Université du Québec à Chicoutimi

Biologie de la reproduction de la Paruline à gorge grise (*Oporornis agilis*) dans les pinèdes grises du Lac-Saint-Jean, Canada

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SOMMAIRE

La Paruline à gorge grise est l'une des espèces les plus méconnue d'Amérique du Nord, sans doute en partie parce qu'elle se déplace, se nourrit et niche au sol. Une meilleure compréhension de sa biologie permettrait de mieux gérer ses populations qui sont peu abondantes et localisées partout dans son aire de distribution. En plus des études comportementales, les outils génétiques sont de plus en plus utilisés pour connaître la structure et la qualité d'une population. Comme le territoire actuel de la Paruline à gorge grise dans le nord du Lac-Saint-Jean subit d'importante fragmentation, il y a lieu de s'assurer qu'il demeure encore viable et que les transformations ne gêneront pas ces oiseaux dans leur reproduction. L'objectif principal vise à mieux comprendre la biologie de la reproduction chez la Paruline à gorge grise. Pour ce faire, nous avons travaillé plus spécifiquement sur deux sous-objectifs; 1) compiler les comportements liés à la reproduction et ce pour les deux sexes et 2) élaborer des méthodes d'analyses génétiques à partir d'ADN sanguin plus précisément en déterminant le sexe des jeunes et évaluant leur affiliation parentale.

L'échantillonnage a été réalisé pendant trois étés, de 2007 à 2009. La fréquence de chant des mâles, diverses mesures morphométriques des oiseaux (jeunes et adultes), les mesures de nids ainsi que des œufs ont été compilés. Des caméras connectées à des DVR (Digital Video Recording) ont enregistré tous les mouvements du comportement des parents pendant l'élevage des jeunes au nid. Du sang a été récolté pour les analyses génétiques chez les jeunes et les adultes.

Les analyses ont démontré que les mâles en couple chantent moins que les mâles seuls. Les célibataires ont un patron de chant très régulier en pleine période de reproduction où les autres mâles (en couple) réalisent d'autres tâches en lien avec la nidification. Ceux qui se reproduisent, ont de quatre à cinq œufs par nid qui sont incubés par la femelle uniquement, et ce pendant 11 jours. Après l'éclosion, les deux parents s'occupent des soins apportés aux jeunes (nourriture, sac fécal, thermorégulation), mais la nuit, il n'y a que la femelle qui les couve. Les jeunes quittent le nid vers huit jours. L'activité principale de la femelle est de couver les jeunes et celle du mâle est de chercher de la nourriture (hors du nid). Cela change pour la femelle à mesure que les jeunes grandissent, elle a moins besoin de réchauffer les jeunes et s'implique plus dans la recherche de nourriture. Les jeunes semblent être en mesure de réguler seul leur température corporelle vers l'âge de sept jours.

Les deux adultes visitent le nid à un taux similaire et celui-ci augmente en même temps que les jeunes vieillissent, car ils ont besoin de plus en plus de nourriture. Les mâles retirent beaucoup plus de sacs fécaux que les femelles et le nombre de sacs augmentent avec l'âge des jeunes, puisqu'ils mangent davantage. Les adultes ingurgitent les sacs fécaux surtout lorsque les oisillons sont jeunes et les transportent de plus en plus à mesure qu'ils grandissent.

Pour le second sous-objectif, nous avons utilisé des microsatellites créés pour deux autres espèces et qui amplifiaient également avec des oiseaux du genre *Oporornis*. Cinq *loci* ont

été utilisé pour déterminer l'affiliation parentale malgré le fait que l'équilibre d'Hardy-Weinberg n'était pas rencontré (sans doute en raison du faible échantillonnage). Une étude plus approfondie avec davantage d'échantillons et en ciblant d'autres loci permettrait de réaliser plus efficacement l'affiliation parentale. Néanmoins, avec 5 loci et seulement 3 nids testés nos résultats suggèrent que les pères sociaux étaient également les géniteurs. Ce qui signifie qu'aucune copulation extra-couple ayant donnée un jeune n'a été reconnue pour ces nids. Pour ce qui est de la détermination du sexe des individus, nous avons utilisé une technique connue, mais non évaluée sur la Paruline à gorge grise jusqu'à ce jour et démontré que cette méthode fonctionne avec cette espèce.

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INTRODUCTION

La Paruline à gorge grise (*Oporornis agilis*) est un oiseau discret. Il a été observé pour la première fois en 1812 par Alexander Wilson (Huff 1929). Malgré qu'il se soit écoulé près de 200 ans depuis sa découverte, cette espèce n'a jamais été l'objet principal d'une recherche de sorte que les connaissances dont nous disposons sont le fruit d'observations opportunistes, généralement accumulées lors d'études portant sur d'autres espèces (Pitochelli *et al.* 1997). De ce fait, elle est l'un des passereaux les moins bien connus d'Amérique du Nord (Pitochelli *et al.* 1997). Le premier nid a été trouvé 71 ans après la découverte de l'espèce (Seton 1884). Pour ce qui est du Québec, on ne connaît l'existence que d'un seul nid découvert et c'était à McWatters (Lac Joannès), en Abitibi, le 26 juin 1976 (Chabot et Préfontaine 1976). Selon Pitochelli *et al.* (1997), une étude sur n'importe quel aspect de sa biologie générale apporterait une contribution importante à la connaissance de cette espèce.

La Paruline à gorge grise

Cette paruline effectue sa nidification à travers le Canada, de la Colombie-Britannique au Québec et quelques États américains (Michigan, Wisconsin et Minnesota), mais possède une population faible et localisée (Cooper *et al.* 1997; Pitochelli *et al.* 1997). Elle est une migratrice néo-tropicale qui arrive tard dans la saison de reproduction et le mâle arrive sur le territoire de nidification avant la femelle (Walkinshaw et Dyer 1961). Cet oiseau niche et se nourrit au sol à travers le couvert végétal (Huff 1929; Pitochelli *et al.* 1997).

Le mâle possède un chant caractéristique et puissant. Il permet de détecter l'oiseau dans les arbres, car la paruline ne chante pas au sol, mais à la cime des arbres. Selon Walkinshaw (1936), le mâle peut chanter à un taux de six fois par minute et le taux le plus élevé survient le matin. Huff (1929) a noté que les mâles pouvaient chanter plusieurs heures perchés sur un arbre avec des périodes de repos allant de cinq à 10 minutes. Le domaine vital de cette espèce, lorsqu'il est déterminé avec les chants des mâles, peut s'étendre entre 0,24 et 0,48 hectare (Pitochelli *et al.* 1997). Par contre, on ignore le

territoire réellement utilisé par le mâle et la femelle lorsqu'ils se trouvent au sol. De plus, aucune information n'est disponible sur la fidélité ou non au site de reproduction chez les deux sexes ni si c'est une espèce philopatrique.

Bien que peu d'information soit rassemblée sur sa nidification, on sait que seule la femelle s'occupe des œufs et couve les jeunes pendant la nuit (Kells 1889; dans Pitochelli *et al.* 1997). Les deux parents participent à l'alimentation des jeunes (Walkinshaw et Dyer 1961). Selon Curson *et al.* (1994), la femelle pond en moyenne quatre œufs sur un suivi de six nids. Les jeunes sont nidicoles et quittent le nid vers 9 ou 10 jours selon des observations basées sur deux nids (Walkinshaw et Dyer 1961; Parmelee et Oehlenschlager 1972; dans Pitochelli *et al.* 1997). Pour le reste, les textes qui décrivent la biologie de la reproduction de la Paruline à gorge grise supposent que le comportement va ressembler à celui d'autres espèces du genre *Oporornis*, plus particulièrement la Paruline triste (*Oporornis philadelphia*) et la P. des buissons (*Oporornis tolmiei*) (Pitochelli 1993; Pitochelli 1995). Ces deux espèces pondent un œuf par jour et commencent à couver immédiatement (Bent 1953; Hofslund 1954; Cox 1960). De façon générale elles couvent les œufs pendant 12 jours, les jeunes quittent le nid vers 8 et 9 jours et sont indépendants 3 semaines après l'éclosion (Bent 1953; Hofslund 1954; Cox 1960; Semenchuk 1993).

De récentes recherches en génétique ont démontré, à l'aide de l'ADN mitochondrial, que le genre *Oporornis* devrait être inclus dans le genre *Geothlypis* (Escalante *et al.* 2009; Lovette *et al.* 2010) ce qui pourrait laisser supposer des affinités physiologiques, biologiques et comportementales avec d'autres espèces comme la Paruline masquée (*Geothlypis trichas*). Au cours de l'été 2011, il y a eu remaniement dans la classification des oiseaux. Toutes les parulines du genre *Oporornis* (*philadelphia, tolmiei et formosus*) ont été transférées dans le genre *Geothlypis* sauf la Paruline à gorge grise (Chesser *et al.* 2011).

Statut

La paruline appartient à deux catégories d'oiseaux qui subissent un déclin, les insectivores et les migrateurs sur longue distance (Bohninggaese *et al.* 1993; Archaux 2003). Les insectivores sont affectés par l'utilisation de pesticides en agriculture et en foresterie, car leur usage diminue la quantité d'insectes présents dans l'environnement (Benton *et al.* 2002). Les migrateurs eux, sont dépendants de leur aire d'hivernage, de leur aire de reproduction et de leurs couloirs migratoires. Il est difficile de protéger tant de territoire pour assurer la viabilité de populations saines. Bien que peu d'informations soient rassemblées sur la Paruline à gorge grise, on la retrouve toujours en faible densité de population (Cooper *et al.* 1997; Pitochelli *et al.* 1997). Elle est donc sur la liste rouge des espèces menacées depuis 2004 pour l'Union internationale pour la conservation de la nature (UICN 2010). Selon NatureServe, le statut global serait apparemment stable, même si l'espèce est considérée menacée sur un tiers de son aire de nidification (Comlombie-Britannique, Saskatchewan, Wisconsin et Minnesota: NatureServe 2010). En ce qui concerne les provinces, la Colombie-Britannique considère cette espèce comme menacée (Cooper *et al.* 1997; Cooper et Beauchesne 2004) et elle se retrouve aussi sur la liste des espèces prioritaires en Ontario (Ontario Partners in Flight, 2006). La Paruline à gorge grise n'a pas fait l'objet d'un rapport par le Comité sur la situation des espèces en péril au Canada (COSEPAC 2010) ni par le gouvernement du Québec puisque ce n'est pas une espèce de juridiction provinciale.

Territoire

Au Québec, à la limite Est de l'aire de nidification de la Paruline à gorge grise, l'espèce occupe surtout les pinèdes grises du Saguenay–Lac-Saint-Jean et de l'Abitibi et plus rarement dans des tourbières (Abitibi-Témiscaminque). La Paruline à gorge grise n'est pas considérée comme un oiseau des milieux agricoles, mais une partie de son habitat soit les tourbières et les pinèdes grises est régulièrement convoitée pour la transformation. En Ontario, elle fréquente régulièrement les tourbières (Cadman *et al.* 2008). Ces habitats préférentiels peuvent faire l'objet d'une production agricole comme celle de la canneberge et du bleuet sauvage. Il y a quelques décennies, notamment au Saguenay–Lac-Saint-Jean

de vastes terres occupées principalement par le pin gris sur sol sablonneux ont également été converties en milieu agricole entre autres pour la culture de la pomme de terre.

Ces pinèdes grises offrent également une couche d'éricacée en sous-étage (*Kalmia, Ledum et Vaccinum*) (Pitochelli *et al.* 1997). Ce type d'environnement est idéal pour l'exploitation agricole du bleuet par le biais de la création d'une bleuetière. L'engouement actuel pour ce fruit riche en antioxydants incite de plus en plus à la conversion de ces forêts en terres agricoles. Puisqu'au Lac-Saint-Jean, tous les lots intra municipaux potentiels ont déjà été transformés en bleuetières, l'industrie se tourne maintenant vers les territoires forestiers destinés à la production ligneuse. C'est sur le territoire de la Corporation d'Aménagement Forêt de Normandin (CAFN) qu'a été créé un nouvel aménagement agro-forestier : la «forêt/bleuet». Cela permet de maintenir la sylviculture tout en cultivant aussi le bleuet. Le concept est le suivant; des bandes de bleuetière variant de 40 à 60 m sont séparées par des bandes de forêts de largeur comparable. De plus, les bandes forestières subissent un aménagement intensif, chaque bande est subdivisée en trois sous-bandes et chacune d'elle sera récoltée dans un intervalle de 17 ans. Entre les récoltes, il est planifié de préparer le sol (scarifiage), d'effectuer la plantation de Pin gris et d'entretenir la plantation avec de l'aménagement sylvicole (éclaircie pré-commerciale et commerciale). Il est probable que l'impact soit moins grand qu'une bleuetière conventionnelle puisque dans ce paysage on maintien du couvert forestier. Toutefois, les bandes de forêts subissent une sylviculture intensive qui perturbera la présence de la faune (Lavoie 2009). Cela amènera une fragmentation importante du territoire soulevant des interrogations sur l'effet que cela aura sur le maintien des populations de Parulines à gorge grise à moyen et long terme.

Comportement reproducteur

Le comportement des oiseaux lors de la période de reproduction est dirigé vers un seul et unique but, obtenir une descendance; que ce soit par l'activité vocale des mâles jusqu'à l'apport en nourriture aux jeunes en passant par la construction d'un nid. Puisque les oiseaux utilisent un système d'anisogamie, c'est-à-dire que les femelles produisent de gros gamètes alors que les mâles produisent des gamètes contenant uniquement le

complément génétique, les mâles ont tendance à vouloir augmenter leur descendance en fertilisant plusieurs femelles (Giraldeau et Dubois 2009). De ce fait, il existe une compétition intra-sexuelle chez le sexe qui investi le moins dans la production des jeunes (Giraldeau et Dubois 2009). Chez les oiseaux territoriaux dont les deux sexes s'investissent auprès des jeunes, cela se concrétise par des comportements tels que l'agression d'intrus et des vocalisations dans les limites de leur domaine. Ces activités vocales démontrent la présence d'un mâle mature sexuellement qui tente d'attirer une femelle et de protéger son territoire (Gil et Gahr 2002). De façon générale, un mâle avec une femelle aura une activité de chant moins élevée qu'un mâle célibataire (Hayes *et al.* 1986; Hennin *et al.* 2009).

