a lieu dans cette zone que fréquentent de nombreuses espèces protégées par les lois nationales congolaises, comme les tortues marines, les baleines et les dauphins à bosse, mais également plusieurs espèces de requins (Ministère du Développement durable de l'Économie forestière et de l'Environnement, 2011). Il convient de noter que ces prises accessoires, outre leur impact négatif sur la biodiversité, posent un problème

pour l'activité économique de la pêche. En effet, les prises accessoires peuvent endommager les filets et être un danger pour les pêcheurs lors de la libération de l'animal, car les pirogues manquent de stabilité (Bourget *et al.*, 2012).

Pour remédier à cette situation, RENATURA Congo a initié en 2005 un programme de libération des captures accidentelles de tortues marines dans les filets de pêche artisanale (Bal *et al.*, 2007). Une mesure d'accompagnement consiste à fournir le matériel nécessaire à la réparation du filet endommagé en échange de la libération de l'animal. Plus de 1500 relâchers sont ainsi effectués annuellement depuis huit ans par les agents de terrain de cette ONG (Godgenger *et al.*, 2009). La libération des tortues ne constitue cependant pas une solution à long terme et devant la fréquence de leurs captures accidentelles, il est apparu nécessaire de chercher à réduire le nombre de captures dans les filets. C'est pourquoi il a été entrepris de recenser les engins et les techniques de pêche artisanale utilisés au Congo et de les comparer afin de mettre en évidence ceux qui minimisent les captures acci-

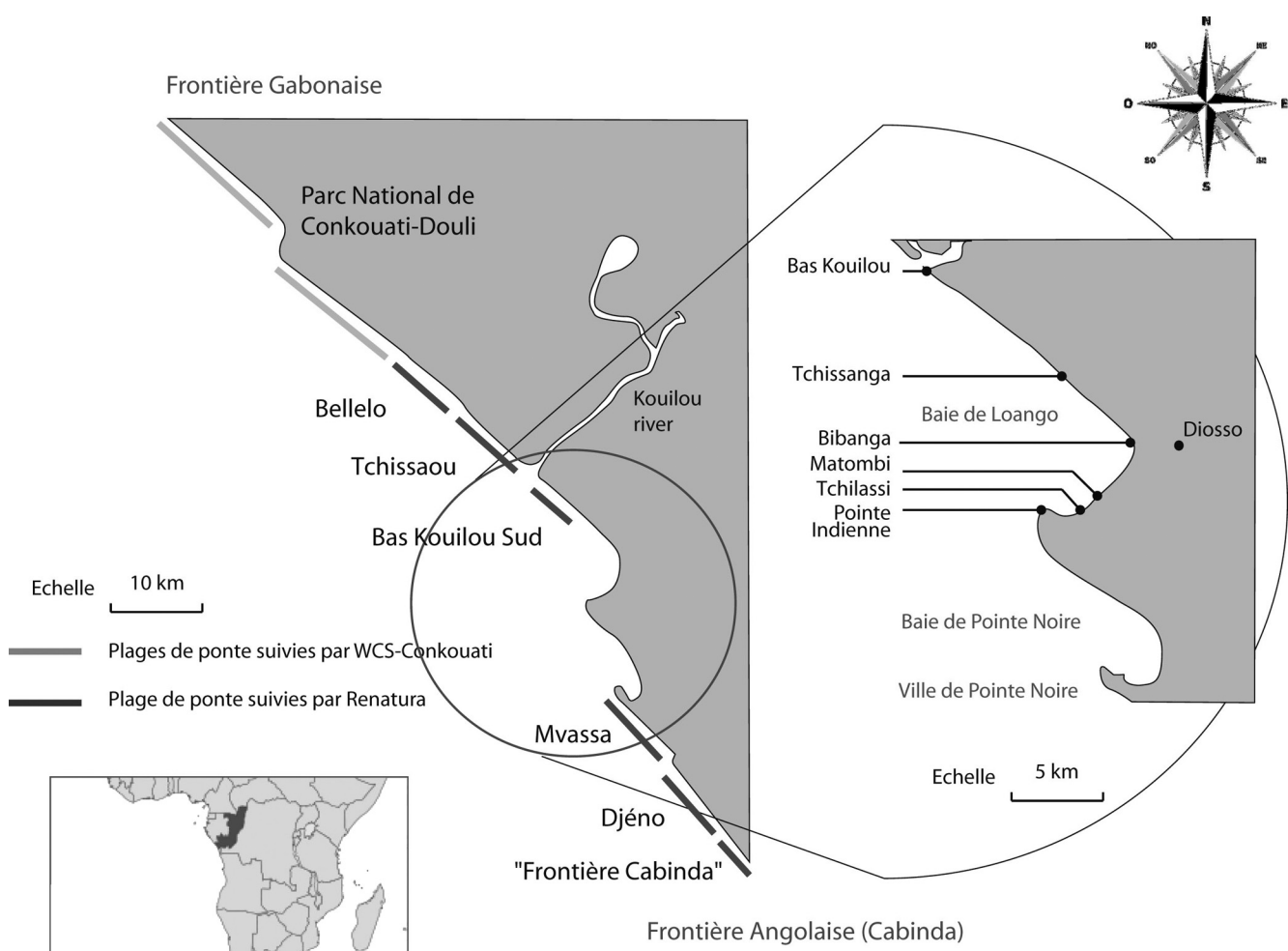


Figure 1. - Carte du littoral congolais (République du Congo) et de la zone d'étude : la baie de Loango. [Map of the Congo coastline (Republic of Congo) and of the study area: Loango Bay.]

dentelles de tortues tout en maintenant un niveau de prise acceptable sur les espèces cibles. Ces résultats seront mis à profit pour proposer une évolution des techniques de pêche rentable économiquement et limitant les prises accessoires.

MATÉRIELS ET MÉTHODES

L'étude s'est déroulée d'octobre 2010 à mai 2011 à la Pointe Indienne, un quartier côtier du village de Loango dont l'économie est principalement tournée vers la mer (Fig. 1). Environ 100 pêcheurs sont officiellement enregistrés dans ce quartier qui compte 460 habitants adultes (Direction Département de la Pêche et d'Aquaculture du Kouilou, 2010).

Enquête et entretiens

Une enquête a été menée sur la base du volontariat auprès de la communauté de pêcheurs à l'aide d'un questionnaire. Cette enquête a permis de décrire les différents engins et techniques de pêche utilisés dans la zone afin de déterminer pour chacun d'eux les milieux, les périodes, les espèces cibles et les fréquences d'utilisation. Il a également été demandé aux professionnels de la pêche d'évaluer les fréquences de capture et de mortalité des tortues marines pour chacune des techniques recensées.

Suivi des captures accessoires

Dans le cadre du programme de libération des captures accidentelles de tortues marines dans les filets de pêche artisanale, les agents Rénatura ont répertorié toutes les captures accessoires observées. Pour chaque événement de capture d'une tortue marine, ils ont ainsi enregistré quels étaient la technique de pêche et le type de filet en cause, et si la tortue capturée était retrouvée morte ou vivante à la levée du filet.

Les résultats de l'enquête, complétés par l'analyse rétrospective des données collectées dans le cadre du programme de libération des tortues marines, ont permis d'identifier les techniques les plus nocives pour les tortues marines afin d'orienter la suite de l'étude.

Expérimentation des techniques de pêche

Les techniques de pêche les plus souvent impliquées dans les prises accessoires de tortues marines ont été comparées expérimentalement. Deux équipes, composées chacune de deux pêcheurs, ont été mobilisées. L'une d'elle a utilisé des filets dormants avec des mailles de 6 à 8 cm (maille étirée) et l'autre des filets dormants à grandes mailles (12 à 20 cm de maille étirée). Dans la mesure du possible, ces filets ont été déployés de façon couplée, à faible distance l'un de l'autre, pendant les mêmes périodes, dans les mêmes zones, sur des fonds et à des profondeurs identiques. Selon les périodes, ils étaient utilisés flottants ou calés au fond.

Pour chaque événement de pêche, les coordonnées GPS et la profondeur du lieu où le filet était posé ont été relevées. Les heures de pose et de relevé ont également été notées afin de déterminer la durée pendant laquelle le filet était resté calé.

Les filets ont été déployés dans des eaux de 4 à 93 m de profondeur. Le temps de calée variait de 2 h à 16 h. Trente relevés ont été suivis pour chaque filet.

À l'issue de chaque événement de pêche, les poissons et les crustacés capturés ont été identifiés. Les prises étaient pesées individuellement ou par espèces pour les plus petites. Le nombre de tortues vivantes et mortes capturées par événement de pêche était enregistré.

Les résultats obtenus ont permis de comparer l'efficacité des engins et techniques de pêche ainsi que leur nocivité pour les tortues marines. Afin d'évaluer la valeur commerciale d'une pêche, un indice semi-quantitatif (de 1 à 3) de valeur commerciale a été attribué à chaque espèce ciblée. Ces indices ont été établis sur le terrain avec l'aide des pêcheurs et des intermédiaires achetant les poissons sur les sites de débarquement. Un indice de la valeur d'une pêche a été ensuite calculé en multipliant la masse d'une espèce pour un événement de pêche par son indice de valeur commerciale, puis en additionnant les valeurs obtenues pour chaque espèce capturée.

L'efficacité d'une technique de pêche a été évaluée à travers deux paramètres : la biomasse de poissons et de crustacés capturés par événement de pêche, et la valeur commerciale estimée des prises par événement de pêche. Les prises accessoires n'ont pas été comptabilisées dans cette estimation.

La nocivité des techniques de pêche a été estimée à travers, d'une part, le nombre moyen de tortues marines prises accidentellement par événement de pêche et, d'autre part, la proportion de tortues retrouvées mortes lors du relevé du filet.

Traitement statistique

Un modèle de régression linéaire est utilisé afin de rechercher, parmi les différents facteurs liés à la technique de pêche et au matériel utilisé, lesquels ont une influence significative sur les performances de la pêche puis sa nocivité en termes de nombre et de survie des captures accessoires.

Sachant que l'on ne peut pas présumer de la distribution normale des variables à expliquer et que le modèle inclut une mixité de variables explicatives qui peuvent être continues, discrètes, binomiales ou catégorielles, un modèle linéaire généralisé (GLM) a été utilisé. Quatre variables ont été successivement testées à l'aide de ce modèle : la masse de poissons et de crustacés capturés, la valeur commerciale estimée des prises, le nombre de tortues marines capturées, la proportion de tortues retrouvées mortes à la levée du filet. Les variables explicatives retenues pour le modèle GLM sont :

la technique de pêche utilisée, filet calé au fond ou flottant ; la taille des mailles du filet, mesurée maille étirée en cm ; la longueur du filet en mètres ; la hauteur de la colonne d'eau sur le lieu de déploiement du filet ; la proportion de colonne d'eau couverte par la hauteur de la partie maillée du filet ; le temps de calée, c'est-à-dire la durée écoulée en heures entre le filage et le virage du filet ; le temps linéaire sous la forme du nombre de jours écoulés depuis le début de l'étude ; la saisonnalité après transformation de la variable temps par une transformée de Fourier.

L'analyse a été réalisée à l'aide du logiciel libre R et du package "Mass" pour la fonction "glm", ainsi que le package "car" pour les fonctions diagnostique, notamment pour la détection des valeurs aberrantes et la vérification de la qualité de l'ajustement.

Les variables à expliquer continues "masse de poissons et crustacés" et "valeur commerciale des prises" ont été modélisées à l'aide d'une loi Gamma. Une fonction de lien inverse a été utilisée dans le modèle GLM.

La distribution du nombre de tortues capturées par événement de pêche a été modélisée à l'aide d'une loi de Poisson, l'observation d'une tortue prise dans un filet de pêche pouvant être considérée comme un événement rare. Une fonction de lien logarithmique a été utilisée dans le modèle GLM.

Le nombre de tortues retrouvées mortes parmi les tortues capturées a été modélisé à l'aide d'une loi binomiale (fonction de lien logit dans le modèle GLM).

Le meilleur modèle a été sélectionné par comparaison des "Akaike Information Content" AIC (Akaike, 1974), selon une approche pas-à-pas ("backward", "forward" et "stepwise") à l'aide de la commande "stepAIC" du package "Mass" dans le logiciel R.

Nous avons ensuite testé l'hypothèse selon laquelle la masse des prises commerciales a une influence sur la probabilité de prises accidentelles de tortues. Deux scénarios étaient possibles : soit la masse des poissons et des crustacés et le nombre de tortues sont deux événements indépendants, soit ces deux événements sont liés par la relation suivante : $y = a + bx$ (y = nombre de tortues, x = masse de poissons et crustacés).

La sélection du modèle utilise l'AIC qui est une méthode de classement qui prend en compte la qualité du modèle en le comparant aux données et en pénalisant par le nombre de paramètres utilisés :

$$AIC = -2\ln L + 2N$$

L correspond au maximum de vraisemblance et N au nombre de paramètres. Le modèle qui présente la valeur d'AIC la plus basse est retenu comme le meilleur modèle.

L'Akaike weight ω est utilisé pour comparer les modèles entre eux :

Soit ω_i l'Akaike weight du modèle i et ΔAIC la différence entre l'AIC du modèle i et l'AIC du meilleur modèle :

$$\omega_i = \exp(-\Delta AIC / 2)$$

L'Akaike weight normalisé à 1 peut être directement interprété comme la probabilité relative d'un modèle parmi les n modèles candidats comparés (Burnham et Anderson, 2002).

$$P = \omega_i / \sum \omega$$

Le modèle avec l'AIC le plus bas est sélectionné pour être éventuellement de nouveau comparé à d'autres modèles. Les modèles avec des Akaike weights similaires sont retenus puis discutés.

RÉSULTATS

Engins et techniques de pêches utilisés à la Pointe Indienne

Vingt pêcheurs, trois ramendeurs et sept autres acteurs en lien avec le monde de la pêche ont accepté de participer à l'enquête.

Quatre grandes catégories d'engins et de techniques de pêche ont ainsi été répertoriées : les filets droits, les sennes, les plateaux et les lignes à hameçon. Le tableau I précise, pour chaque technique inventoriée, le milieu, la période pendant laquelle il est pratiqué, ainsi qu'une évaluation de sa fréquence d'utilisation sur la base des interviews.

Les pêches au filet droit sont les plus répandues au Congo. Elles sont utilisées toute l'année et les produits de pêche sont principalement destinés à la commercialisation. Les filets peuvent atteindre 100 à 350 m de longueur avec une hauteur de 1,5 à 6 m, voire plus. Le filet est fabriqué à partir du fil en nylon le plus souvent tressé, parfois monobrin.

Ce type de filet est constitué d'un maillage uniforme. La taille des mailles est très variable selon les espèces ciblées, variant de 6 cm à 24 cm, bien que la législation congolaise fixe la taille minimale autorisée à 10 cm, maille étirée.

Les pêcheurs interviewés font la différence entre les filets dont la maille étirée mesure moins de 10 cm, dits filets à petites mailles, et ceux dont la taille de la maille étirée est supérieure à 10 cm, dits filets à grandes mailles. Les filets à grandes mailles sont parfois appelés "folles". Ces deux types de filets sont utilisés dans des buts différents. Les filets à petites mailles (entre 5 et 10 cm maille étirée) visent les poissons de petites tailles. Les filets à grandes mailles (> 10 cm et jusqu'à 24 cm maille étirée) ciblent les plus gros spécimens ainsi que les crustacés. Le filet est équipé de flotteurs sur la ralingue supérieure et de lests sur la ralingue inférieure. L'armement sur compas, qui consiste à prendre un certain nombre de mailles pour amarrer la nappe aux ralingues, est le plus utilisé. La souplesse du filet est définie par la quantité de flotteurs et de lests utilisés ainsi que le rapport d'armement, c'est-à-dire la fréquence de mailles amarrées aux ralingues relativement à la longueur du filet. Les informa-

Tableau I. - Les engins et techniques de pêche utilisés à la Pointe Indienne et dans la baie de Loango : milieux, périodes, espèces ciblées et fréquence d'utilisation. * : Voir les noms scientifiques Tab. V. [Fishing gears and techniques used at the Pointe Indienne and in the Loango Bay: area of use, periods, targeted species and frequency of use. *: See scientific names Tab. V.]

Engins et techniques de pêche		Milieu de pêche	Période de pêche	Espèces cibles*	Fréquence d'utilisation
Filets maillants	Filet à petites mailles calé au fond	Dans la baie et au large, sur les fonds rocheux et sableux.	Toute l'année	Varie selon les saisons. Orphie et bar en saisons de pluie	Élevée
	Folle calée au fond	Dans la baie, sur les fonds rocheux et sableux	Toute l'année, beaucoup plus en saison sèche et en décembre	Bar, requin, raie, capitaine	Élevée
	Folle flottante	Au large, sur les fonds rocheux et sableux.	Saison sèche	Bar, requin, raie, capitaine, mérrou	Faible
Sennes	Senne de plage ou filet tiré	Dans la baie, sur les fonds rocheux et sableux.	Toute l'année	Toutes les espèces se trouvant sur le plateau	Faible
	Filet encerclant	Dans la baie et au large, sur les fonds rocheux et sableux.	Saison des pluies	Aiguillette	Élevée
Nasses ou plateaux		Dans la baie, sur les fonds rocheux et sableux.	Saison sèche	Sardinelle	Élevée de mai à septembre
Lignes à hameçons		Dans la baie et au large	Toute l'année	Murène, orphie, dorade	Faible

teurs indiquent que la souplesse du filet aurait un effet sur la prise de poisson car elle semble favoriser l'emmêlement. L'armement maille à maille, qui tend mieux le filet est plus rare.