La littérature scientifique assume la monogamie du genre *Oporornis*, mais dans les faits nous ignorons le véritable système d'appariement de la Paruline à gorge grise, car il peut y avoir présence de copulation extra-couple. Il se peut que ce soit un régime de polygynie, où un mâle peut fertiliser plusieurs femelles pour augmenter sa descendance (Giraldeau et Dubois 2009). La polygynandrie peut également être une option, car bien qu'en apparence ces oiseaux soient en couple, il se peut que la femelle aille se faire fertiliser par d'autres mâles ayant des gènes de grande qualité pour améliorer la génétique de sa descendance. Dans cette étude, nous nous concentrerons sur les soins parentaux définis par l'ensemble des comportements réalisés pour augmenter la survie des jeunes, contrairement à l'investissement parental qui calcule les coûts immédiats de la production de jeunes sur ses reproductions futures (McNamara *et al.* 1999; Houston *et al.* 2005). Chez la Paruline à gorge grise, le mâle s'investit dans le soin des jeunes (Pitochelli *et al.* 1997). Les soins parentaux sont très importants pour les passereaux, car chez la plupart d'entre eux, les oisillons sont nidicoles (Gill 1995). Par contre, il se peut que les comportements des deux sexes diffèrent autant dans leur proportion que dans leur nature (Pechacek *et al.* 2005; Barba *et al.* 2009; Mitrus *et al.* 2010).

Outils génétiques

Chez les oiseaux, contrairement aux mammifères, ce sont les femelles qui sont hétérogamétiques (ZW) et les mâles qui sont homogamétiques (ZZ) (Ellegren 2001). Une

des méthodes pour faire l'identification sexuelle est l'amplification d'une partie du gène CHD (Chromobox-helicase-DNA-binding) situé sur le chromosome sexuel (Cerit et Avanus 2007). Si le résultat démontre deux fragments de longueurs différentes, il s'agit d'une femelle et si les fragments sont de même taille nous avons un mâle (Marshall Graves 2009). La méthode pour déterminer le sexe chez plusieurs espèces d'oiseaux basée sur le gène CHD est celle de Griffiths *et al.* (1998). À l'aide de marqueurs moléculaires spécifiques, il est possible d'isoler par réaction de polymérase en chaîne (PCR) les portions du gène CHD qui nous intéresse et ainsi déterminer la longueur des fragments isolés pour savoir de quel sexe il s'agit. La technique n'a jamais été réalisée pour la Paruline à gorge grise, mais elle fonctionne pour de nombreux passereaux (Jensen *et al.* 2003).

La Paruline à gorge grise est un oiseau peu connu dans tous les domaines, autant pour le comportement que pour la génétique. Avec les outils moléculaires, on peut connaître les liens génétiques qui unissent les individus ainsi que la qualité d'une population (Si une population est en déclin, en expansion ou stable: Hedrick 1999). Les microsatellites sont une bonne façon d'évaluer la qualité d'une population et l'affiliation parentale (Jones et Ardren 2003) avec un simple échantillon d'ADN (Dawson *et al.* 2010). Les microsatellites sont des marqueurs dont les allèles présentent des variations dans le nombre de répétitions d'un motif de base généralement située dans l'ADN non codant (Ellegren 2004). Ils ont pris de l'importance et sont devenus les marqueurs génétiques les plus utilisés, car ils sont fréquents dans le génome, ont une grande proportion d'individus hétérozygotes et présentent une grande variabilité (Jarne et Lagoda 1996; Ellegren 2004; Nunome *et al.* 2006; Selkoe et Toonen 2006). La longueur des fragments de microsatellites varie beaucoup, car ceux-ci subissent beaucoup de mutations, cela permet de distinguer les individus lorsque le nombre de loci et leur variabilité sont adéquats (Jarne et Lagoda 1996).

Objectifs

L'objectif général poursuivi dans cette recherche vise à mieux comprendre la biologie de la reproduction de la Paruline à gorge grise (*Oporornis agilis*) sur les territoires utilisés

pour l'aménagement forêts/bleuets au nord du Lac-Saint-Jean au Québec, Canada. Tout d'abord, nous déterminerons le comportement reproducteur de cette espèce et les soins parentaux apportés par les deux sexes, dont l'activité de chant des mâles, pour évaluer s'il existe des différences entre les nicheurs et les non-nicheurs. Finalement, il y aura réalisation d'analyses génétiques pour élaborer des méthodes qui permettront de déterminer l'affiliation parentale ainsi que le sexe ratio au sein des couvées.

Cette étude est la première fondée exclusivement sur le comportement de la Paruline à gorge grise ayant comme objectif de mieux comprendre la biologie de sa reproduction. Les articles qui parlaient de comportement relié à la nidification étaient des observations faites sur un nid trouvé par hasard. Nous voulons suivre les adultes ainsi que plusieurs nids sans arrêt pour connaître tous les aspects du comportement reproducteur. Les informations récoltées lors de la collecte de données devraient se comparer aux observations ponctuelles recensées dans la littérature et le cas échéant aux autres espèces du genre *Oporonis*.

De plus, nous ferons des analyses génétiques dans le but de trouver des outils moléculaires pour étudier la génétique de la Paruline à gorge grise. Comme aucune étude n'a été réalisée sur cette espèce, nos devrons rechercher des microsatellites déjà existants qui pourraient amplifier avec des espèces du genre *Oporornis* et être séquencés. Il faudra également identifier des protocoles qui fonctionnent pour déterminer le sexe de nos individus. La technique la plus utilisée à l'heure actuelle soit celle de Griffiths *et al.* (1998) sera testée.

CHAPITRE 1

Breeding behavior and male status determination of the Connecticut Warbler (*Oporornis agilis*) in Jack pine (*Pinus banksiana*) forests of Quebec.

Authors: Marie-Christine Saulnier, Jacques Ibarzabal and Jean-Pierre Savard

ABSTRACT

The Connecticut Warbler is a poorly known and secretive species. Understanding of its behavior is essential to monitor and manage this species. Our objective was to study the breeding behavior of the Connecticut warbler. Data were collected during the breeding period by monitoring adult male song frequency and nest activities of both sexes. Cameras were connected to a Digital Video Recording (DVR) working in motion detection capability mode and installed above the nest to continuously monitor breeding behavior throughout the nestling period. Paired males sang less often than unpaired males. Females incubated four to five eggs alone during 11 days. After hatching, both sexes provided parental care but females alone incubated and brooded during night-time. Nestlings left the nest at eight day old with a success rate of 8 / 18 eggs (44.4%) among four nests. During the first days after hatching, females mainly brooded nestlings whereas males fed them. As nestlings aged, females spent less time brooding and more time foraging. According to brooding behavior we suggest nestlings could thermoregulate near seven days old. Both sexes visited the nest at the same frequency and increasingly as nestlings aged. Sanitary activities increased with nestling age and were mainly accomplished by the male. Nevertheless, both parents ate fecal sacs, but at lower frequency as nestlings aged possibly because of the decreasing amount of nutrients in the faeces of young as their digestive system developed. This research is the first entirely about the biology of the Connecticut Warbler.

Key words: Parental care, nestling growth, breeding ecology, reproductive success, clutch size, singing rate, male status determination

INTRODUCTION

The Connecticut Warbler is a small neotropical late migrant. It is a very secretive bird that feeds and nests on the ground (Pitochelli *et al.* 1997). This warbler is poorly known and recent research has not addressed specifically its reproductive behavior (Pitochelli *et al.* 1997). The breeding range is a boreal band crossing Canada, from British Colombia to Quebec including north central United-States (Michigan, Wisconsin and Minnesota). Throughout this large breeding range, population densities are low and geographically localised (Cooper *et al.* 1997; Pitochelli *et al.* 1997). Long-distance migratory birds, particularly insectivorous species (Bohninggaese *et al.* 1993; Benton *et al.* 2002; Archaux 2003) like the Connecticut Warbler are declining.

Given its secretive habits and low population densities little is known of its population status. It has not been evaluated in Canada (COSEWIC 2010) but is considered least of concern since 2004 by IUCN (2010) and not at risk by NatureServe (2010). However, regionally, the Connecticut Warbler is imperilled in large parts of its breeding area (British Colombia, Saskatchewan, Michigan and Wisconsin; NatureServe (2010). Moreover, it has been identified as a priority species in Ontario (Ontario Partners in Flight, 2006) and it is on the British Colombia red list with the "threatened" status (Cooper *et al.* 1997; Cooper et Beauchesne 2004).

Breeding Bird Atlases from many provinces and states highlighted the difficulties in confirming the breeding status of Connecticut Warblers (Cadman *et al.* 2008; Gauthier et Aubry 1995; Penner et Wagner 2005). During the Quebec Breeding Bird Atlas (5 509 parcels of 100 km^2), the Connecticut Warbler was detected in 21 parcels, but nesting was only confirmed in only 3 (Ibarzabal *et al.* 1995). Comparatively, the Yellow-rumped Warbler (*Dendroica coronata*), an other conifer associated warbler, had a confirmation rate of 33 % (507 on 1539 parcels; Letourneau et Lafontaine 1995).

The number of documented nests in the literature is small (Seton 1884; Huff 1929; Kilgore et Breckenridge 1929; Stone 1929; Gromme 1942; Bent 1953; Walkinshaw et

Dyer 1961) and not much information has been compiled on the time budget allowed by adults during breeding. Parental care is important in atricial species. Parents need to defend brood and sanitize their young as well as forage for them. The frequency of these behaviors may vary between sexes (Verner et Willson 1969; Pechacek *et al.* 2005; Barba *et al.* 2009; Mitrus *et al.* 2010). For males, reproductive efforts begin with singing activities to attract mates and defend territories (Gil et Gahr 2002). A singing male is not a guaranty of nesting and once the male is paired, its singing activity may decline (Thusius *et al.* 2001; Hennin *et al.* 2009).

In Quebec, the Connecticut Warbler is mainly found in Jack Pine forests located on flat sand deposits covered by ericaceous plants, a habitat favourable to the implantation of blueberry fields (Chagnon 1970; Savard 2001). The rapid growth of the blueberry industry may impact populations of Connecticut Warblers (habitat loss). To protect both wildlife and logging, a new agro-forested development has been implanted: the "forest/blueberry management" which alternated 40-60m blueberry and forest bands. This research was done to evaluate if this management would indeed accommodate Connecticut Warblers.

Our main objective was to document the reproductive behavior of the Connecticut Warbler in "forest/blueberry management landscapes". Specifics objectives were 1) to document the breeding behavior and implication of both sexes in parental care; and 2) to determine if song patterns of paired and unpaired males differed and could be used to determine breeding status. We predicted that paired males would sang less often than unpaired males (Hayes *et al.* 1986; Hennin *et al.* 2009) and that the breeding behavior and parental care will be similar to those of other *Oporornis* species, like the Mourning (*Oporornis philadelphia*) and MacGillivray's warblers (*Oporornis tolmiei*) (Verner et Willson 1969; Pitochelli 1993; Pitochelli 1995) as well as to those of other Connecticut Warbler populations (Huff 1929; Walkinshaw et Dyer 1961).

METHOD

Field area

The study was conducted north of the Lac-Saint-Jean, Quebec, Canada, more accurately at Normandin (48°50'99''N, 72°37'21''W) on the CAFN (corporation d'aménagement forestier de Normandin) territory during three years (2007 to 2009). For the last year, 2009, Albanel was added (48°55'34''N, 72°23'73''W) on the DAFTA (Développement et aménagement d'une forêt touristique à Albanel) territory. Sites were composed of blueberry fields dispersed within a matrix of mature Jack pine (*Pinus banksiana*) forests. The ground was covered by *Ericaceous* species, mainly *Kalmia angustifolia, Vaccinium* sp. and *Ledum groenlandicum*. The site was located on fine fluvioglacial material like sand, in this case dating from the last deglaciation, and without significant relief (Chagnon 1970; Savard 2001). Connecticut Warblers were captured at their arrival from wintering areas from 30th May to 14th of June. We also sampled nestlings from the ends of June to the end of July.

Capture, bird measurements and male status

All Connecticut Warblers on the study area were located and captured if possible. The capture array was composed of two mist nets (12 meters; 30 mm mesh) placed at 90° near a singing male. Playbacks of territorial calls (Pitochelli *et al.* 1997), recorded on site, were broadcasted over ericaceous plants until the defending male was captured. Special efforts were made to capture females because they were very useful for finding nests. Females, walking on the ground, were driven into nets. Wing length, tail and under tail covers were measured with standard wing rules of 15 cm (± 0.5 mm; Avinet Inc.). Here the term "under tail cover" (Uc tail) corresponds to the length of tail not covered by under tail covers. Exposed culmen was measured with an electronic calliper (± 0.01 mm; Mitutoyo); mass recorded with a digital balance (± 0.1g; Ohaus CS200); and fat scores noted according to DeSante *et al* (2008). Darvic color rings were installed for individual identification. In 2008 and 2009 each individual, but preferentially females had a BD2-A radio-transmitter (Holohil Ltd.), weighing 0.63 g fitted with a harness (Rappole et Tipton 1991).

A STR-1000 telemetry receiver (Lotek engineering inc.) was used to follow males and females and to locate nest. For each male captured, we determined his status by the presence or absence of breeding activity. A male was considered paired when he was seen with a female at several observations, or transporting material or food. The singing behavior of the male was monitored regularly and the numbers of songs emitted by 15-minute periods were noted.

Nest

When a nest was discovered, a surveillance system (Digital video recorder; CPT-SDR40/160) with infrared camera (Dual power true day and night IR dome camera; w/ 40 IR LEDs and vari-focal lens) was installed to monitor the nesting process. The system started recording ten seconds before and after movements were detected approximately 0.3 m around the nest. The system was powered by a low decibel Yamaha generator (EF1000is; 47 dB at 7 m) set at 75 m of the nest. Finally, eggs were measured when the nest was found before hatching and, when possible, without disturbing adults. An observer watched videos and quantified male, female and nestling behaviors. Nestlings were banded when 5 or 6 day old and biometrical measures were taken as for the adults.

Nests were collected at least one week after departure of fledglings. We took the nest and all plants within a ten centimetre radius. After deposing the nest on a plane surface, parts that were not attached to the nest were removed. Nest composition, diameter (two perpendicular measures) and depth were noted for the outside and the inside walls. Nest dry weight was obtained after six hours in a stove at 65° C.

Video

Nest monitoring was analysed and all information compiled in a data base for both sexes. For the incubating period, number of visits, time spent at the nest (TSN: time to incubate eggs) and time spent out of the nest (TON) were noted. After hatching, four behaviors

were compiled: time spent out of the nest (TON), time spent at the nest (TSN; combination of time spent brooding (TB) and time spent taking care of nestlings (TCN; feeding, sanitary activities and watching nestlings next to the nest). Also, the frequencies of nestling feeding and fecal sac removal were recorded. A visit with sanitary activity meant that fecal sacs were removed (1, 2 or 3).

The compilation of activity was divided in nocturnal and diurnal periods. Before hatching, we fixed the beginning and the end of the diurnal period of activity at 06:00 and 20:00 respectively (14 hours). After hatching, the diurnal period of activity started as females left the nest in the morning and finished when they returned at the nest for the night. Considering this, we fixed the diurnal period of activity between 05:00 and 21:00 to have full hours (16 hours).

Statistics

All analysis were run with SAS 9.1.3 (SAS 2003). Means are presented ± SE and compared using *t*-tests for parametric distribution. We used chi-square tests for frequency analysis. As adult culmen measures did not have a normal distribution, nonparametric tests (Kolmogorov-Smirnov) were used. GLMs were used to compare the effect of pairing status and time of day on singing activity and *t*-tests to compare status of male between each hour. For the incubating period, *t*-tests between mean values and frequencies were done to compare behaviors (TSN and TON). ANOVAs were used to analyse behaviors among nestling ages and the hours of the day. We used chi-squares to test for differences in number of visits between nesting ages and times of day (hours).