La technique la plus fréquemment utilisée est le filet "dormant et maillant" calé au fond. La méthode consiste à déposer longitudinalement le filet qui vient se déposer au fond afin de cibler des espèces benthiques. Le filet est dit "dormant et maillant" car il est déposé pour une durée déterminée (jusqu'à 24 h) et les poissons qui rencontrent le filet s'emmêlent dans les mailles par les ouïes ou les mâchoires.

Ces filets peuvent être déployés au large, calés au fond. Ils sont alors destinés à attraper de gros poissons démersaux.

La seconde technique, le filet "dormant et maillant" flottant, consiste à suffisamment délester le filet de manière à le laisser flotter entre deux eaux ou à la surface. Cette technique cible les espèces benthopélagiques et pélagiques. L'engin est davantage sujet aux mouvements provoqués par les courants. Les filets dormants flottants sont fréquemment utilisés à de faibles profondeurs, la partie maillée du filet couvrant alors toute la hauteur de la colonne d'eau et le filet se comportant comme un filet à la fois flottant et calé au fond.

La troisième technique, le filet "dormant et dérivant", est la moins utilisée car elle s'exerce principalement au large. Cette technique n'est pas interdite par la loi Congolaise. Le principe est semblable à la technique précédente à la différence que le filet est attaché à la pirogue, et non fixé à l'ancre. Le filet suit donc les mouvements de la pirogue et des courants.

Ces filets sont dits "dormants" parce qu'ils sont immergés pendant une durée déterminée. Les filets maillants dormants restent en place pour une période qui peut correspondre à un flux de marées, "flot ou jusant" de quelques heures à quelques jours avant d'être levés selon la fragilité des captures (Le Douguet, 2009). En pratique, le temps de calée excède rarement 24 h. A la Pointe Indienne, les temps de mouillage observés dans le cadre de notre étude variaient de 1 h 30 à 16 h.

Un même filet peut être aménagé et modifié pour adapter la technique de pêche en fonction des saisons. Ainsi, une "folle" calée au fond peut être modifiée en folle flottante en l'équipant de flotteurs disposés de telle sorte que le filet est maintenu plus vertical, et en surface. La folle flottante est déployée le plus souvent au large pour une durée variant de 4 h à quelques jours selon les pêcheurs. Cette technique nécessite deux pêcheurs et ne capture que des gros poissons pélagiques. De même, les filets dormants à petites mailles qui sont utilisés calés au fond toute l'année à des profondeurs variables, sont transformés en filets flottants pour la pêche au large en saison sèche.

La pêche à la senne est également utilisée toute l'année mais occasionnellement car les prises sont destinées à la consommation locale. Deux techniques utilisent la senne : sennes de plages et filets encerclant.

Le principe d'utilisation de la senne est actif. Le filet ne reste pas dans l'eau pour une durée déterminée. La technique repose sur trois étapes : le repérage du banc de poissons et/ou le moment favorable de la marée ; l'encercllement du banc de

Tableau II. - Engins et techniques de pêches impliqués dans les captures et la mortalité des tortues marines. Résultats du programme de libération des captures accidentelles des tortues marines (2008). [*Fishing gears and techniques involved in sea turtle by-catch and mortality. Results of the sea turtle by-catch release program (2008).*]

Engins et techniques de pêche	Effort de pêche	Nombre de tortues capturées	Proportion des tortues capturées par une technique identifiée et rang	Nombre de tortues retrouvées mortes	Taux de mortalité des tortues capturées (IC 95%)
Filets à petites mailles calés au fond	Élevé	27	2,49% (3)	5	19% (6,3-38,1)
Filets à grandes mailles calés au fond	Élevé	310	28,62% (1)	67	21% (17,2-26,6)
Filets à grandes mailles flottants	Faible	307	28,35% (2)	NA	NA
Senne de plage	Faible	13	1,20% (4)	0	0
Filet encerclant	Faible	0	0 (5)	0	0
Nasses ou plateaux	Élevé certains mois	0	0 (5)	0	0
Lignes et hameçons	Faible	0	0 (5)	0	0
Technique non renseignée		426			

Tableau III. - Récapitulatif des espèces de tortues capturées dans la baie de Loango (2008- 2011). [*Overview of the sea turtle species captured in the Loango Bay (2008-2011).*]

Espèces Plages	<i>Chelonia mydas</i> (Linnaeus, 1758)	<i>Lepidochelys olivacea</i> (Eschscholtz, 1829)	<i>Eretmochelys imbricata</i> (Linnaeus, 1766)	<i>Dermochelys coriacea</i> (Vandelli, 1761)	Total
Pointe-Indienne	4152	23	59	3	4237
Tchilassi	17	3	0	3	23
Matombi	35	2	0	10	47
Bibanga	52	8	0	6	66
Tchissanga	0	1	0	1	2
Bas- Kouilou	23	7	1	15	46
Total	4279	44	60	38	4421

Tableau IV. - Stades de vie des individus capturés par espèce. [*Living stages of captured sea turtles according to species.*]

	<i>Chelonia mydas</i>	<i>Lepidochelys olivacea</i>	<i>Eretmochelys imbricata</i>	<i>Dermochelys coriacea</i>	Total
Juveniles	2814	0	32	0	2846
Adultes	80	43	5	26	154
Indéterminés/Subadultes (individus de taille intermédiaire)	1385	1	23	12	1421
Total	4279	44	60	38	4421

poisson visualisé ou présumé ; et l'opération de "virage" qui consiste à rapporter les prises en diminuant le filet.

La senne de plage consiste à déposer le filet en mer depuis la plage à un moment favorable, le plus souvent lors d'une marée descendante et le tirer pour piéger le poisson. Son utilisation nécessite 6 à 8 personnes.

Généralement, les sennes de plage sont des filets d'une longueur de 150 à 200 m, mais ils peuvent atteindre plus de

500 m. Le filet est constitué de quatre parties qui ont chacune un maillage, une longueur et une chute propre. Une longue corde de 100 à 150 m est attachée à chaque extrémité du filet. Les mailles sont larges aux extrémités et se rétrécissent au centre ce qui forme une sorte de poche. Le filet est fabriqué à partir de fils de coton tressés. Les mailles sont très fines, entre 1 et 2 cm de longueur pour une maille étirée, ce qui est très inférieur à la taille minimale autorisée par la

Tableau V. - Espèces de poissons et de crustacés observées dans les filets et leurs indices de valeurs commerciales. [Fish and shellfish species observed in fishing nets and their commercial value indexes.]

Noms pilotes	Noms vernaculaires	Famille	Nom scientifique	Coeff. de valeur commerciale
Alose rasoir	Tchibeli	Pristigasteridae	<i>Ilisha africana</i> (Bloch, 1795)	1
Bar	Likalala	Sciaenidae	<i>Pseudotolithus senegalensis</i> (Valenciennes, 1833)	2
Bar royal	Tchilondo	Sciaenidae	<i>Pseudotolithus typus</i> Bleeker, 1863	3
Maigre (Bar noir)	Nkokoto	Sciaenidae	<i>Argyrosomus regius</i> (Asso, 1801)	1
Barbillon	N'kang	Polynemidae	<i>Pentanemus quinquarius</i> (Linnaeus, 1758)	1
Barracuda	Mouéndji	Paralepididae	<i>Sphyaena</i> sp.	3
Capitaine	Likuimbimbi	Polynemidae	<i>Polydactylus quadrifilis</i> (Cuvier, 1829)	3
Carangue jaune	Nkaba	Carangidae	<i>Caranx hippos</i> (Linnaeus, 1766)	3
Carangue noire	Nkaba	Carangidae	<i>Caranx senegallus</i> Cuvier, 1833	3
Carpe	Tiale	Haemulidae	<i>Plectorhynchus macrolepis</i> (Boulenger, 1899)	2
Ceinture	Nongou	Trichiuridae	<i>Trichiurus lepturus</i> Linnaeus, 1758	2
Disque	Tchipaple Pangou	Drepaneidae	<i>Drepane africana</i> Osório, 1892	1
Dorade grise	Tchikuate	Haemulidae	<i>Pomadasys jubelini</i> (Cuvier, 1830)	2
Ethmalose	Manzi	Clupeidae	<i>Ethmalosa fimbriata</i> (Bowdich, 1825)	1
Fiatole	Likouf	Stromateidae	<i>Stromateus fiatola</i> Linnaeus, 1758	2
Hareng (sardinelle)	Makouala	Clupeidae	<i>Sardinella maderensis</i> (Lowe, 1838) <i>S. aurita</i> Valenciennes, 1847	1
Mâchoiron	Nchiendo	Ariidae	<i>Arius africanus</i> Günther, 1867	2
Mérou	–	Serranidae	<i>Epinephelus</i> spp. Bloch, 1793	3
Ladyfish d'Afrique de l'Ouest	Sounze	Elopidae	<i>Elops lacerta</i> Valenciennes, 1847	2
Murène	'Livil	Muraenidae	<i>Muraena helena</i> Linnaeus, 1758	1
Orphie ou Aiguillette	Moeho	Belonidae	<i>Belone belone</i> (Linnaeus, 1761)	1
Pelon	Lindongo	Haemulidae	<i>Brachydeuterus auritus</i> (Valenciennes, 1832)	1
Plat-plat ou Sélène	Mboulou	Carangidae	<i>Selene dorsalis</i> (Gill, 1863)	1
Poisson perroquet vert	–	Scaridae	<i>Scarus hoefleri</i> (Steindachner, 1881)	1
Poisson volant	–	Exocoetidae	<i>Cheilopogon</i> sp.	1
Pastenague marguerite	Tchibouel	Dasyatidae	<i>Dasyatis margarita</i> (Günther, 1870)	2
Requin soyeux	Dukudaka	Carcharhinidae	<i>Carcharhinus falciformis</i> (Müller & Henle, 1839)	2
Requin renard	Dukudaka	Alopiidae	<i>Alopias pelagicus</i> Nakamura, 1935	2
Remora	Lubana		<i>Remora remora</i> (Linnaeus, 1758)	
Sardine	Nveni	Clupeidae	<i>Sardina pilchardus</i> (Walbaum, 1792)	3
Sole	Libondo	Cynoglossidae	<i>Cynoglossus senegalensis</i> (Kaup, 1858)	3
Tarpon	–	Megalopidae	<i>Megalops atlanticus</i> Valenciennes, 1847	3
Thon	Mouémbila	Scombridae	<i>Thunnus</i> sp.	2
Crabe fantôme	Oural (N'kala ?)	Ocypodidae	<i>Ocypode gaudichaudii</i> H. Milne-Edwards & Lucas, 1843	1
Crabe bleu	Oural (N'kala)	Portunidae	<i>Callinectes sapidus</i> Rathbun, 1896	2
Crabe marbré	Oural (N'kala)	Portunidae	<i>Portunus hastatus</i> (Linnaeus, 1767)	1
Langouste royale	Nkosso	Palinuridae	<i>Panulirus regius</i> De Brito Capello, 1864	3

réglementation congolaise (10 cm maille étirée).

Le filet encerclant (ou senne tournante) consiste à réaliser la même manœuvre d'encerclant actif d'un banc de poissons, mais à partir d'une pirogue en mer. Avant de lancer

le filet, les pêcheurs suivent le mouvement des poissons. Le filet est déployé en encerclant le banc de poissons. Le ramassage s'effectue 2 à 4 h plus tard. Les filets encerclant sont des engins qui restent dans l'eau pendant 5 h au maximum.

Tableau VI. - Espèces de poissons, de tortues et de crustacés observées dans les filets ayant un statut dans la liste rouge de l'IUCN. [Fish, Turtles and shellfish species observed in fishing nets with a particular status in the IUCN red listing.]

Nom commun	Nom scientifique	Statut liste rouge	Critères liste rouge (ver 3.1)	Tendance de la population
Poissons				
Ethmalose	<i>Ethmalosa fimbriata</i>	Least concern	–	Unknown
Ladyfish d'Afrique de l'Ouest	<i>Elops lacerta</i>	Least concern	–	Unknown
Poisson perroquet vert	<i>Scarus hoefleri</i>	Least concern	–	Unknown
Raie pastenague	<i>Dasyatis margarita</i>	Endangered	A2bd+3bd+4bd	Decreasing
Requin soyeux	<i>Carcharhinus falciformis</i>	Near Threatened	–	Decreasing
Requin renard	<i>Alopias pelagicus</i>	Vulnerable	A2d+4d	Decreasing
Tarpon	<i>Megalops atlanticus</i>	Vulnerable	A2bd	Decreasing
Thon albacore	<i>Thunnus alalunga</i> (Bonnaterre, 1788)	Near threatened	–	Decreasing
Thon jaune	<i>Thunnus albacares</i> (Bonnaterre, 1788)	Near threatened	–	Decreasing
Thon obèse	<i>Thunnus obesus</i> (Lowe, 1839)	Vulnerable	A2bd	Decreasing
Tortues marines				
Tortue luth	<i>Dermochelys coriacea</i>	Vulnerable	A2bd	Decreasing
Tortue olivâtre	<i>Lepidochelys olivacea</i>	Vulnerable	A2bd	Decreasing
Tortue verte	<i>Chelonia mydas</i>	Endangered	A2bd	Decreasing
Tortue imbriquée	<i>Eretmochelys imbricata</i>	Critically endangered	A2bd	Decreasing
Crustacés				
Langouste royale	<i>Panulirus regius</i>	Data Deficient	–	Decreasing

Cette technique cible principalement les aiguillettes (*Belone belone*) pendant la saison des pluies. Ces filets de surface ont plus de flotteurs que de lests et une longueur de 100 à 250 m environ, avec une chute de 4 à 10 m. La taille de la maille est généralement de 2 cm (maille étirée).

La pêche au plateau est, quant à elle, saisonnière. Elle vise exclusivement les sardinelles (*Sardinella* sp.) destinées à la commercialisation, et qui sont présentes pendant la saison sèche. Le plateau est un engin de pêche composé d'un filet au maillage très fin (entre 0,5 et 1 cm de longueur pour une maille étirée) fixé sur un cadre en bois rectangulaire pouvant mesurer jusqu'à 8 m de long pour 5 m de large, soit une surface de 40 m². Le milieu du plateau forme une poche dont la profondeur n'excède pas 1 m et où se concentrent les poissons. Cette pêche est pratiquée depuis la plage, elle ne nécessite pas de pirogue. La technique consiste à immerger le plateau, tenu aux extrémités par au moins deux pêcheurs, puis de le soulever lors du passage d'un banc de sardinelles pour les récolter.

La pêche à la traîne utilise une ligne munie d'un ou plusieurs hameçons. Elle est pratiquée au large en ciblant un banc de poissons mais aussi depuis le bord, notamment par les enfants. Cette technique est utilisée toute l'année par les pêcheurs du village principalement pour l'autoconsom-

mation. Il existe aussi une pêche de loisir pratiquée par des expatriés. Il s'agit d'une pêche à la canne. La ligne est équipée d'un leurre ou d'appâts.

Techniques responsables des prises accessoires de tortues marines

D'après les données issues du suivi des captures accessoires (Tab. II), les techniques de pêche impliquées dans les captures accidentelles de tortues marines sont par ordre décroissant du pourcentage de capture et en relation avec la fréquence d'utilisation de la technique (Tab. I) : 28% : filets à grandes mailles calés au fond (technique très utilisée) ; 28% : folles flottantes (technique peu utilisée) ; 2,5% : filets à petites mailles calés au fond (technique fréquemment utilisée).

Environ une tortue sur cinq capturées dans les filets calés au fond, petites mailles et grosses mailles confondues, est retrouvée morte dans le filet (Tab. II).

Les tortues sont essentiellement capturées à la Pointe Indienne (Tab. III). Il s'agit majoritairement de tortues vertes, le plus fréquemment au stade juvénile [longueur courbe de carapace inférieure à 80 cm (Godley *et al.*, 2002)] (Tab. IV).

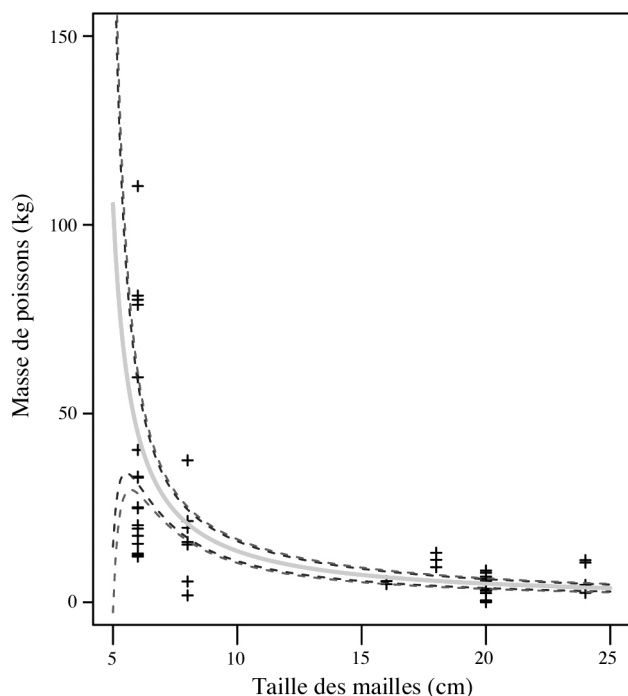


Figure 2. - Courbe réponse de la masse de poissons et crustacés (kg) en fonction de la taille des mailles (cm, maille étirée). L'intervalle de confiance à 95% apparaît en pointillés. + : valeurs observées. [Response curve of fish weight (kg) by fishing event according to mesh size (in cm mesh stretched). The 95% confidence interval appears in dotted lines. + : observed values.]