After hatching, four variables were measured (TSN, TB, TCN and TON). The means of the different behaviors and visits were compared with *t* and chi-square tests between different nests to verify if they could be pooled together. After that, GLMs (relations with nestling age) and ANOVAs (relations with hours of the day) were done. An lsmeans for GLM and a Tuckey test for ANOVA revealed where differences occurred. In this context, *t*-tests were done to compare both sexes day by day and hour by hour. The Pearson's correlation coefficient was used for the sum of diurnal time spent brooding for

males and females and to quantify relations between times spent brooding and nestling age.

The frequency of all visits and visits with brooding were analysed with chi-square tests for both sexes and between them. Numbers of visits with food and with fecal sac removal were expressed in percentage of total number of visits. The percentage of frequency of feeding and fecal sac removal activities were compared for each sex among the age of nestlings and the time of the day with chi-square tests. Percentages of frequencies were also compared between sexes with chi-square. At each visit with fecal sac removal, adults ate them or transported them out of the nest. Numbers of fecal sacs eaten and transported were analysed in function nestling age for both sexes using chi-square tests. Means numbers of visits in function of the hour were compared with t-test.

RESULTS

Date and bird measurements

In three years, nine females and 31 males were captured on their breeding territory (13 in 2007; 10 in 2008 and 17 in 2009). We also recaptured four males in 2008 and three in 2009. Results will be present in a chronological sequence, starting with adult measurements through the singing activity of male and ending with adult behavior for nestling.

Males arrived at the end of May (27-29 May) and females at the beginning of June (3-7 June). Weight and body measurements were taken a few days after bird detection (Table 1).

Table 1: Measurements of adult body parts and weight of Connecticut Warbler males (n = 31) and females (In parenthesis; n = 9).

	Wing (mm)	Weight (g)	Culmen (mm)	Tail (mm)	Uc tail (mm)
Mean ± SE	71.8 ± 0.6 (69.2 ± 1.3)	13.6 ± 0.1 (14.6 ± 0.7)	11.27 ± 0.10 (11.25 ± 0.32)	48.7 ± 0.4 (47.6 ± 0.5)	11.5 ± 0.5 (14.6 ± 1.9)
Min	65.9 (65.5)	12.3 (10.3)	9.57 (9.13)	40.7 (46.0)	7.0 (10.0)
Max	82.0 (79.0)	15.7 (17.3)	12.60 (12.62)	52.0 (50.0)	20.0 (26.0)

Most birds detected on the area were males (78 %; $X^2 = 12.10$; DF = 1; P = 0.0005). Differences between sexes were significant for weight (t = 2.21; DF = 37; P = 0.0336; Table 1) and for under tail covers (t = -2.04; DF = 38; P = 0.0489; Table 1). Paired males were heavier than unpaired males (paired = 13.9 ± 0.3g, n = 9; unpaired = 13.0 ± 0.2g, n = 13; t = -2.97; DF = 10.8; P = 0.0129). Sixty five percent of captured individuals had no fat accumulation and traces were found on 18 % of specimens. Female had higher fat scores, but more samples are needed to compare sexes.

Singing activity

The singing activity of 22 males (6 in 2007, 7 in 2008 and 9 in 2009) was recorded for a total of 149 hours of observation (76 h for paired and 73 h for unpaired). Observations were done from 11th to 30th of June and between 5:30 to 14:00 (Figure 1). Connecticut Warblers sang 27.0 ± 32.9 times in fifteen minutes when considering all observations including empty period with a maximum value of 116 /15-min. However, male status affected singing activity as paired males sang three times less than unpaired males (paired males = 13.7 ± 1.4 /15-min, n = 9; unpaired males = 40.4 ± 2.0 /15-min, n = 13; Table 2). Male status influenced singing rate (Table 2). Differences between the singing activities of paired and unpaired males were more important early in the morning as suggested by the time distribution of the data (Figure 1). Daily singing patterns were similar between male status but singing rates decreased during the day.

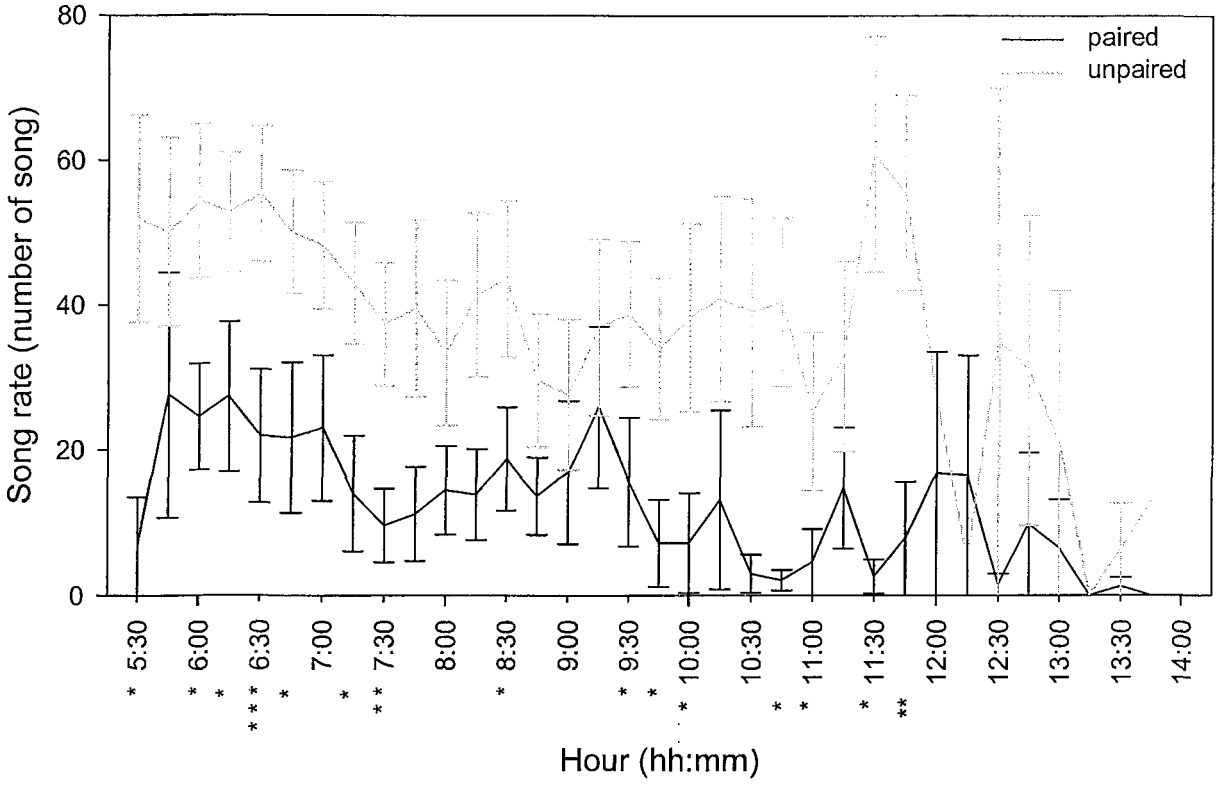

Figure 1: Singing activity patterns of 9 paired (black) and 13 unpaired males (gray) during the time of the day (mean ± SE). Significance level by hour are indicated with an asterisk (*P*: * ≥0.05, ** ≥0.01 and *** ≥0.001).

Table 2: Statistical analysis (GLMs) of the interaction between male status (paired and unpaired) and time of day for the singing activity of Figure 1.

Source of variation	DF	F	P
Status	1	56.64	<0.0001
Time of the day	34	1.22	0.1841
Status*Time of the day	33	0.45	0.9969

Nest

Seven nests were found on the ground in small depressions (3 in 2008, #1 to 3 and 4 in 2009, #4 to 7), all were located near an opening in forest cover. Nests were composed of a matrix of herbaceous twigs with some inserted roots; Pine needles and leaves were present in different proportions for each nest. Nests were characterised by a circle-shape as no difference was detected between both perpendicular measures of the outside diameter (length = 84.14 ± 3.45 mm, width = 75.72 ± 2.97 mm; t = -1.85; DF = 11.7; P = 0.0896). Nests had an inside diameter of 54.61 ± 2.15 mm and an inside depth of 27.82 ± 3.56 mm (Table 3).

Table 3: Dry weight and inside and outside measurements of seven Connecticut Warbler nests.

	Dry weight (g)	Inside		Outside	
		Diameter (mm)	Depth (mm)	Diameter (mm)	Depth (mm)
Mean ± SE	5.3 ± 1.0	54.61 ± 2.15	27.83 ± 3.56	79.96 ± 3.12	48.98 ± 4.94
Min	2.0	46.99	20.00	71.48	34.00
Max	8.3	61.65	44.00	94.74	70.93

Nest contained an average of 4.4 ± 0.5 eggs (range 4-5) for a total of 32 eggs in seven nests. The length and width were measured on 20 eggs from 5 nests (Table 4). In one case, five eggs were taken after the female abandoned the nest and the weight (mean = 0.098 ± 0.003 g) and thickness (mean = 0.09 ± 0.01 mm) of empty shells were noted. Eggs were creamy with "old rose" spots, which were more intense at the larger

end. Six females started to incubate between 20 and 24 of June and one started the 10 July.

Table 4: Measurements of Connecticut Warbler eggs.

	Weight (g)	Width (mm)	Lenght (mm)
n	16	20	20
Mean ± SE	1.7 ± 0.1	14.24 ± 0.12	18.95 ± 0.25
Min	1.2	13.37	17.43
Max	2.3	15.2	21.01

Adult behavior at nest

Of the seven nests, three were abandoned (nest #1, 5 and 6; with 5, 5 and 4 eggs), two were depredated (nest # 2 and 7; with respectively 5 nestlings and 4 eggs) and two arrived at term (nest #3 and 4; from 4 and 5 eggs). Predator identity was unknown for both nests but Eastern Chipmunk (*Tamias striatus*) was suspected for nest #2. The two last nests produced respectively three and five nestlings which were measured at seven and six day old without differences between nestlings of both nests, thus data were pooled (Table 5). However, there were fat traces on the five nestlings in the same nest and the other three were each one in the categories "light", "half" and "filled".

Table 5: Measurements of Connecticut Warbler nestlings at six (5 nestlings) and seven day old (3 nestlings).

	Tarsus m (mm)	Tarsus (mm)	Wing (mm)	Weight (g)	Culmen (mm)	Tail (mm)	Uc tail (mm)	Primary (mm)
N	8	8	8	3	8	8	8	8
Mean	20.4	18.4	27.8	10.9	6.30	3.6	1.8	16.8
SE	0.4	0.6	2.0	0.8	0.25	0.7	0.5	2.2
Min	19.0	15.0	18.0	9.8	5.10	2.0	0	6.0
Max	23.0	20.0	35.0	12.4	7.44	7.0	3.0	25.0

Activity at nests (#3 and #4) was recorded for approximately 29 days (including 10 days of incubating, only at nest #3) for a total of 170 hours of video recordings (video recorded only when movements occurred). The data base contained 1222 observations of adults visits at the nest. There are 29 sex identity missing values, 30 food information missing values and 193 fecal bag information missing values.

The incubation lasted 11 days (information taken on video for nest #3 and observation for nest #4) and juveniles left the nest at seven (1 nestling) and eight days old (7 nestlings) without fully completing the development of their tail and wing feathers (Table 5). The female of the nest #3 ate the empty shell of the eggs.

Nocturnal activity

Based on two nests, night-time incubating and brooding was assumed by the female only, (Table 6). This activity started at twilight and ended at dawn like in the Mourning Warbler (Cox 1960). There was no difference in brooding time in relation to nestling age. Only seven nights were compiled before hatching because the camera sensibility was not well adjusted and the video had not recorded all female activities.

Table 6: Night-time spent at the nest as well as arrival and departure times of female Connecticut Warblers before hatching for nests #3 ($n = 7$ nights) and after hatching for nests #3 and #4 ($n = 16$ nights).

Period	Time at nest	Arrival	Departure
Before hatching			
Mean ± SE	09:04:05 ± 00:42:05	20:38:28 ± 00:25:37	05:42:33 ± 00:26:40
Min	08:12:14	20:02:33	05:12:45
Max	10:03:39	21:03:15	06:26:10
After hatching			
Mean ± SE	07:48:05 ± 00:05:59	21:01:17 ± 00:03:54	04:49:22 ± 00:03:19
Min	07:13:13	20:28:12	04:10:00
Max	08:34:12	21:24:00	05:06:17

Time expressed in: hh:mm:ss

Diurnal activity

Before hatching

Only females attended the nest before hatching. They incubated for 09:43:37 ± 00:20:20 hours each day (between 06:00 and 20:00; Figure 2). However, they left the nest regularly totalising 03:12:29 ± 00:07:09 hours away from the nest. At each visit

(n = 108), females spent 51:54 ± 01:59 min at the nest and left it for 19:46 ± 01:45 min in average demonstrating a larger allocation of time to incubation ($t = 10.73$; $DF = 24.4$; $P < 0.0001$; Figure 3). Time at and out of the nest did not change throughout the incubation period (Figure 4; Table 7a). In average, females visited the nest 10.8 times per day and the number of visits did not change during the incubation period ($X^2 = 2.1852$; $DF = 9$; $P = 0.9882$; Figure 5). Time spent away from the nest did not differ throughout the day but time spent at the nest did, with less time spent at the end of the morning (Figure 6; Table 7b). The frequency of female visits in the day was 0.77/hour and there was no variation among the time of the day ($X^2 = 9.4444$; $DF = 13$; $P = 0.7386$).

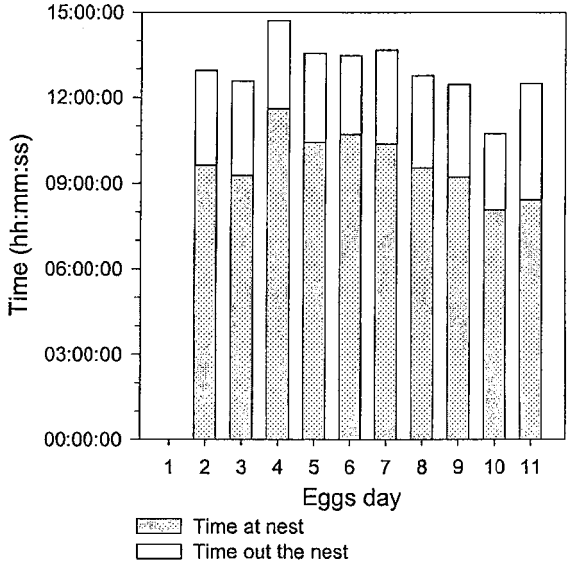

Figure 2: Time invested in diurnal activities by females before hatching in relation to incubation stage (eggs day). Time spent at the nest (gray bar) and out of the nest (white bar). Time for a given behavior was compiled in the hour it began and for this reason it is possible for it to exceed 14 hours.