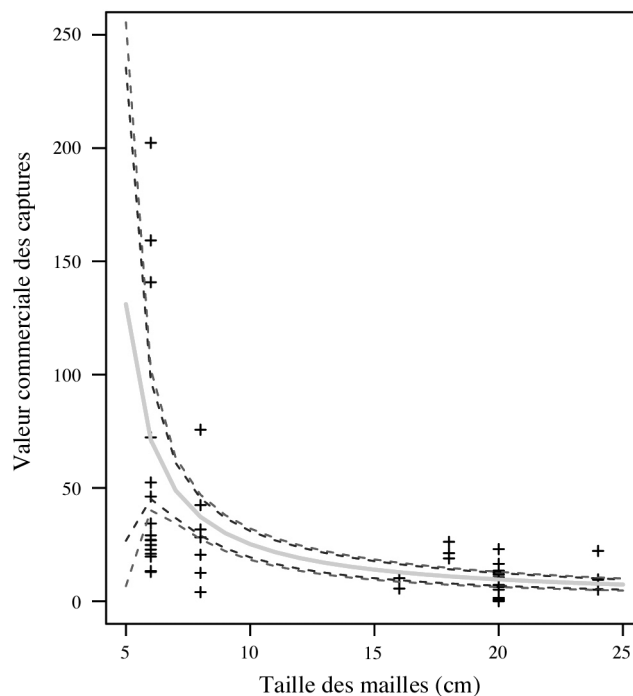


Figure 3. - Courbe réponse de la valeur commerciale des prises en fonction de la taille des mailles (cm, maille étirée). L'intervalle de confiance à 95% apparaît en pointillés. + : valeurs observées. [Response curve of commercial value of the catches by fishing event according to mesh size (in cm mesh stretched). The 95% confidence interval appears in dotted lines. + : observed values.]

Comparaison expérimentale des techniques

L'efficacité des filets testés est comparée en termes de masse d'espèces ciblées capturées par événement de pêche, puis en termes de valeur commerciale des prises. Nous n'avons pas réalisé d'appariement pour les tests dans la mesure où, pour des raisons logistiques, dans quelques cas la pose des filets n'a pas été réalisée en couple.

Au cours de la phase expérimentale, 33 espèces de poissons pélagiques et démersaux et quatre espèces de crustacés (une espèce de langouste et trois espèces de crabes) ont été observées (Tab. V). Seuls quatre des taxons rencontrés ont un statut documenté dans la liste rouge de l'IUCN (Tab. VI).

La modélisation GLM de la variable à expliquer "Masse de poissons et crustacés" met en évidence un effet hautement significatif de la taille des mailles ($p < 0,01$) (Fig. 2). La masse de poissons et de crustacés capturés par événement de pêche augmente quand la taille de la maille diminue.

Pour le facteur "Valeur commerciale des prises", deux variables explicatives ont un effet : la taille des mailles (significatif, $p < 0,05$) (Fig. 3) et la proportion de la colonne d'eau couverte par la partie maillante du filet (faiblement significatif, $p < 0,1$) (Fig. 4). La valeur commerciale des prises évolue en sens inverse de la taille des mailles et de la proportion de colonne d'eau occupée. La valeur de prises

diminue lorsque la taille des mailles augmente et lorsque la proportion de colonne d'eau augmente.

Captures accessoires de tortues marines

Le modèle linéaire GLM appliqué à la variable à expliquer "Nombre de tortues par événement de pêche" détecte un effet significatif ($p < 0,05$) de la variable "taille de maille" (Fig. 5). Le nombre de prises accessoires de tortues marines augmente avec la taille des mailles. Sur les 31 événements de pêche étudiés avec les filets à grosses mailles, déployés sur les fonds rocheux peu profonds et rarement sableux, 23 tortues ont été capturées soit une moyenne de 0,74 tortue par événement de pêche. Sur les 27 événements de pêche réalisés avec les petites mailles, seules deux tortues ont été capturées, soit une moyenne de 0,07 tortue par événement de pêche. Selon que l'on utilise le filet à grandes mailles ou le filet à petites mailles, la fréquence des captures de tortues marines est significativement différente ($p < 0,05$) (Fig. 6).

La modélisation GLM de la variable à expliquer "Proportion de tortues retrouvées mortes" ne met en évidence aucun effet significatif. La variable temps de calée a l'effet le plus fort ($p = 0,16$) mais il n'est pas significatif (Fig. 7). Le nombre d'événements de pêche comptabilisant des captures de tortues marines est insuffisant pour permettre une

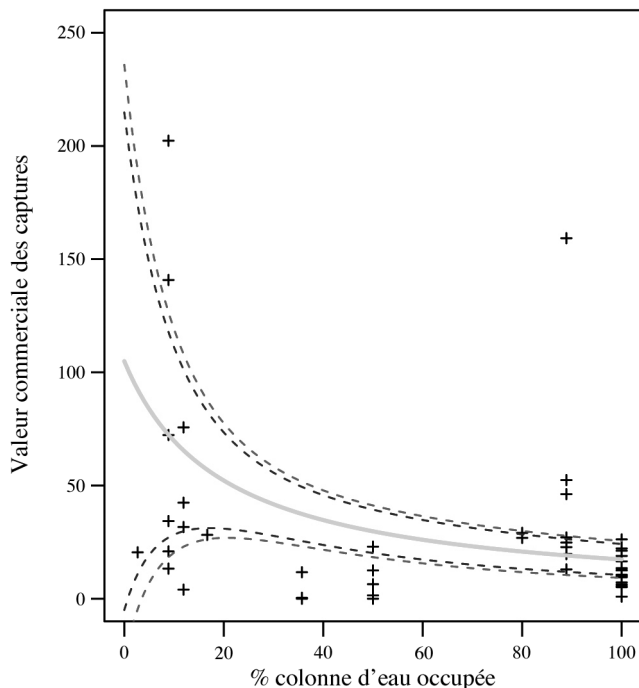


Figure 4. - Courbe réponse de la valeur commerciale des prises en fonction de la proportion de colonne d'eau couverte par la partie maillante du filet. L'intervalle de confiance à 95% apparaît en pointillés. + : valeurs observées. [Response curve of the commercial value of the catches by fishing event according to the height of water column covered by the meshes. The 95% confidence interval appears in dotted lines. + : observed values.]

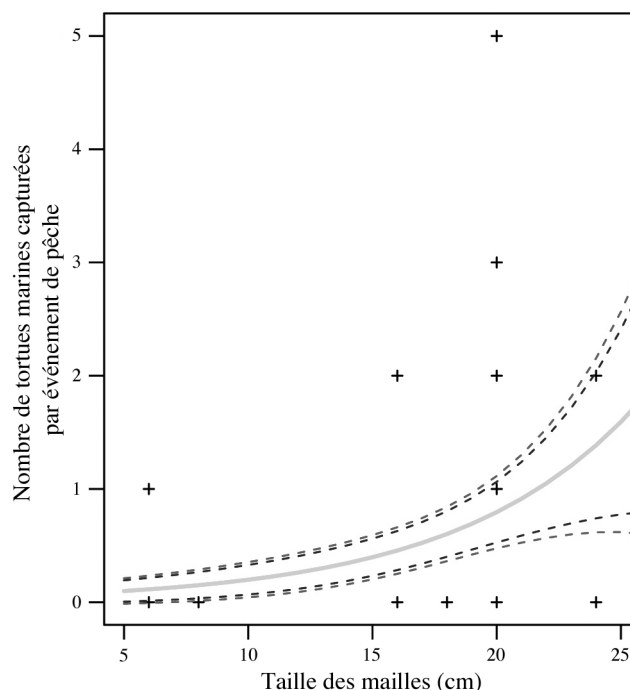


Figure 5. - Courbe réponse du nombre de tortues capturées par événement de pêche en fonction de la taille des mailles (cm, maille étirée). L'intervalle de confiance à 95% apparaît en pointillés. + : valeurs observées. [Response curve of the number of sea turtles caught by fishing event according to mesh size (in cm mesh stretched). The 95% confidence interval appears in dotted lines. + : observed values.]

recherche efficace des facteurs influençant la proportion de tortues retrouvées mortes.