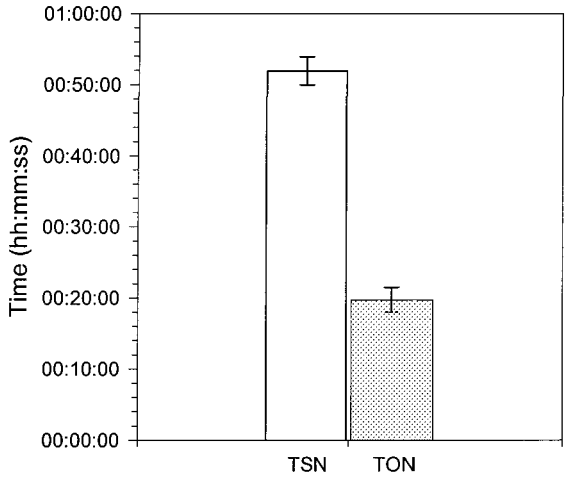

Figure 3: Average time spent at the nest (TSN: white) and time spent out of the nest (TON: gray) by female Connecticut Warblers (mean ± SE; $n = 108$ TSN and TON).

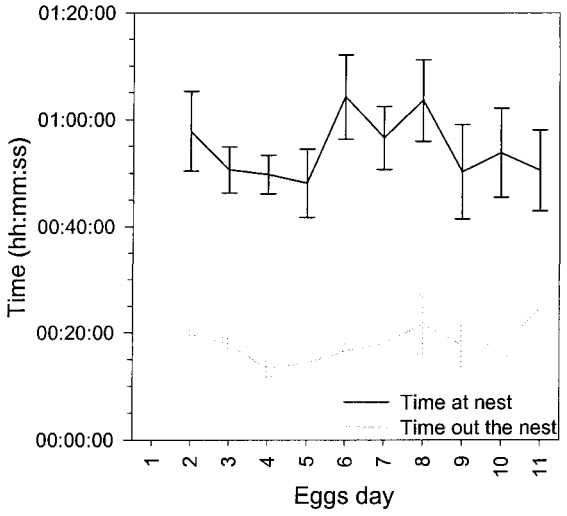

Figure 4: Average time spent at the nest (black) and time spent out (gray) by female Connecticut Warblers during the incubation period (eggs day; mean ± SE).

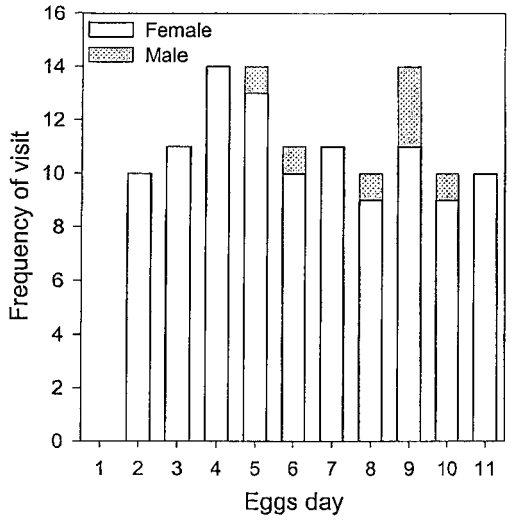

Figure 5: Nest visit frequency by male and female Connecticut Warblers in relation to incubation stage (egg day; female in white and male in gray).

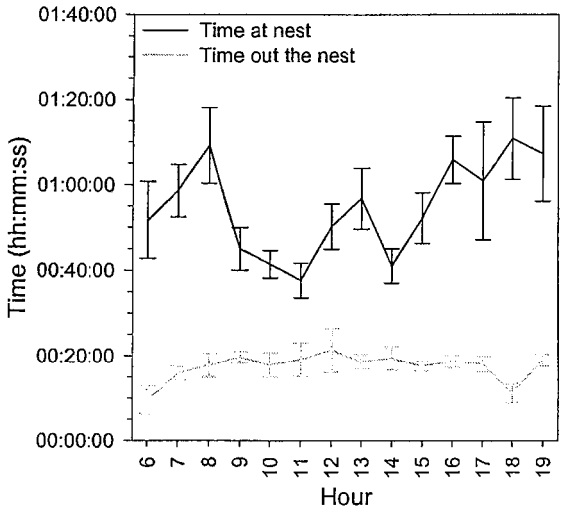

Figure 6: Average time spent at nest (black) and time spent out of the nest (gray) for female Connecticut Warblers in relation to time of the day (hour) before hatching (mean ± SE). The time for a given behavior was compiled in the hour it began and for this reason it is possible it exceed 60 min.

Table 7: Comparison of time spent at the nest (TSN) and time spent out of the nest (TON) in relation to incubation stage (a) (eggs day) and time of day (b).

Source of variation	DF	F	P
a) Eggs day			
TSN	9	0.72	0.6856
TON	9	1.69	0.1009
b) Time of the day			
TSN	13	2.57	0.0044
TON	13	0.81	0.6490

After hatching

No differences were found between the two males and females of different nests (Figure 7; Table 8 a-b) but differences were found between sexes (Figure 7; Table 8 c).

Females did not spent the same amount of time brooding, caring for young and out of the nest (Table 8 c; $F = 182.87$; $DF = 2$; $P < 0.0001$). Males, spent more time out of the nest than for brooding or caring for the young ($F = 1076.88$; $DF = 2$; $P < 0.0001$; Table 8 c).

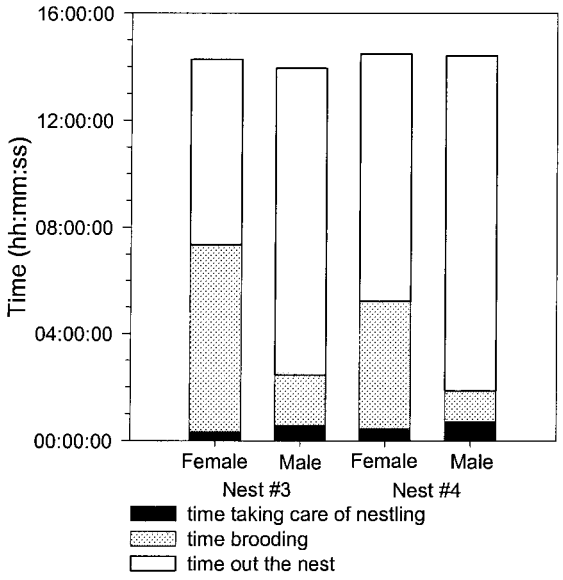

Figure 7: Time invested in diurnal activities after hatching in relation to sex and nest. Time spent during the day taking care of nestlings (black), brooding (gray) and away from the nest (white)

Table 8: Comparison of daily time allocated to nestling care (TCN), brooding (TB) and out of the nest (TON) for males and females in two nests (a-b) and between sexes (c).

Behavior	Mean ± SE		DF	t	P
a) Female	Nest #3	Nest #4			
TCN	00:19:50 ± 00:05:12	00:26:47 ± 00:03:40	12.9	-1.09	0.2959
TB	07:01:23 ± 01:17:17	04:47:19 ± 01:17:04	12.9	1.23	0.2413
TON	06:54:52 ± 01:29:34	09:14:27 ± 01:25:41	14.8	-1.13	0.2781
b) Male	Nest #3	Nest #4			
TCN	00:34:25 ± 00:08:58	00:42:31 ± 00:06:52	15.1	-0.73	0.4745
TB	01:52:21 ± 00:33:57	01:09:16 ± 00:28:02	8.95	0.98	0.3535
TON	11:29:44 ± 00:59:58	12:32:51 ± 01:17:36	15.0	-0.64	0.5309
c) Total	Female	Male			
TCN	00:23:31 ± 00:03:08	00:38:28 ± 00:05:33	33.0	-2.31	0.0274
TB	05:49:53 ± 00:55:34	01:32:46 ± 00:23:56	24.0	3.78	0.0009
TON	08:08:46 ± 01:02:26	12:01:18 ± 00:48:11	30.5	-2.95	0.0061

Time expressed in: hh:mm:ss

The mean times allocated were calculated for both nests by sex and even if differences were observed, tendencies look similar between nests (Figure 8). Males and females from the same nest differed in time allocated to each behavior except for the time spent to care for nestling. Time brooding (Figure 8 B) was longer for females than males and time out of the nest shorter for females than males (Figure 8 C). Also, time spent taking care of nestlings was similar for the same sex at both nests, although differed between sexes in nest # 4 (Figure 8 D).

There was no difference in behavior frequencies between the females and males of both nests (TSN, TON and TCN: Female: $X^2 = 0.5634$; DF = 1; P = 0.4529; Male: $X^2 = 3.3612$; DF = 1; P = 0.0667) (TB: Female: $X^2 = 1.6177$; DF = 1; P = 0.2034; Male: $X^2 = 3.6446$; DF = 1; P = 0.0563).

For the rest of the results, both nests will be pooled together to analyse differences in behavior duration and frequencies between sex in relation to nestling age and time of day.

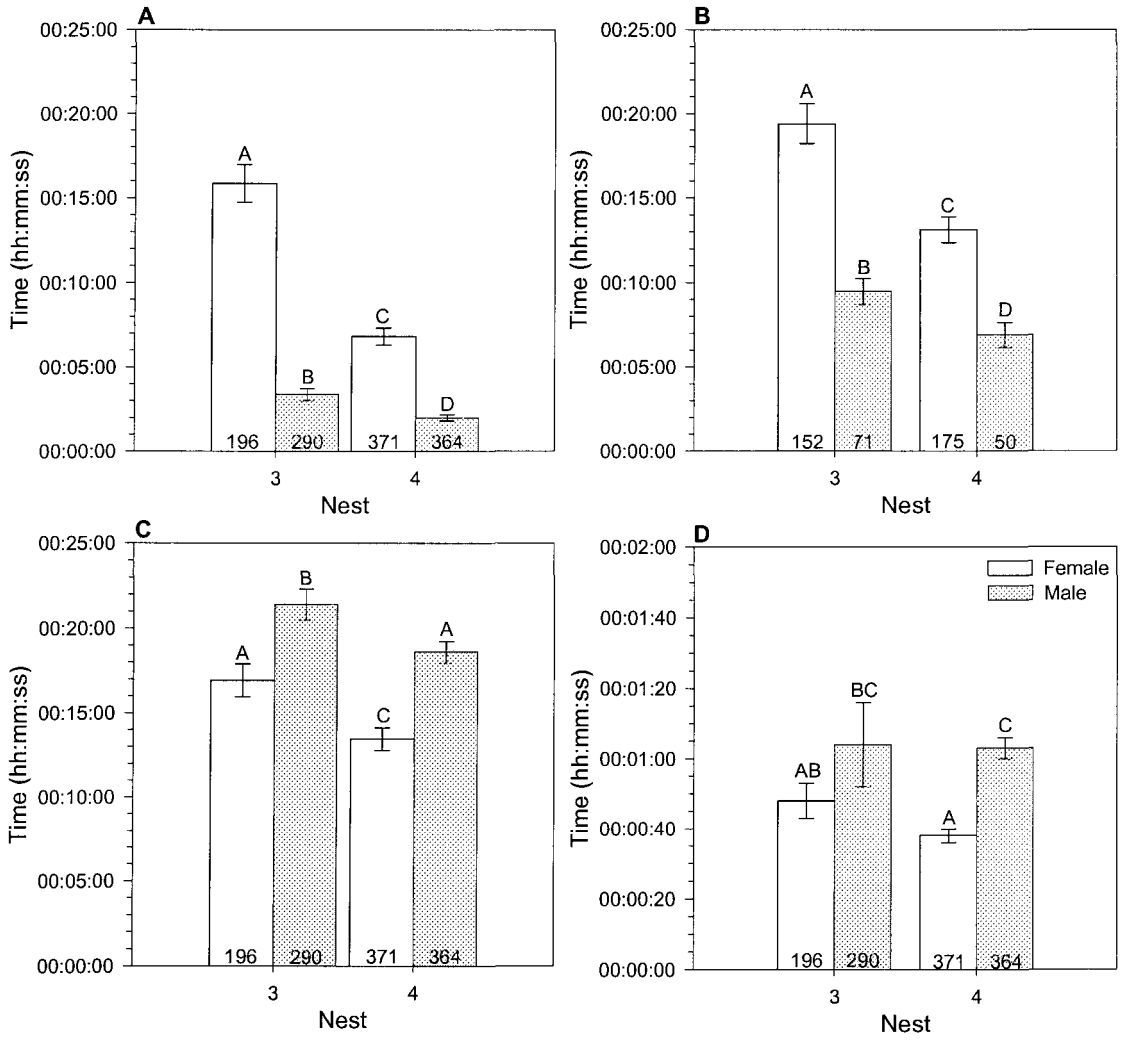

Figure 8: Average duration (in hour:minute:second; means ± SE) of time spent at the nest (A), brooding (B), out of the nest (C) and taking care of nestlings (D) in relation to sexes and nests (# 3 and # 4). *n* values appear at the bottom of each column and significances differences are represented by a letter above.

Nestling age

As hatching hour and the hour when nestlings left the nest were not controlled, days 0 and 8 did not totalised 16 hours (Figure 9). Time spent brooding decreased for females and males as nestlings got older (Figure 9 A; females: r = -0.9173, P = 0.0013; B; males: r = -0.7820, P = 0.0661).

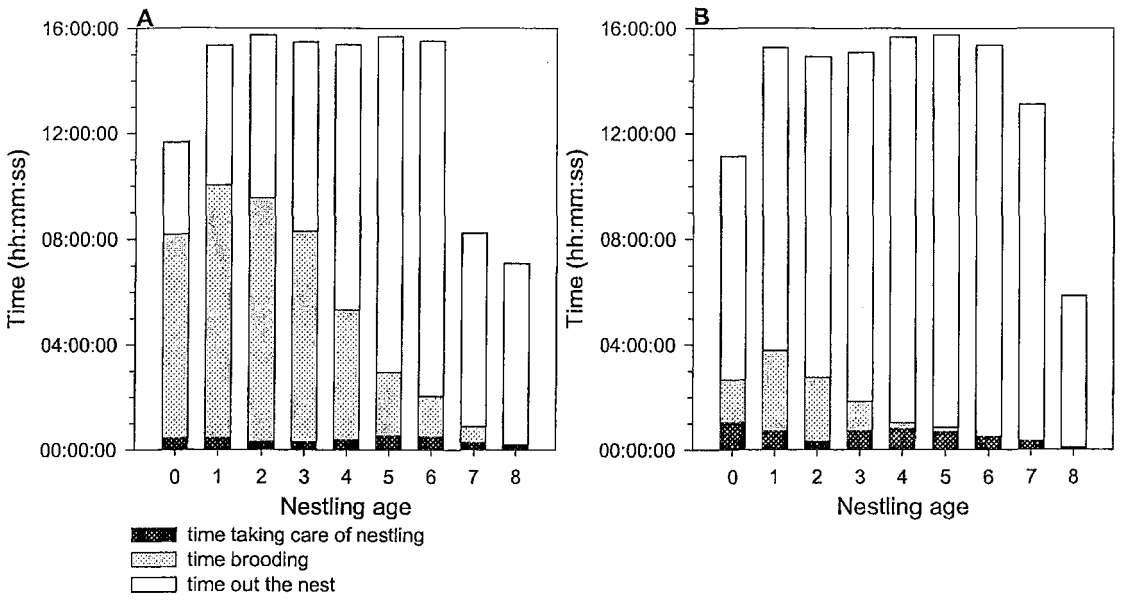

Figure 9: Time invested in diurnal activities in relation of the nestling age. Time spent taking care of nestlings (black), brooding (gray), and out of the nest (white) in relation to nestling age (Female: A; Male: B).