Dans les filets à petites mailles, aucune tortue morte n'a été observée, mais il n'y avait que deux événements. Les cas de mortalité ne sont observés que lorsque le filet à grandes mailles reste déployé plus de 12 h (Fig. 6).

Un éventuel lien a aussi été recherché entre la masse de poisson et le nombre de prises accessoires. Les résultats montrent que la probabilité P du modèle «événement indépendant» ($p = 0,73$) est supérieure à celle du modèle «événement lié» ($p = 0,27$) (Tab. VII). Il n'existe donc pas de

lien entre la masse de prises de poissons et de crustacés et le nombre de tortues capturées dans les filets à grosses mailles.

DISCUSSION

Les engins et techniques de pêche

Les différentes techniques de pêche se distinguent principalement par la taille des mailles utilisées, la position de la partie maillante dans la colonne d'eau, le temps de calé et le mode actif ou passif de la pêche. Ainsi le filet encerclant a de petites mailles (1 à 2 cm), le filet droit a des mailles de 4 à 8 cm et la "folle" a des grandes mailles 12 à 20 cm. Cependant, les dimensions des mailles sont réglementées par la loi congolaise en matière de pêche maritime. La taille minimale est de 10 cm maille étirée pour les filets dormants de fond et 6 cm maille étirée pour les filets encerclants (Décret n° 2009-33 du 6 février 2009) (Ministère de la Pêche et de l'Aquaculture, 2009). Les filets à grosses mailles (folles) utilisés à la Pointe Indienne ont donc des mailles dont la dimension est conforme à la réglementation congolaise. En revanche certains filets droits et certains filets encerclants ont des mailles de dimensions inférieures à celles qui sont autorisées par la réglementation.

Tableau VII. - Recherche d'un éventuel lien entre masse de poissons et nombre de prises accessoires de tortues marines. [Analysis of a putative link between fish weight and number of sea turtle by-catch.]

	Modèle : événements liés	Modèle : événements indépendants
a	0,902521074	0,741935483
b	-0,025847858	0
x	0,548806869	0,546654729
Δ AIC	1,943289666	0
p	0,274552773	0,725447227

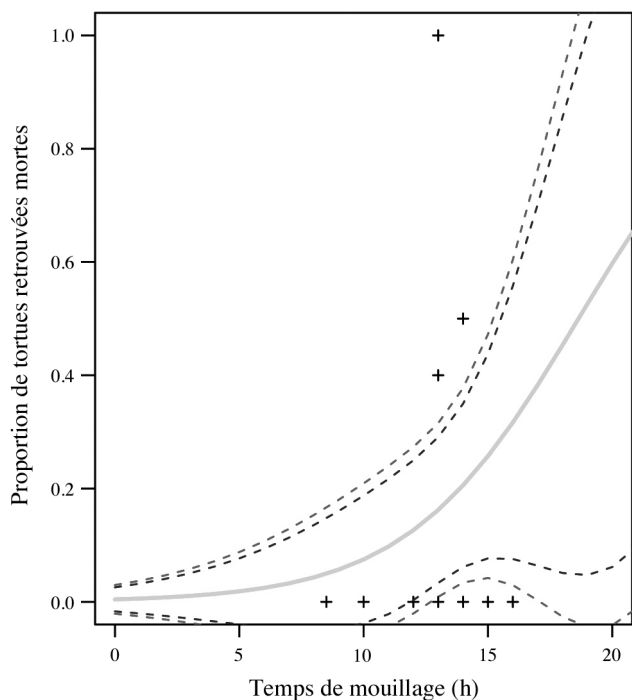


Figure 6. - Courbe réponse de la proportion de tortues retrouvées mortes par événement de pêche en fonction du temps de calée (en heures). L'intervalle de confiance à 95% apparaît en pointillés. + : valeurs observées. [Response curve of the proportion of sea turtles found dead per fishing event according to soaking time (in hours). The 95% confidence interval appears in dotted lines. +: observed values.]

Les engins peuvent être utilisés de différentes manières : calé au fond, flottant encerclant, pêche au plateau, et pêche à la ligne. De toutes ces techniques, les plus utilisées sont les filets calés au fond qui pêchent en profondeur principalement les poissons démersaux très recherchés pour leur qualité, et les filets flottants qui capturent les poissons pélagiques souvent très abondants sur les côtes congolaises (Nguigiri, 1995).

Les techniques responsables des prises accessoires

Les résultats de l'enquête auprès des professionnels de la pêche, associés à l'analyse des données collectées dans le cadre du programme de libération ont permis de classer les techniques de pêche en fonction de leur fréquence d'utilisation et de leur implication dans les captures accessoires de tortues marines (Tab. II). Les filets maillants dormants sont majoritairement impliqués ; la pêche au filet à grandes mailles calé au fond est au premier rang, suivie de la pêche au filet à grandes mailles flottant, puis de celle au filet à petites mailles calé au fond. En revanche, si l'on considère la fréquence d'utilisation de ces techniques, la pêche au filet à petites mailles calé au fond et celle au filet à grandes mailles calé au fond sont fréquentes, tandis que l'utilisation du filet à grande maille flottant est plus occasionnelle. Ces

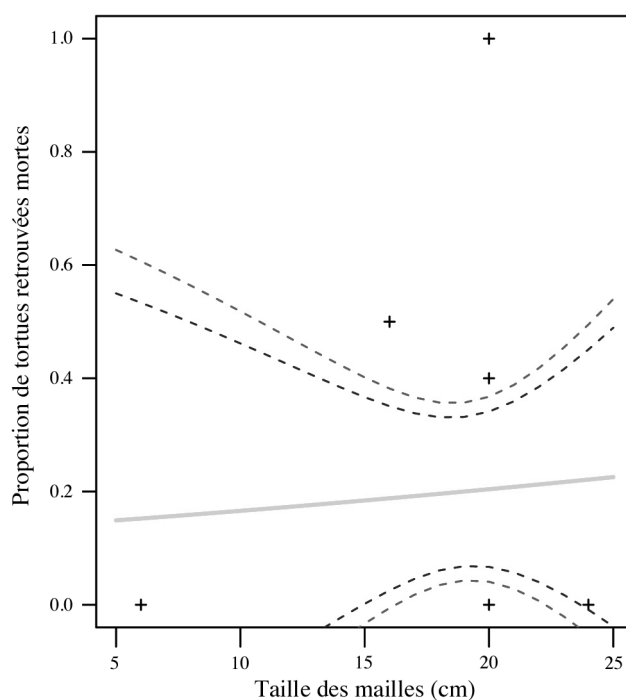


Figure 7. - Courbe réponse de la proportion de tortues retrouvées mortes par événement de pêche en fonction de la taille des mailles (cm, maille étirée). L'intervalle de confiance à 95% apparaît en pointillés. + : valeurs observées. [Response curve of the proportion of sea turtles found dead per fishing event according to mesh size (cm mesh stretched). The 95% confidence interval appears in dotted lines. +: observed values.]

résultats suggèrent que les grandes mailles sont plus nocives pour les tortues marines que les petites mailles. En effet, un grand nombre de captures impliquent les filets à grosses mailles, calés au fond ou flottant. Dans le même temps, les filets droits à petites mailles calés au fond sont moins souvent impliqués dans les captures accessoires alors que leur fréquence d'utilisation est élevée.

Les données issues du programme de libération ne renseignent toutefois pas sur l'effort de pêche déployé pour chacune des techniques de pêche répertoriées. Il n'était donc pas possible d'apprécier et de comparer précisément le risque de prises accessoires selon les différentes techniques d'après ces seuls résultats. Pour compléter l'étude, nous avons donc choisi de comparer expérimentalement les techniques de pêche les plus souvent impliquées dans les prises accessoires. Cette étude expérimentale a permis de mettre en évidence un lien significatif entre la taille des mailles et le nombre de captures accessoires. Le risque de captures accessoires de tortues marines est significativement plus élevé dans les filets à grandes mailles par rapport aux filets à petites mailles.

D'autres études ont montré que la largeur des mailles a une influence sur les prises des tortues marines. Les pêcheurs ciblant les tortues marines utilisent d'ailleurs des filets dont

les mailles mesurent de 20 à 60 cm (Gilman *et al.*, 2010). Acosta et Appeldoorn (1995) indiquent que les gros spécimens comme les requins, les raies et les tortues marines s’emmêlent d’autant plus facilement que la maille est large.

Les filets maillants calés au fond sont décrits comme les plus nuisibles par différents auteurs. Selon Delcroix (2003), tous les filets maillants calés sur le fond (filets droits, folles et trémaills) sont impliqués dans la capture des tortues marines.

Les sennes peuvent également occasionner des captures de tortues marines. Elles n’apparaissent cependant pas dans les données du suivi du programme de libération (Tab. II) car les pêcheurs ne sollicitent pas les équipes de Rénatura dans ce contexte. Soit la capture est évitée en raison de la visualisation de la tortue, soit la tortue est relâchée immédiatement. Le risque de mortalité est donc minime.

La littérature montre que les lignes d’hameçons, notamment les palangres sont responsables de la capture de nombreuses tortues marines dans les océans du monde (Casale, 2008 ; Wallace *et al.*, 2010a). Au Congo, les tortues marines sont périodiquement pêchées par les lignes équipées d’hameçons. Il arrive souvent que la ligne, voire la canne, casse sous le poids de la tortue. Des hameçons sont parfois retrouvés sur les individus observés dans les filets de pêche. Ils sont alors le plus souvent plantés au niveau des palettes natatoires plutôt que dans la bouche.

Espèces et stades de vie des tortues marines capturées

Quatre espèces de tortues marines sont capturées par les pêcheurs de la baie de Loango. Ce sont principalement des tortues vertes (*Chelonia mydas*) mais également des tortues luths (*Dermochelys coriacea*), des tortues olivâtres (*Lepidochelys olivacea*) et des tortues imbriquées (*Eretmochelys imbricata*) (Tab. IV). La grande majorité des tortues vertes et des tortues imbriquées observées dans les filets de pêche artisanale sont des individus juvéniles.

Dans la baie de Loango, la Pointe Indienne (Fig. 1 ; Tab. III) est le lieu où la majorité des captures accessoires de tortues marines est observée. La prédominance des captures à la Pointe Indienne est certainement le résultat de la conjonction de plusieurs facteurs. Cette zone a un intérêt en terme de biodiversité marine, elle est constituée d’une bande de rochers riche en plantes, algues et animaux benthiques marins ce qui favorise le développement de la faune aquatique (Fontana, 1981). Les tortues vertes y trouvent les végétaux et les petits animaux benthiques qui constituent leur alimentation. Les tortues imbriquées y recherchent certainement des éponges, base de leur régime alimentaire. La richesse liée à la bande rocheuse est également la raison d’une concentration des activités de pêche. La forte concentration de tortues marines, notamment des tortues vertes juvéniles, sur les rochers, associée à une pression de pêche particulièrement élevée, favorise les captures accessoires à la Pointe Indienne.

Si les tortues vertes et imbriquées sont essentiellement juvéniles sur les rochers de la Pointe Indienne, les tortues olivâtres et les tortues luths capturées sont des adultes qui fréquentent les côtes congolaises pour la reproduction. Ces deux espèces pondent régulièrement de septembre à mars sur les plages de sable du Congo et fréquentent les eaux côtières à partir du mois d’août quand débutent les accouplements. C’est pourquoi les tortues luths et olivâtres capturées le long des côtes sont des adultes, essentiellement des femelles et quelques mâles.

Les captures accidentelles observées ont un impact sur ces espèces classées sur la liste rouge de l’IUCN (IUCN, 2013) : la tortue imbriquée (*Eretmochelys imbricata*) est classée “en danger critique d’extinction” (Cr) ; la tortue verte (*Chelonia mydas*) est classée “en danger” (En) ; la tortue luth (*Dermochelys coriacea*) et la tortue olivâtre (*Lepidochelys olivacea*) sont classées “vulnérables” (Vu). Il est intéressant de mettre en rapport le nombre de tortues marines relâchées dans le cadre du programme Rénatura avec les estimations de taille des populations connues sur les côtes occidentales de l’Afrique. Entre 2008 et 2011, plus de 4000 tortues vertes ont été libérées. Il convient de nuancer ce chiffre car il s’agit parfois d’une même tortue relâchée plusieurs fois. Toutefois, la libération de plus de 4000 individus essentiellement juvéniles (seulement 2% d’adultes, Tab. IV) semble pouvoir avoir un impact réel sur la tendance des populations lorsqu’on compare ce chiffre aux 3 à 5000 femelles nidifiantes estimées sur les plages des îles d’Ascension au centre de l’océan Atlantique (Godley *et al.*, 2001), et aux 7000 nids comptabilisés en une saison sur l’îlot de Poilao, dans l’archipel des Bijagos en Guinée Bissau (Catry *et al.*, 2002). En Afrique centrale, les sites de ponte de tortues vertes les plus importants sont l’île de Bioko (800 à 1700 nids par an) (Tomás *et al.*, 1999 ; Rader *et al.*, 2006 ; Tomás *et al.*, 2010) et l’île de Principe (89-151 nids : intervalle de confiance à 95% sur Praia Jalé ; 230-407 nids : IC à 95% sur Praia Grande) à Sao Tome-et-Principe (Godgenger *et al.*, 2008 ; Loureiro *et al.*, 2011). Au Congo les pontes de tortues vertes sont rares.

La libération de 60 tortues imbriquées semble également importante lorsque l’on sait que la population nidifiante de tortues imbriquées recensées dans l’Atlantique Est ne dépasse pas une centaine d’individus (soit un équivalent de 350 nids par an ; Mortimer et Donnelly, 2007). En Afrique centrale, le plus important site de ponte pour la tortue imbriquée est constitué des archipels de Sao Tome-et-Principe et de Bioko en Guinée équatoriale (Fretay, 2001 ; Abreu-Grobois et Le Roux, 2008 ; Mortimer et Donnelly, 2007). L’île de Bioko a accueilli moins de 10 femelles en ponte par an depuis 1997 (soit environ 35 nids par saison ; Tomás *et al.*, 2010). Le suivi est irrégulier à Sao Tome-et-Principe mais on s’accorde pour estimer que l’île de Principe accueille moins de 50 femelles nidifiantes chaque année (soit environ 175 nids par saison ; Loureiro, com. pers.).

Promouvoir des filets minimisant les captures accessoires

Cette étude des techniques de pêches artisanale utilisées au Congo et de leur incidence sur les tortues marines au Congo a été réalisée avec un objectif plus large : proposer une stratégie de conservation et des solutions pratiques afin de limiter les captures accessoires de tortues marines. Le programme de relâcher des tortues marines mis en place par Rénatura est coûteux et ne constitue pas une solution viable sur le long terme. L'étude présentée ici pourrait permettre de trouver une solution plus pérenne. L'identification des pratiques les plus nocives est un préalable nécessaire pour envisager de limiter l'utilisation des techniques qui occasionnent le plus de prises accessoires et de promouvoir des techniques qui capturent peu de tortues marines. Or, une technique peu nocive pour les tortues ne pourra être raisonnablement proposée aux pêcheurs et adoptée par eux que si elle se révèle au moins aussi efficace et économiquement rentable que les autres techniques. L'étude expérimentale incluait donc le recensement des prises commerciales. En termes de biomasses de poissons et crustacés capturés par événement de pêche, le modèle linéaire généralisé a montré l'influence hautement significative de la taille des mailles. Les filets à petites mailles se sont révélés plus efficaces que les filets à grandes mailles : 32,38 kg en moyenne par événement de pêche contre 7,83 kg.

En termes d'indice de valeurs commerciales des prises par événement de pêche, la taille des mailles avait également un effet significatif, la valeur de la pêche augmentant lorsque la taille des mailles diminue. Les filets dormants à petites mailles sont donc les plus efficaces et également ceux qui capturent le moins fréquemment les tortues marines. Il semble donc intéressant d'encourager cette technique et de limiter l'utilisation des filets à grandes mailles.

Toutefois, la technique utilisant les filets droits à petites mailles est connue pour affecter les ressources marines avec des captures considérables de poissons (Gobert, 1992). Dans cette étude, la taille des mailles des filets dits "à petites mailles" n'excédait pas 8 cm (maille étirée), une taille de maille non réglementaire. Ces filets, bien que productifs, pêchent des espèces de poissons sans distinction de taille et sont donc potentiellement impliqués dans la dégradation des ressources marines.

Afin de concilier les exigences réglementaires, la gestion de la ressource et la minimisation des risques de captures de tortues marines, il paraît raisonnable d'envisager de promouvoir l'usage des filets dormants dont la taille des mailles sera proche du minimal réglementaire, c'est-à-dire 10 cm de maille étirée. Concomitamment, la limitation de l'usage des filets dormants à grandes mailles, et leur interdiction pendant le pic de la saison de ponte des tortues olivâtres et des tortues luths, est susceptible de limiter les prises accessoires.

Co-facteurs susceptibles de modifier le risque de captures accessoires

Outre la promotion ou la limitation de l'utilisation des techniques existantes en fonction de leur efficacité et de leur innocuité, nous envisageons de créer un prototype de filet dormant amélioré pour limiter les prises accessoires. La création d'un tel filet requiert d'identifier les cofacteurs liés au matériel susceptibles de minimiser les prises accessoires.