Mean time allocated at each visit for a given behavior generally differed according to nestling age (Figure 10). However, brooding time per visit for males was constant throughout the nestling period (Figure 10 B). The interaction of sex and the nestling age was significant for all behaviors (Table 12) suggesting both sexes varied differently according to nestling age. Time spent brooding was longer for females especially from day 0 to 3 whereas time spent out of the nest was shorter. Males stopped brooding at day five and females reduced it significantly from day 2 and stopped at day 7. The average time spent taking care of nestlings at each visit was short, generally under 1 min for both parents. In general, all averages decreased as nestlings grew older.

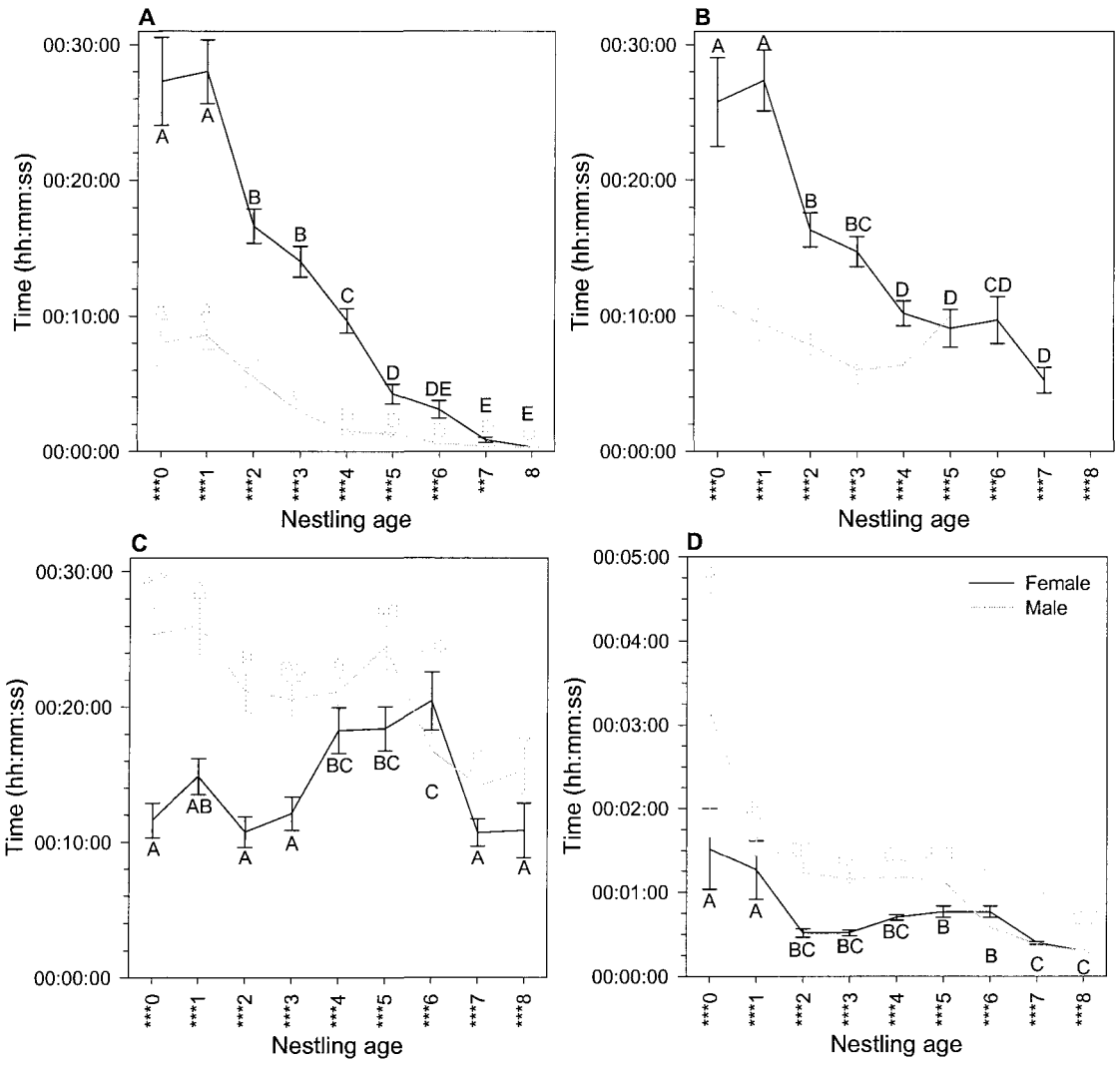

Figure 10: Time spent at the nest (A), brooding (B), out of the nest (C) and of taking care of nestling (D) in hour:minute:second for both parents (mean ± SE) in relation to nestling age. Differences within a curve are indicated by different letters and significant differences between sexes are shown at the bottom of the graph (*P*: * ≥0.05, ** ≥0.01 and *** ≥0.001).

Table 9: Comparison of time spent at the nest (TSN), brooding (TB), taking care of nestlings (TCN), and out of the nest (TON) by sex, nestling age and their interaction.

Source of variation	DF	F	P
a) TSN			
Sex	1	388.12	□ 0.0001
Nestling age	8	106.12	□ 0.0001
Sex*Nestling age	8	31.36	□ 0.0001
b) TB			
Sex	1	17.51	□ 0.0001
Nestling age	7	8.10	□ 0.0001
Sex*Nestling age	5	3.27	0.0066
c) TON			
Sex	1	63.15	□ 0.0001
Nestling age	8	8.19	□ 0.0001
Sex*Nestling age	8	5.39	□ 0.0001
d) TCN			
Sex	1	14.19	0.0002
Nestling age	8	8.65	□ 0.0001
Sex*Nestling age	8	1.95	0.0496

The number of visits at the nest increased with nestling age for both sexes (Female: $X^2 = 45.7143$; DF = 8; $P < 0.0001$; Male: $X^2 = 64.9266$; DF = 8; $P < 0.0001$; Figure 11). The frequency of visits at the nest for both adults increased similarly for both sexes with nestling age until mid-age ($X^2 = 5.9963$; DF = 8; $P = 0.6476$; Figure 11). Brooding frequency increased as nestling became older and decreased at the end of the nestling stage (Female: $X^2 = 81.7339$; DF = 7; $P < 0.0001$; Male: $X^2 = 63.2149$; DF = 5; $P < 0.0001$). Males did less brooding visits than females ($X^2 = 55.7299$; DF = 7; $P < 0.0001$).

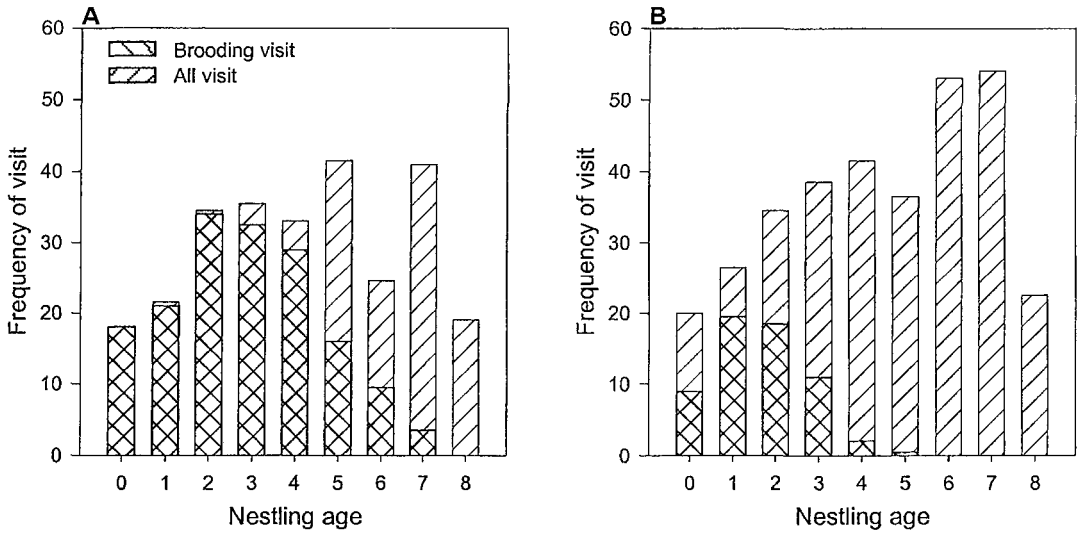

Figure 11: Frequency of number of visits (right diagonal) and of brooding visits (left diagonal) for Connecticut Warbler nests in relation to nestling age for both sexes (Female: A; Male: B).

The proportion of visits with food was very high for the males and this proportion did not changed accordingly to nestling age ($X^2 = 13.9224$; DF = 8; P = 0.0838; Figure 12 B). For females, an increase occurred as they switched progressively from brooding to feeding activity ($X^2 = 34.1668$; DF = 8; $P < 0.0001$; Figure 12 A). There was no difference between sexes and nestling ages for the number of visit with food ($X^2 = 6.2254$; DF = 8; P = 0.6220).

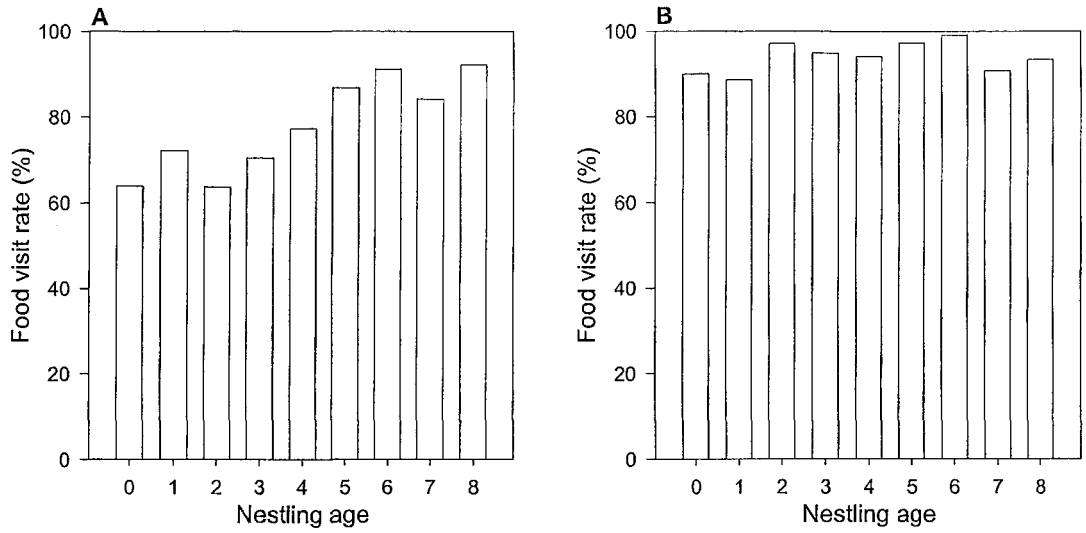

Figure 12: Percentage of the visit with food provision in function of nestling age for both sexes (Female: A; Male: B).

Males and females fecal sac removal patterns differed slightly (% visit with fecal sac removal: $X^2 = 13.3770$; DF = 8; P = 0.0995; Figure 13; nb of fecal sac total: $X^2 = 15.0669$; DF = 8; P = 0.0579; Figure 14) but increased in frequency as nestling aged (Female: $X^2 = 34.9053$; DF = 8; P < 0.0001; Male: $X^2 = 57.2731$; DF = 8; P < 0.0001; Figure 13A-B).

It was the same when the number of fecal sacs removed throughout nestling period was considered (Female: $X^2 = 67.6604$; DF = 8; P < 0.0001; Male: $X^2 = 107.5111$; DF = 8; P < 0.0001; Figure 14 A-B). Moreover, at each visit to clean the nest there were two outcomes for a fecal sac (eaten or transported). At the beginning of the nestling period, adults ate all fecal sacs and began transporting them when nestlings were three days old The number of fecal sac eaten diminished progressively until fledgling departure (Female: $X^2 = 142.7504$; DF = 8; P < 0.0001; Male: $X^2 = 195.5513$; DF = 8; P < 0.0001). One nestling had already left the nest by day 8 and the remaining nestlings left in the middle of day 8.

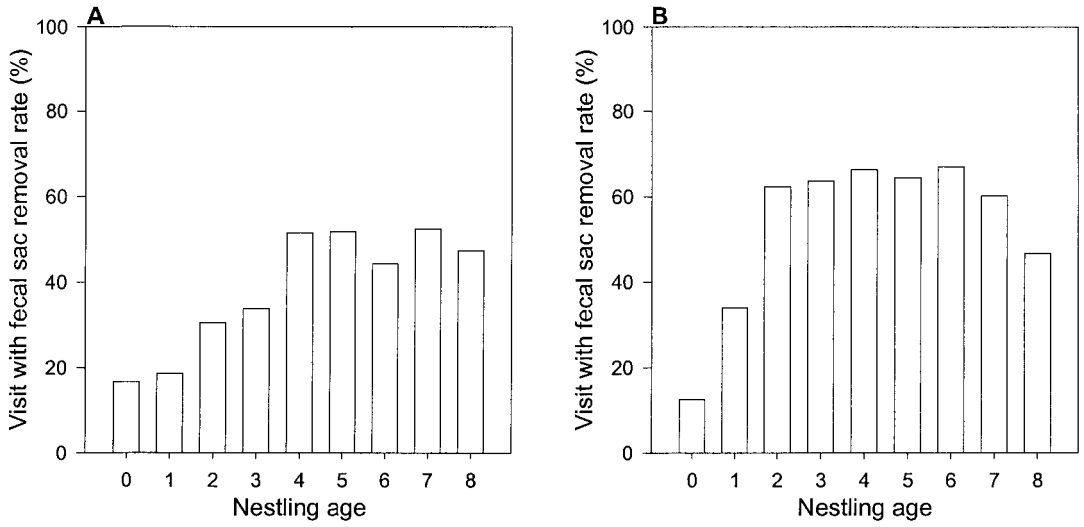

Figure 13: Percentage of visits with fecal sac removal in relation to nestling age for females (A) and males (B).