Dans la présente étude, nous avons pu montrer que la biomasse de poisson capturé n'était pas corrélée au risque de capture de tortue marine. Les deux phénomènes étant indépendants, il devient possible de chercher à aménager le matériel et/ou la technique de pêche pour réduire le risque de prise des tortues sans réduire la quantité des espèces cibles pêchées.

La souplesse et le rapport d'armement peuvent aussi favoriser l'emmêlement des tortues marines. L'armement sur "compas" de la nappe de maille sur la ralingue supérieure est la méthode la plus utilisée par les pêcheurs. Le tissage d'une maille sur quatre amarrée à la ralingue accentue la souplesse de la nappe de mailles et provoque un effet de festonnage. Modifier ce rapport d'armement en tissant la nappe mailles à mailles permettrait de faire varier la souplesse en conférant plus de rigidité aux filets.

Le comportement de ces filets droits varie également en fonction de la quantité de bouées et de lests utilisés. Réduire le nombre de flotteurs au niveau des lignes de flottaison horizontale et verticale permet de réduire le taux de captures accidentelles de tortues marines (Gilman *et al.*, 2010). L'utilisation de barres verticales de maintien du filet a aussi un effet sur le risque d'emmêlement des tortues marines. Un effet "sac" est créé par les courants marins lorsque la barre de maintien utilisée est moins longue que la hauteur du filet. Utiliser une barre de maintien aussi longue que la hauteur du filet ou ne pas appliquer de barres permet de conférer plus de rigidité à l'engin et ainsi permettre aux tortues de le contourner (Gilman *et al.*, 2010).

Les pêcheurs utilisent parfois des filets tissés à l'aide de monofilaments de nylon, peu coûteux, au détriment des filaments tressés synthétiques ou en coton. Les monofilaments de nylon semblent favoriser l'effet emmêlement et présentent deux inconvénients majeurs : leur effet coupant peut causer de graves blessures aux tortues capturées et aussi aux prises commerciales, et étant non biodégradables, ces filets augmentent le risque de pêche "fantôme" lorsqu'ils sont perdus et persistent sur les fonds marins (Eckert *et al.*, 1999).

L'utilisation d'avertissements visuels sur les engins de pêches peut être une amélioration intéressante. Des expérimentations ont montré une diminution prometteuse des captures accessoires dans les filets traditionnels de pêche côtière ainsi équipés (Wang *et al.*, 2010).

CONCLUSION

Les tortues marines qui fréquentent le littoral congolais font, depuis le 9 avril 2011, l'objet d'une législation établie par le ministère du Développement durable, de l'Économie forestière et de l'Environnement (MDDEFE). Un arrêté les classe comme espèces intégralement protégées (MDDEFE, 2011). Les individus capturés accidentellement dans les engins de pêche sont donc maintenant considérés comme captures accessoires. Selon ces conditions, toute tortue capturée doit être relâchée morte ou vivante.

La pêche artisanale au Congo fait, depuis le 26 avril 2011, l'objet de décrets établis par le ministère de la Pêche et de l'Aquaculture déterminant les conditions et la législation relatives à l'exercice de la pêche maritime artisanale professionnelle. L'utilisation de filets de plus de 500 m est interdite. La zone de pêche réservée à la pêche maritime artisanale professionnelle va de 0 à 6 miles marins (10 km) (Décret n° 2011-317 du 26 avril 2011 : déterminant les conditions d'exercice de la pêche maritime artisanale professionnelle). Des suivis Argos® nous ont permis de mettre en évidence que les tortues vertes juvéniles restent la plupart du temps inféodées aux zones rocheuses très proches de la côte. Elles sont donc uniquement soumises à la pression de pêche artisanale dans la zone de 6 miles. En revanche, les tortues olivâtres et les tortues luths évoluent plus au large. Il est fort probable que la pêche industrielle ait un impact sur ces dernières. Les chalutiers utilisent en effet des filets peu sélectifs et peuvent également impacter les populations de tortues marines. Rénatura a établi un premier contact avec les armateurs de pêche industrielle du Congo afin de créer le Programme d'Accompagnement des Pratiques de Pêche au Congo (PAPPC). Ce projet vise à sensibiliser et former les pêcheurs sur les mesures à prendre en vue de limiter la mortalité des tortues marines dans les engins de pêche (Gerosa et Aureggi, 2001). Une alternative envisageable est la mise en application de Turtle Excluder Devices (TED) sur les chaluts. Cette modification devra toutefois être évaluée dans le contexte particulier du Congo car elle peut avoir des impacts négatifs notamment sur la prise des espèces ciblées. Le TED a été évalué sur les pêcheries industrielles au Gabon, dans des conditions proches de celles du Congo. Les essais ont été globalement convaincants (Nalovic *et al.*, 2014) et des démarches sont en cours pour reproduire au Congo les ateliers réalisés par la National Oceanic and Atmospheric Administration (NOAA) au Gabon. L'évaluation du TED en collaboration avec les pêcheries industrielles locales permettra aussi de mieux faire accepter la mesure si elle s'avérait efficace.

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Titre : Développement embryonnaire, détermination du sexe sensible à la température et phénologie des pontes sous contrainte du changement climatique : le cas de la tortue Caouanne (*Caretta caretta*)

Mots clés : Plasticité phénotypique, norme de réaction, incubation, reptile, tortue marine

Résumé : Le climat affecte entre autre la phénologie, l'aire de distribution, le comportement et la physiologie des espèces. Le changement climatique a donc des répercussions sur chacun de ces facteurs. L'augmentation globale des températures prévue d'ici 2100 pourrait profondément modifier la biodiversité de l'échelle des espèces jusqu'à celle des écosystèmes. Les ectothermes, et en particulier les reptiles ovipares à détermination du sexe sensible à la température, font partie des organismes susceptibles d'être les plus vulnérables au réchauffement du climat puisque quasiment tous leurs traits d'histoire de vie dépendent de la température. L'origine et le maintien de ce mécanisme de détermination du sexe, pouvant conduire à un sex ratio fortement biaisé à l'échelle d'une population, reste une énigme pour les écologues. Parmi les nombreuses questions soulevées par la présence de ce mécanisme de détermination du sexe, la signification adaptative, s'il y en a une, de ce mécanisme est cruciale. Ce mécanisme de détermination du sexe rend-il les espèces plus vulnérables dans le contexte actuel du changement du climat ?

Plusieurs hypothèses évolutives ont été proposées et des modèles de dynamique des populations sont disponibles pour répondre à ces questions. Cependant, prédire le sex ratio primaire en conditions naturelles, c'est-à-dire le sex ratio des nouveaux nés, reste un défi majeur à l'heure actuel. Ce manuscrit vise à apporter de nouveaux outils méthodologiques afin de correctement prédire le sex ratio d'une ponte en fonction de la température ressentie par les embryons au cours de l'incubation. Les tortues marines, quasiment toutes menacées, sont des espèces migratrices présentant toute ce mécanisme de détermination du sexe. Chez ces espèces, la phénologie des pontes est aussi sensible à la température du milieu. Ce type de plasticité phénotypique est probablement la stratégie la plus efficace pour pallier à un changement rapide du climat. Ce manuscrit apporte quelques éléments de réponse quant au potentiel adaptatif des tortues marines face au réchauffement climatique avec l'exemple de plusieurs populations de tortues Caouanne (*Caretta caretta*).

Title: Embryonic development, temperature-dependent sex determination and nesting phenology under climate change constraints: the case of the loggerhead turtle (*Caretta caretta*)

Keywords: Phenotypic plasticity, reaction norm, incubation, reptile, sea turtle

Abstract: Climate affects, among other things, species' phenology, distribution range, behavior and physiology. Climate change thus impacts each of these factors. Global warming expected by 2100 might profoundly modify biodiversity from species to ecosystems. Ectotherms, and in particular oviparous reptiles with temperature-dependent sex determination, are thought to be among the most vulnerable in the face of global warming because virtually all their life history traits depend on temperature. The origin and the persistence of temperature-dependent sex determination, which could lead to heavily biased population sex ratios, is still an enigma for ecologists. Among numerous issues related to this sex determining mechanism, understanding its adaptive significance, if there is one, is crucial. At another level, does this sex determining mechanism make species more vulnerable in the context of contemporary climate change?

Several evolutionary hypotheses have been proposed and population dynamic models are available to address these issues. However, predicting primary sex ratio, i.e., the sex ratio of hatchlings, in natural conditions currently remains a challenge. This manuscript aims to bring new methodological tools to properly predict sex ratio of a clutch depending on temperature experienced by embryos throughout incubation. Marine turtles, almost all being threatened, are migratory species that all exhibit this sex determining mechanism. For those species, nesting phenology is also sensitive to environmental temperature. This type of phenotypic plasticity is probably the most efficient strategy to keep up with rapid climate change. This manuscript provides some elements for understanding the adaptive potential of sea turtles in the face of global warming with the example of several populations of the loggerhead turtle (*Caretta caretta*).