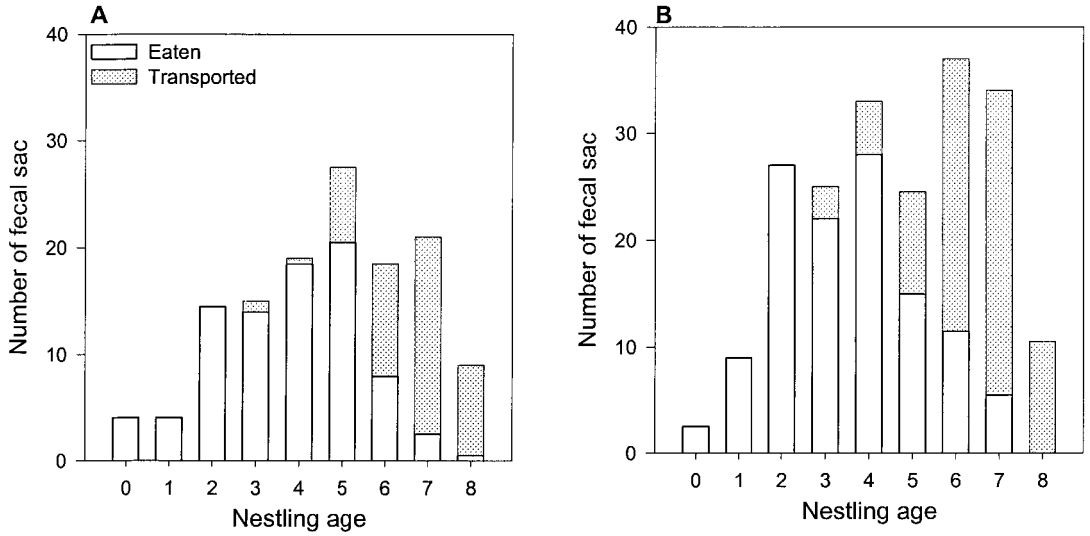

Figure 14: Frequency of fecal sacs eaten (white) and transported (gray) for Connecticut Warbler nests in relation to nestling age for females (A) and males (B).

Time of day

Time spent out of the nest and time taking care of nestlings differed throughout the day for both sexes (TON: Females: $F = 2.96$; $DF = 15$; $P < 0.0001$; Males: $F = 1.82$; $DF = 15$; $P = 0.0282$; Figure 15C). Average time spent out of the nest was shorter at the beginning

and end of the day and longer in the middle. Time spent brooding was longer for females than males in mid-day whereas time spent out of the nest was shorter for females than males. Effectively, males spent more time out of the nest than females in the morning and in the evening. At each visit, the time spent taking care of nestlings was short and stable during all hours of the day for both sexes, but an outlier was present at 18:00 hour for the male.

Males and females had similar numbers of visits and brooding visits by hour during the day (Table 10). On average the female did 2.36 ± 0.016 visit total/hour (1.36 ± 0.048 brood visit/hour) and the male did 2.56 ± 0.076 visit total/hour (0.47 ± 0.032 brood visit/hour). Frequencies (%) of visits with food and with fecal sac removal for both sexes were similar and did not vary during the day (Female 79.5 % and male 94.3 %; Table 10).

Table 10: Comparison of visit frequency (a) and the percentage of activity realised (b) for each sex between hours.

Visit	Sex	DF	X^2	P
a)				
Total	Female	15	17.1552	0.3090
	Male	15	8.7034	0.8925
Brooding	Female	15	15.4441	0.4199
	Male	15	10.2252	0.8053
b)				
Food	Female	15	16.6780	0.3385
	Male	15	12.5607	0.6362
Fecal sac	Female	15	18.7302	0.2262
	Male	15	10.9511	0.7561

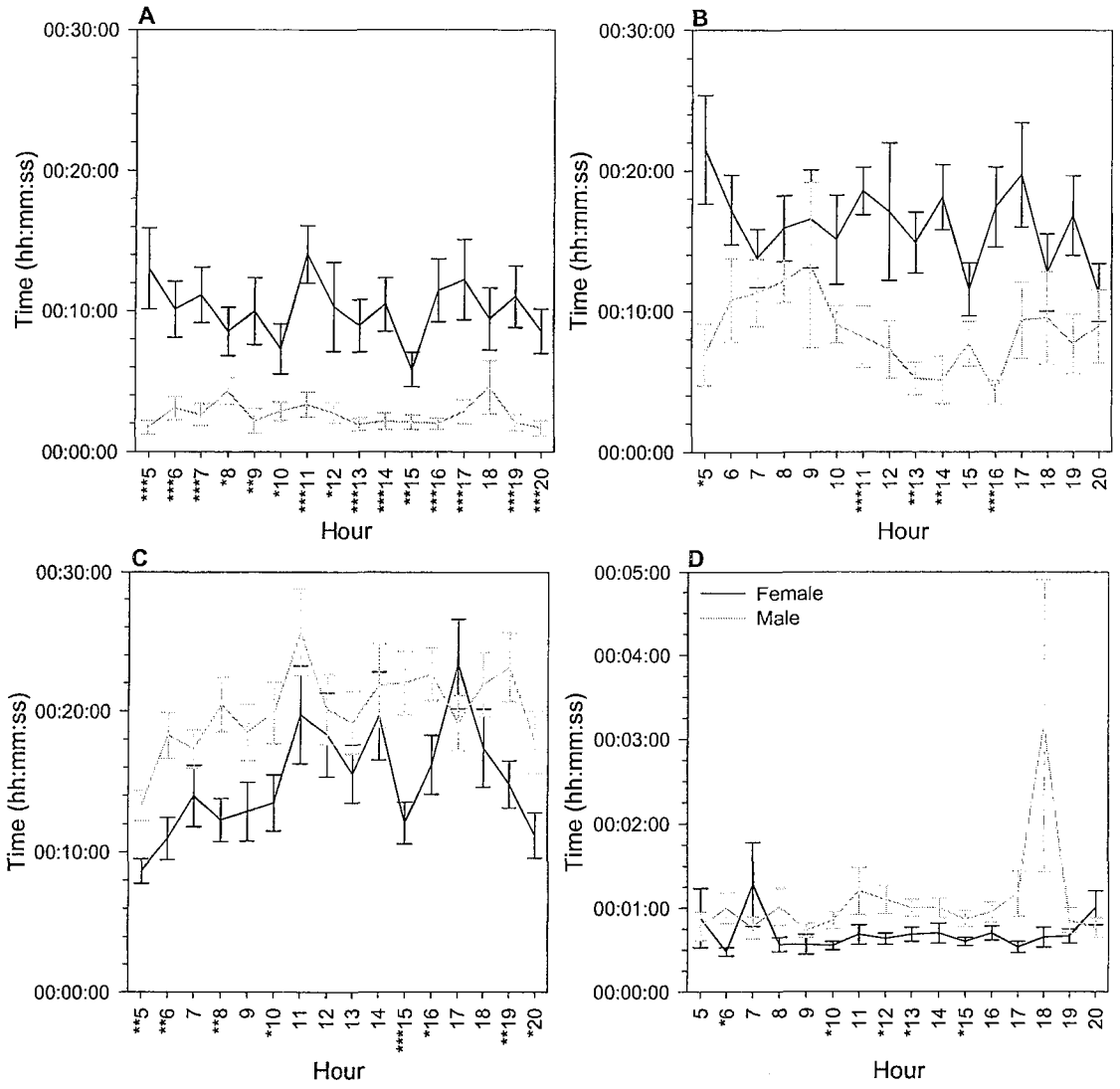

Figure 15: Time spent at the nest (A), brooding (B), out of the nest (C) and taking care of nestling (D) in hour:minute:second by sex (mean ± SE; Female in black; Male in gray) in function of the hour. Each graph presented with standard error and significant differences between sex at the bottom (*P*: * ≥0.05, ** ≥0.01 and *** ≥0.001).

DISCUSSION

The study focussed on the biology and breeding behavior of the Connecticut Warbler. Males had strong site fidelity between years. Paired males were heavier than unpaired males and differed in song patterns. In fact, paired males sang less than unpaired males and the difference was greater early in the morning. Seven nests averaging 4.4 eggs / nest were followed but only two produced fledglings (two were depredated and three abandoned). These two nests produced eight young from nine eggs and nestlings left the nest when eight-day-old. Females spent eleven days incubating the eggs without assistance from the male. Following hatching, both adults took care of the young but only the female spent night-time at the nest. Females spent more time brooding and at the nest than males. For both parents, time spent brooding decreased as nestling aged. Males spent more time out of the nest and taking care of nestlings than females resulting into a greater number of visits when he brought food and when he removed fecal sacs from the nest. The proportion of visits with food did not changed according to nesting age, but visits with fecal sac removal were more frequent as nestlings grew. Globally, more fecal sacs were eaten than transported and a greater proportion of them were eaten in the early days of nestlings.

Date and bird measurements

Males arrived on the breeding ground approximately one week before females a common pattern in several species (Francis et Cooke 1986). In Saguenay–Lac-Saint-Jean, the Connecticut Warbler is considered as one of the last migrating warbler (Savard et Cormier 1995; Saguenay-Lac-Saint-Jean: David 1996). Some males return within a few tens of meters from the first capture site like for the Common Yellowthroat (*Geothlypis trichas*: Klicka 1994).

Males outnumbered females on the study area resulting in a large number of unpaired males. This unbalanced sex ratio may result from sex-biased dispersion. In fact, most bird species have female-biased dispersal (Greenwood 1980; Clarke, Saether *et al.* 1997). Normally, in a healthy population, some individuals migrate toward other reproductive

territories and some arrive from them. The presence of many unpaired males in small isolated populations are symptomatic of a populations near extinction (Dale 2001). When a population is isolated, dispersal of females leads to temporary or permanent lost from the breeding population (Allee and al. 1949; Wells, Strauss and al. 1998). Dispersal of females from their territory could increase when habitat change rapidly by human fragmentation (Dale 2001).

Males arrive earlier in the season than females to establish and defend a breeding territory (Walkinshaw et Dyer 1961). Contrary to most other warbler species, Connecticut Warbler females are heavier than males (Walkinshaw et Dyer 1961; Mills 2008). Body measurements were similar to those found in the literature (Walkinshaw et Dyer 1961; Lanyon et Bull 1967; Jahn *et al.* 1999). Paired males were heavier than unpaired males in mass by nearly 0.9 g for a bird that has a mean mass of 13.6 g. This could be a sign that females choose high quality males or that heavier males can better compete against smaller ones (Dunn *et al.* 2001; Schuett *et al.* 2010).

Fat scores of captured adults were low, possibly a result of their migration. Females tended to have higher fat scores than males but more research is needed to confirm this. This may result from the later migrating females encountering better migration conditions (Smith et Moore 2003).

Song activity

Male Connecticut Warblers sang from 0 to 116 songs in fifteen minutes but averaged only 40 songs/15 min a result of frequent inactive periods taken regularly by the male. Walkinshaw and Dyer (1961) reported two males singing at rate of 90 songs/15 min. This large variability is likely due to the inclusion of inactive periods. Huff (1929) recorded inactive periods of 5 to 10 min between song activity for this species whereas Staicer *et al.* (2006) found that paired males sang more irregularly than unpaired males in American Redstarts (*Setophaga ruticilla*). In fact, male Connecticut Warblers can sing intensely perched on a tree and feed quietly on the ground next moment (Pitochelli *et al.* 1997).

Song is used for territory protection (Searcy *et al.* 2000; Trillo et Vehrencamp 2005) and mate attraction (Nowicki *et al.* 1998; Gil et Gahr 2002). In (Kirtland Warbler (*Dendroica kirtlandii*): Hayes *et al.* 1986; Black-capped Chickadee (*Parus atricapillus*): Otter et Ratcliffe 1993; Common Yellowthroat: Thusius *et al.* 2001; Rufous and white Wren (*Thryophilus rufalbus*): Hennin *et al.* 2009) unpaired males sing also more than paired males probably because they need to attract females. It's a compromise between singing activities and reproductive behavior. Differences were more important in the morning because singing activity was greater at this period (Pitochelli *et al.* 1997; Cerulean Warbler (*Dendroica cerulea*): Robbins *et al.* 2009). This is an interesting finding which could facilitate the determination of pairing status in various populations and between years. More observations in the afternoon and evening are needed to determine whether this could be done throughout the day. Paired males sang differently when they wanted to re-establish contact with their mate. This behavior can be use to locate females.

Nest

Seven nests were found on the ground in a habitat similar to that described by Huff (1929). To our knowledge, this is the first description of Connecticut Warbler nests in the eastern portion of its distribution. Only one nest had been found in Quebec before our study (Chabot et Préfontaine 1976). Nests measurements were similar those reported in Bent (1953), however, a nest found in Michigan was almost twice as big for his outside diameter and his inside depth and had a dry weight nearly four times greater than those of this study (Walkinshaw et Dyer 1961). This difference could be explained by the composition of the nest. In Michigan the major part of the foundation was composed of dead leaf.

Connecticut Warblers lay one egg per day and probably start incubating before all eggs are laid like Mourning and the MacGillivray's warblers (Bent 1953). However, all eggs hatched the same day like in the Kentucky Warbler (*Oporornis formosus*) (MacDonald 1998) who starts brooding at the end of the laying period. Clutch sizes were within the range of those reported by Curson *et al.* (1994). Egg descriptions and measures were similar to those reported by Seton (1884), Stone (1929), Huff (1929) and Bent (1953).

Adult behavior

For the three nest abandoned in this research the presence of biologist (even if egg were not manipulated), around could explain this desertion, particularly if the female had just began incubating. This suggests a high sensitivity to human disturbance. In the study area, predation seems important (2/7 nests) for this species and two reasons could explain that; 1) it is a soil nesting passerine which seems to experience greater predation rate than aerial nests in boreal forest (Ibarzabal et Desrochers 2001) and 2) the study area shows a high level of fragmentation, a variable recognised to increase predation rate (Falk *et al.* 2011). The main suspect for one depredated nest was the Eastern Chipmunk since it was seen flushed and aggressed the female on her nestlings just before a troubleshooting of the camera system which, did not allowed recording the predator in action. After, the nest was empty. Eastern Chipmunk is also a suspected predator of other *Oporornis* species as reported by Cox (1960) on Mourning Warbler.

The incubating period lasted 11 days, similar to Mourning and MacGillivray's Warbler incubating respectively 12 days and 11 to 13 days (Hofslund 1954; Cox 1960; Semenchuk 1993). Nestlings left the nest at 7 or 8-day-old, sooner than at one nest in Michigan followed by Walkinshaw and Dyer (1961) where nestlings stayed at nest until 9 to 10-day-old. Mourning and MacGillivray's warbler nestlings are known to leave the nest at 8 or 9 days, remain on the ground for an indeterminate period (above one week) and become independent after 3 weeks following fledgling (Bent 1953; Hofslund 1954; Cox 1960; Semenchuk 1993). Nestlings measured in this study were smaller than those measured by Walkinshaw and Dyer (1961), but their mass was similar. The difference could be due to a difference in the age of the nestling during the sampling.

Nocturnal activity

Only the female incubated eggs and after hatching, only the female brooded nestlings at night (Kells 1889; In Pitochelli *et al.* 1997; Kentucky Warbler: Verner et Willson 1969).

Diurnal activity

Before hatching

Incubation bouts (female only) averaged 51:54 ± 01:59-min which is longer than 39.4-min spent by the Mourning Warbler (Cox 1960) but is similar to the Common Yellowthroat who spent a average of 61-min on the nest and 16-min out (Stewart 1953). Males came at the nest sometimes, stayed around but never fed females on the nest, in contrast to observations on Mourning Warblers (Cox 1960; Verner et Willson 1969). Incubation bouts were shorter at the end of the morning and during mid afternoon when the temperature was optimal for eggs (Cox 1960) An augmentation of time off of the nest and frequency of visit could explained the decreased of the time past at the nest but the augmentation was not statistically observable.

After hatching

Female spent more time with nestlings but both sexes brooded and there was almost always an adult on the nest following hatching as has been observed in other birds (Verner et Willson 1969). The male spent more time than female out of the nest probably for foraging.

As expected, brooding decreased as nestling became able to thermoregulate, a common feature in atricial birds (Figure 9; Royama 1966; Wiebe et Elchuk 2003). Also, as brooding visits decreased, visits with food increased (Ovenbird (*Seiurus aurocapilla*): Porneluzi *et al.* 2011). Like for Mourning Warblers (Cox 1960), adults spent more time off the nest in the afternoon when temperature was higher.

Food was delivered by both adults like in other *Oporornis* warblers (Burtt 1969; Verner et Willson 1969; In Pitochelli 1993). Males fed nestlings more often than females which is a common pattern in birds (Figure 12; Mourning Warbler: Cox 1960; Three-Toed Woodpecker (*Picoides tridactylus*): Pechacek *et al.* 2005; Great Tit: Barba *et al.* 2009; Red-Breasted Flycatcher (*Ficedula parva*): Mitrus *et al.* 2010). Nevertheless, in some species like Buff-breasted wrens (*Cantorchilus leucotis*) and Superb Fairy-wrens

(*Malurus cyaneus*)), both sexes bring the same quantity of food (Gill et Stutchbury 2005; Colombelli-Negrel et Kleindorfer 2010).

Fecal sacs were removed more often by males (Figure 13). Both sexes cleaned the nest more often as nestlings grew old, eating more and expulsing more fecal sacs (Figure 14). Initially, fecal sacs were entirely eaten but as nestlings reached four-day-old they tended to be removed and transported out of the nest like the Common Yellowthroat (Stewart 1953). Young nestlings do not digest efficiently and adults may recover nutrients by ingesting fecal sacs (Calder 1968, Gill 1995). As nestling digestion becomes more efficient, adults transport fecal sacs out of the nest (Dell'omo *et al.* 1998).

The information obtained in this research should be interpreted carefully because of the small sample. Not many females have been missed during capture because special efforts were done for them. Only two nests were monitored until fledgling stage. Effectively, Connecticut Warbler was very sensitive, too much human intrusion led the bird to abandon the nest, particularly in the first days of brooding. Differentiation of singing rates of paired and unpaired males should be easier after hatching.

This study was inserted into a research program focussing on the impact of a new type of culture for blueberry production: the "forest/blueberry management" concept. Even if this study allows better understanding the Connecticut warbler reproduction, it has limitation to judge the impact of this management. Some males were loyal to their territories through the years even if their territory was close or within the management area. Nevertheless, of the seven nests found, two were located directly inside the larger 60m wide forest band. Other warbler nests were located at the periphery of the management but closer to the forest matrix. In the case, where the landscape matrix conserves a forested status, we think that this kind of management could support Connecticut Warblers, contrary to conventional blueberry crop. Moreover, nest position tended to be placed near the edge of the open area (V. Blais pers. comm.). In this case, alternation of forest and blueberry bands could offer suitable habitat for Connecticut Warblers. However, within an agricultural landscape matrix, the habitat may not be considered

suitable as many birds install their territory within forested areas but close to open areas. Long term research is needed to determine the suitability of forested bands in a context of partial harvesting (1/3) within a 17 years framework. Larger bands (60 m) with a third harvested (20 m) could support Connecticut warblers but a band with 20 m of mature forest, 20 m of young forest (17 years) and 20 m of harvested forest may not be, more researches are needed.

This research brings a great quantity of information about the reproduction of the Connecticut Warbler and it was probably the only one from the north-east range of its distribution. The nest localisation was very hard and maybe it was why only few researchers worked on this bird reproduction. Those works are important because the Connecticut Warbler is a rare species in Quebec and its habitat is threatened by the blueberry industry. This kind of agriculture is thriving and it is important to determine if the new concept like the "forest-blueberry" can support the population and the reproduction of this species. For that, it was essential to start at the beginning and improve our knowledge about the breeding behavior of the Connecticut Warbler.

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CHAPITRE 2

Microsatellite identification of extra-pair paternity and sex determination for the Connecticut Warbler (*Oporornis agilis*).

Authors: Marie-Christine Saulnier, Jacques Ibarzabal and Catherine Laprise

ABSTRACT

Genetic studies offer many different non invasive ways for conducting biological and ecological research. The Connecticut Warbler (*Oporornis agilis*) is a poorly known species and more information is needed to better evaluate its status. Our objectives were to validate a method for sexual determination and to find microsatellites allowing assignment of nestlings to their parents. Sexual identification was realised with a technique elaborated to sex most birds developed by Griffiths *et al.* (1998). We used microsatellites found and amplified from other species included in the genus *Oporornis* or in closely related genus. Over the nine *loci* tested, five were good for parental assignment and there were no extra-pair paternity in the three nests sampled, which included 11 nestlings.

Key words: DNA, PCR amplification CHD-gene, microsatellites, extra-pair paternity, passerine, Oporornis agilis

INTRODUCTION

The Connecticut Warbler (*Oporornis agilis*) is a secretive passerine and many aspects of its reproductive behavior are unknown. Pitochelli *et al.* (1997) wrote in their review that: "*It has not been the primary subject of any biological research*". This bird is a late neotropical migrant who forages and breeds on the ground but sings at the top of trees (Pitochelli *et al.* 1997). A large part of its breeding range occurs in Canada, from British Columbia to Quebec and south of the Great Lakes region of the United-States (Pitochelli *et al.* 1997). However, population density is low within this large range (Cadman *et al.* 2008; Cooper *et al.* 1997). Internationally, the species is considered least of concern by the IUCN (2010) and apparently secured by NatureServe (2010). However, nationally it is considered threatened by the province of British Columbia (Cooper *et al.* 1997; Cooper et Beauchesne 2004). NatureServe Explorer also lists it as: imperilled (S2) in British Columbia, Saskatchewan, Wisconsin and Minnesota and unranked for Michigan [between S2 and S3(vulnerable)] (NatureServe 2010). Globally, it is perceived as vulnerable or imperilled in at least the third of its breeding range.

The Connecticut Warbler is an insectivorous long-distance migrant bird, a category that is most affected by anthropogenic changes (Bohninggaese *et al.* 1993). Even if Canada represents 80% of world breeding area, its status has yet to be formally evaluated (COSEWIC 2010).

An important aspect in the biology of the Connecticut Warbler is its large versatility in breeding habitat selection: Poplar trees (*Populus* sp) stands in Western Canada, Black Spuce (*Picea mariana*) and Tamarack Larch (*Larix laricina*) fens in Minnesota, Manitoba and Ontario, and mainly in Jack Pine (*Pinus banksiana*) in Quebec (Pitochelli *et al.* 1997). This is particularly intriguing as all these habitats are generally present across the breeding range. Reasons for these geographical habitat preferences could be genetics. The presence of genetic variation could be explained by subspecies or ecotype in the Connecticut Warbler population with no phenotypic variation.

Little is known of the biology and population genetic of this bird. Microsatellite techniques which began in 1989 (Schlotterer 1998), provide an easy way to evaluate population genetic and assess parentage with simple DNA sampling (Dawson *et al.* 2010). Microsatellites are the most popular genetic markers used in the molecular genome by ecologists (Nunome *et al.* 2006; Selkoe et Toonen 2006). No study had yet identified or used microsatellites in this species. Identification of microsatellites would be useful for population studies across the breeding range, determination of nestling sex ratios, and understanding of mating strategies.

In bird genomes, females are heterogametic (ZW) and males homogametic (ZZ) (Ellegren 2001). For sex determination, a part of the Chromobox-helicase-DNA-binding gene situated on the sexual chromosome is amplified, generating two fragments of the same size for the male and of different sizes for the female (Cerit et Avanus 2007; Marshall Graves 2009). The technique for sex determination of many birds species based on the CHD gene is the one developed by Griffiths *et al.* (1998; Jensen *et al.* 2003).

Our objectives were to test markers for parental assignment and sexing using microsatellites. The identification of microsatellite *loci* that could be amplified in Connecticut Warbler could prepare the field to further population or mating strategy studies. For sexual identification, the technique developed by Griffiths (1998) will be tested. To determine parental assignment, the microsatellites used will be chosen from those developed for other bird species but research will demonstrate amplification on the species of the genus *Oporonis*.

METHOD

Field area

Field work was done northwest of Lac-Saint-Jean, Quebec, Canada (48°50'99''N, 72°37'21"W) on the CAFN (corporation d'aménagement forestier de Normandin) territory during the 2008 and 2009 breeding seasons (end of May to July). In 2009, the DAFTA territory (Développement et Aménagement de la Forêt Touristique d'Albanel) in Albanel (48°55'34''N, 72°23'73"W) was added. Field areas were composed mostly of low density Jack Pine stands growing on large terraces of fluvio-glacial sand deposits (Chagnon 1970; Savard 2001). Understory was composed of ericaceous shrubs dominated by *Vaccinium angustifolia*, *Kalmia angustifolia* and *Ledum groenlandica*. Most forest stands were managed under the concept "forest/Blueberry management" consisting of alternating 40-60 m Jack Pine forested and 40-60 m blueberry field bands. Connecticut Warblers were found near or inside these managements units.

Blood sampling

Adults were captured from 30th May to the 14th June 2008-9 on their territory with 30 mm mesh mist nets using playbacks of an aggressive male. Blood samples were taken from the brachial vein with a sterile needle and stored in an EDTA tampon at 4°C. When a nest was discovered, DNA of nestlings was also sampled after they reached 6-7 days old by either collecting blood as in adults or feather parts stored in ethanol 95%. We collected embryos in abandoned eggs (5) from a single nest and stored them in ethanol 95%.

DNA extraction and analysis

DNA was extracted using a DNeasy Blood and tissue kit (# 69504; Quiagen). We diluted the DNA of each subject to 5 ng/µl. We used nine microsatellites, six from Stenzler and al. (2004) created for the Golden-Winged Warbler (*Chrysoptera vermivora*) and three from Winker and al. (1999; 2008) developed for the Swainson's Warbler (*Limnothlypis swainsonii*) and amplified in species of the genus *Oporornis* (Table 11). Each PCR for Stenzler (2004) microsatellites were run in a total volume of 10 µl (8 mM Tris-HCl

(pH 9.0); 40 mM KCl; 0,08% Triton X-100; 1.0-2.5 mM $MgCl_2$; 125 mM dNTPs; 2 pmol of each primer; 0,5 U Amplitaq Gold (Applied Biosystems) and 2 µl DNA template). The three microsatellites of Winker and al. (1999; 2008) were put in 8 mM Tris-HCl (pH 9.0); 40 mM KCl; 0,08% Triton X-100; 1.0-2.5 mM $MgCl_2$; 100 mM dNTPs; 5 or 6 pmol of each primer; 0,5 U Amplitaq Gold (Applied Biosystems) and 2 µl DNA in a reaction of 10 µl. All reactions were performed in a PCR GeneAmp 9700 thermalcycler (Applied Biosystems) via 40 cycles of 94°C for 60 s, the annealing temperature for 60 s, and 72°C for 30 s, with an initial denaturation step of 94°C for 10 min and a final incubation of 72°C for 5 min. Annealing temperatures are mentioned in table 11. Allele sizes were found by AB-3130 automated DNA sequencer (Applied Biosystems) and scored with GENEMAPPER 4.0 (Applied Biosystems).

Table 11: Characteristics of microsatellite loci from *Oporornis agilis*. T_a, optimized annealing temperature and $MgCl_2$, optimized concentration.

Locus	Repeat motif	T_a (°C)	$MgCl_2$ (mM)	Allele size range (bp)	Primer sequence (5′–3′)	GenBank Accession no.
VeCr02	$(TCA)_7$	58	1.5	242-287	F*: AATAGGCTTTGAGGAGGAATCC R: AGCCCCAAAGTGCTGAAATA	AY542875
VeCr04	$(CAT)_9$	58	1.5	105-139	F*: TGCAGGGATGTTGTGACCA R: TGTCTCCTGTACCCTGCAC	AY542877
VeCr07	$(CA)_9$	58	1.5	114-154	F*: CTCGGTATGTGTCCCTGCCTTA R: TTATTCCCTGCAGTTGCTGTGA	AY542880
VeCr08	$(CA)_{14}$	56	2.0	160-192	F*: TCACCTCTGATGGGAAATCCTC R: TCACTGGCCTTTGTGTCCATAA	AY542881
VeCr14	$(ATG)_{13}$	56	2.0	246-268	F*: GTTATACCTGCGTGAGTGT R: AGCCTTGTTATCCTTCTTC	AY542884
VeCr16	$(CAA)_5...(CAA)_4$	58	2.0	382-391	F*: TAAAACTTCCCTGCAATAACCT R: GCCGATGTAGACAAAGAAAG	AY542885
Lsw µ7	$(GT)_{14}$	56	2.0	147-185	F*: GATGTGACAAGTGTGCTCTCC R: TTTATATCTAGTGACGCTCTA	AF129091
Lsw µ14	$(AT)_6(GT)_1$	56	2.0	187-191	F*:GTTATGCTCCAACAAAATAGATA R: AGGTTTTRAAGGATAGATTTATA	AF129095
Lsw µ18	$(AT)_{14}$	58	2.0	141-293	F*:TTGCTGAAAGAAGTACTAAGA R: CTGKTTGCAGGATATGTATAC	AF129096

*Indicates a labelled primer.

Sexual identification was performed on adults first to adjust the protocol and then, on juveniles. Chromobox-helicase-DNA-binding genes (CHD gene, ZW for females and ZZ

for males (Marshall Graves 2009) were used to sex Connecticut Warblers with the primers P2 and P8 developed by Griffiths et al (1998). PCRs recipes followed the protocol of Porlier *et al.* (2009).

Statistical analysis

Genalex 6, was used to measure observed heterozygosity, expected heterozygosity, allele frequency, heterozygosity deficit and Hardy-Weinberg equilibrium (HWE) (Peakall et Smouse 2006). After that, Genepop 4.0.10 gave the linkage disequilibrium (LD) (Raymond et Rousset 1995; Rousset 2008). The last step to determine if microsatellites were usable was the presence of null alleles which was evaluated by Cervus 3.0 (Marshall *et al.* 1998; Slate *et al.* 2000; Kalinowski *et al.* 2007). All those measures could affect the powerful of parental assignment. Cervus 3.0 also gave probabilities of exclusion (the probability of excluding the true father) for the first parent (father) of each locus with a minimum of five loci and paternity function of 2 levels: strict (95%) and relaxed (80%) confidence intervals. If those probabilities had low values, the proportion of successful parental allocation and the father identity for all nestling could be tested.

RESULTS

In 2008, 20 individuals were sampled (11 males; 3 females; 3 nestlings; 3 embryos) and 24 news individuals (14 males; 5 females; 5 nestlings) in 2009. Analyses were done on 41 individuals and 3 embryos.

Sex determination

Three nestlings (Figure 16) were sexed as females (#11, 14 and 15), five as males (#9, 10, 12, 13 and 16). Fragment lengths were between 200 and 500 base pairs for the CHD-W (first band when it was a female ≈380 bp) and CHD-Z (second band for females and the only one for males ≈350 bp).

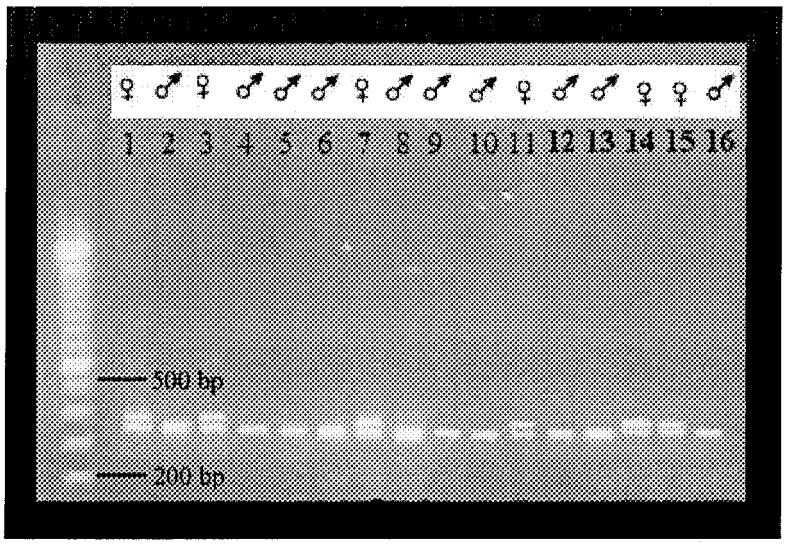

Figure 16: Sex determination of adults (1 to 8) and nestlings (9 to 16) of Connecticut Warblers (*Oporornis agilis*). The fist column is a size marker (100 bp DNA Ladder).

Genetic diversity and equilibrium tests

Alleles for all *loci* ranged from 3 to 34 (mean = 14.78) (Table 12). The mean expected heterozygosity was 0.72 (SE = 0.087; range 0.088 to 0.957; Table 12). Only one locus had no heterozygosity deficit and reached the HWE (VeCr07). This could be explained by the small sample sizes. Two other *loci* (VeCr04 and VeCr14) had almost no heterozygosity deficit. There was no LD in the *loci* tested. Null allele frequency estimates

(Table 12) that there were only five *loci* that could be taken for parental assignment (VeCr02, Vecr04, Vecr07, Vecr14, Lsw μ7). For these five *loci*, probabilities of exclusion of the father were calculated (Table 12). The heterozygosity deficit of most loci could affect the probabilities of exclusion but the test was still achieved because this deficit was probably caused by the small sample sizes.

Table 12: Microsatellite characteristics for *Oporornis agilis*. N, number of individuals genotyped; N_A, number of alleles; H_O, observed heterozygosity and H_E, expected heterozygosity; *Null*, null allele frequency estimate; P_e, probability of exclusion of the father for locus with low null allele.

locus	N	N_A	H_O	H_E	*Null*	P_e
VeCr02	42	23	0.76	0.93	0.0530	0.074
VeCr04	43	9	0.65	0.67	0.0071	0.380
VeCr07	44	19	0.98	0.93	-0.0227	0.070
VeCr08	43	13	0.63	0.82	0.1388	-
VeCr14	44	7	0.66	0.68	0.0147	0.359
VeCr16	41	7	0.32	0.43	0.1260	-
Lsw μ7	43	18	0.74	0.92	0.0767	0.084
Lsw μ14	44	3	0.05	0.09	0.2898	-
Lsw μ18	40	34	0.70	0.96	0.1422	-

Parental assignment

As probabilities of exclusion of the father for the five loci were low, parental assignment could be done. For each of the three broods nestlings, only one mother was assigned and 26 potentials fathers were tested (Table 13). The mother assigned was the social mother observed taking care of nestlings and the genotype was also evaluated between the mother and a young to certify their genetic link. The proportion of successful parental allocation was 0.9913. For two nestlings, father identity could not be assigned because nestling #3 had only four loci and nestling #6 had an error in the sequencer, one locus appeared homozygous but in reality the locus was heterozygous. The father found for

each nestling was the social father and it was the same for all nestlings of the same nest. Those results are preliminary because the heterozygosity deficit could affect the probability of exclusion.

Table 13: Logarithm of the odds (LOD) score for the father of nestlings of all nests with known mother identity and Delta is the difference between the LOD father and the LOD of the other potential father (not in the table).

Year	Nest	Young ID	Mother ID	Father ID	LOD father	Delta
2008	1	1	8	53	5.50	2.16
2008	1	2	8	53	5.63	5.63
2008	1	3	8	-	-	-
2008	3	4	26	3	4.01	0.98
2008	3	5	26	3	4.19	4.19
2008	3	6	26	-	-	-
2009	4	7	48	58	4.50	4.50
2009	4	8	48	58	4.99	4.99
2009	4	9	48	58	3.47	3.47
2009	4	10	48	58	4.16	4.16
2009	4	11	48	58	6.28	6.28

DISCUSSION

This study allowed sexual determination of Connecticut Warbler nestlings. Additionally, nine microsatellite *loci* were amplified and sequenced. Of those, only one had good heterozygosity value and one had Hardy-Weinberg equilibrium (HWE) which is usually related to sub-structure in the population but the small sampling of this research was probably the cause. For parental assignment, only five *loci* did not have null alleles and they were used for statistical analysis. The biological father was found for nine nestlings, and it was always the social father observed on the field.

The Connecticut warbler can be sexed with the primers developed by Griffiths *et al.* (1998) and the protocol of Porlier *et al.* (2009). Fragment lengths were approximately 380 bp for CHD-W and 350 bp for CHD-Z, similar to values expressed in the literature (Griffiths *et al.* 1998).

Nine microsatellites were amplified with different recipes from the originals (VeCr: Stenzler *et al.* 2004) (L*swμ*: Winker *et al.* 1999; Winker et Graves 2008). They were first created for the Golden-Winged (6 VeCr) and the Swaison's warblers (3 *Lswμ*). *Loci* were not too close if they were on the same chromosome because no linkage disequilibrium was observed (Jones et Ardren 2003). Only one locus had Hardy-Weinberg equilibrium explaining the heterozygosity deficit for all the others. No HWE for the eight loci could be explained by a sub-structure in the population (Selkoe et Toonen 2006) but the reason of the non Hardy-Weinberg equilibrium for this research was probably the small sample sizes. Only 5 loci had no null allele, i.e. presence of alleles not amplified by the PCR (Byers *et al.* 2004). The presence of no null alleles is very important for parental assignment because it can cause false exclusion of the parent (Jones et Ardren 2003; Wang 2010).

With 26 potential fathers and five *loci* the Cervus program gave each time the true father, which was the father observed in social behavior. There was no multiple paternity found in the nestlings, but our sample was small. However, a male was seen copulating with

two females, each producing eggs and no other males were noted around them so that it was likely a case of polygyny. Those nestlings were not sampled because one nest was depredated and the other was abandoned. More microsatellites should be amplified and sequenced to have more than five loci to evaluate parental assignment and more nestlings should be sampled to determine whether extra-pair copulation occurs in this species. Recent research with mitochondrial DNA, revealed a close link between the genus *Oporornis* and *Geothlypis* (Escalante *et al.* 2009; Lovette *et al.* 2010). Extra-pair copulation has been documented in Common Yellowthroats (*Geothlypis trichas*: Thusius *et al.* 2001) and for this reason, may occur in Connecticut Warblers. More research is needed to evaluate the parental assignment with more loci but this study demonstrates the possibility with only those loci. Also the HWE should be achieved with larger sample sizes.

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CONCLUSION

L'objectif général de ce projet était de comprendre la biologie de la reproduction de la Paruline à gorge grise. Cette recherche s'est effectuée sur les territoires de l'aménagement «forêt/bleuet» (CAFN) et sur les territoires de la DAFTA au nord du Lac-Saint-Jean au Québec, Canada. Le comportement reproducteur ainsi que les soins parentaux de cette espèce furent évalués. De plus, l'activité de chant des mâles fut analysée pour déceler des différences entre les nicheurs et les non-nicheurs. Pour terminer, les analyses génétiques ont permis d'élaborer des protocoles spécifiques à la Paruline à gorge grise qui détermineront l'affiliation parentale ainsi que le sexe ratio des nichées.

Comportement reproducteur

Le fait que des mâles soient capturés ou revus d'année en année au même endroit a permis de démontrer la fidélité au site de ceux-ci chez la Paruline à gorge grise. Lors de l'analyse, les résultats ont démontré que les mâles nicheurs étaient plus lourds que les non-nicheurs. D'autre part, les mâles nicheurs présentaient une activité de chants différente puisqu'ils chantaient moins fréquemment que les mâles non-nicheurs et cette différence était plus notable le matin. Sept nids ont été découverts avec une moyenne de 4,4 œufs par nid. De ces nids, deux ont subi de la prédation et trois ont été abandonnés. Deux nids sont arrivés à terme. Les jeunes des deux nichées ont quitté le nid à huit jours. Il y a eu une productivité de huit jeunes à partir de neuf œufs. La femelle a été la seule à couver les œufs et ce pendant 11 jours.

Après l'éclosion, les deux adultes s'occupaient des jeunes. Par contre, seule la femelle couvait les jeunes pendant la nuit. La femelle passait plus de temps que le mâle à couver alors que c'était l'inverse pour le temps passé hors du nid et le temps passé à prendre soin des jeunes (nourrir, nettoyer le nid et surveiller). Le temps passé à couver diminuait au fur et à mesure que les jeunes grandissaient. Le temps moyen accordé à chaque activité selon l'âge des jeunes soit: d'être au nid, de couver les jeunes, d'être hors du nid et de

s'occuper des jeunes était significativement différent entre les deux parents et diminuait avec l'âge des jeunes. En ce qui concerne le temps moyen en fonction de l'heure de la journée, il y avait une différence entre les sexes pour tous les comportements (être au nid, couver les jeunes, être hors du nid et s'occuper des jeunes). La femelle passait plus de temps à contrôler la température des jeunes alors que le mâle était plus longtemps à la recherche de nourriture et à prendre soin des jeunes. Pour les deux sexes, la plupart des activités ne variaient pas en fonction de l'heure de la journée excepté pour le temps passé hors du nid qui montre un pic en après-midi attribuable à l'augmentation de la température.

Le nombre de visites total n'était pas différent entre le mâle et la femelle, mais variait en fonction de l'âge des jeunes. Le nombre de visites avec activité de couvaison était plus élevé chez la femelle, mais il se réduisait selon l'âge des jeunes chez les deux adultes. Le mâle effectuait plus de visites avec un apport en nourriture et la proportion des fois où il venait au nid avec de la nourriture restait semblable tout au long de la croissance des jeunes, contrairement à la femelle où cette proportion augmentait avec l'âge des jeunes. Pour ce qui est des sacs fécaux, le nombre de visites avec enlèvement de sacs était plus élevé chez le mâle et la fréquence augmentait avec l'âge des jeunes. Finalement, les adultes nettoyaient le nid soit en mangeant ou en transportant les sacs fécaux.

Outils génétiques

Le protocole utilisé pour déterminer le sexe des individus a fonctionné. Il est dorénavant possible de déterminer le ratio des mâles et des femelles au sein d'une nichée de Paruline à gorge grise, car on peut se fier à la méthode de Griffiths (1998) pour cette espèce. Par la suite, neuf microsatellites ont été amplifiés et génotypés. Ces microsatellites ont d'abord été créés pour la Paruline à aile dorée (6 VeCr) et la Paruline de Swainson (3 *Lswμ*). Pour l'affiliation parentale, seulement cinq microsatellites ont pu être utilisés puisqu'ils avaient de faibles valeurs d'allèle nul. Toutefois, l'ensemble des microsatellites pourrait être utilisé pour de futures études de population avec un échantillonnage plus élevé si l'équilibre Hardy-Weinberg était atteint.

Limite de la recherche

Ce projet de recherche n'a pas permis de comparer la reproduction de la paruline dans une forêt non fragmentée et une forêt fragmentée (aménagement «forêt/bleuet»). Effectivement, malgré nos efforts de recherche, peu de nids ont été trouvés et les adultes se situaient rarement dans ce type d'aménagement. La recherche a permis de récolter des informations importantes sur la reproduction, mais une seule étude longitudinale à long terme permettait d'affirmer que cet aménagement est viable à long terme. Malgré tout, les aménagements existent depuis quelques années et nous y trouvons des individus qui s'y reproduisent avec succès. De plus, le nombre de femelles à l'étude était très faible, bien qu'un effort particulier ait été réalisé pour les capturer. Cela explique le fait que peu de nids aient été suivis entièrement. Les données obtenues proviennent de deux nids et chaque nid compte plusieurs observations. Ces résultats doivent être pris avec précaution, car comme l'échantillonnage est faible l'effet de chaque individu est important. La Paruline à gorge grise semble très sensible à l'intrusion humaine, les recherches doivent être faites avec le plus grand soin, car elles peuvent mener à l'abandon de nids, particulièrement lors de l'incubation des œufs. Pour ces raisons, on questionne l'aménagement intensif des bandes boisées pour le maintien de la Paruline à gorge grise car, 1) la récolte amenuise la surface forestière en ouvrant davantage le milieu, 2) les travaux sylvicoles réguliers dans les sous-bandes récoltées créent du dérangement qui semble être néfaste à la nidification; pour cette raison, les travaux sylvicoles devraient avoir lieu en dehors de la période de nidification et 3) cette sylviculture tend à rajeunir et augmenter la densité du peuplement ce qui à long terme va dans le sens opposé du type de peuplements dans lesquels on les retrouve actuellement.

Pour ce qui est des analyses génétiques, les protocoles ont été élaborés avec succès, par contre il faudrait une étude avec un échantillonnage beaucoup plus élevé pour pouvoir faire une recherche sur le sexe ratio des jeunes et déterminer la présence de copulation extra-couple.

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