Density-dependent age at first reproduction in the eastern kingbird

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Theory predicts that maximal fitness is obtained by individuals who begin to breed immediately upon reaching sexual maturity. However, delayed breeding occurs regularly in some taxa, and in birds and mammals is most often associated with long lifespan and/or limited access to suitable habitats. Delayed breeding is not expected among relatively short-lived species such as migratory passerine birds, but this assumption remains untested in many species. Here we quantify age at first reproduction in an eastern kingbird *Tyrannus tyrannus* population breeding in an ecological island, and through both observational and experimental approaches, investigate the potential causes for the high frequency of delayed breeding that occurs in this population. Nearly half of the fledged nestlings that returned to the breeding grounds did not breed in their first potential breeding season. Some non-breeders occupied territories, for at least some period, but most remained as non-territorial ‘floaters’. Parentage analysis failed to show any reproductive success for female floaters, and only limited success for male floaters, indicating that floating was not a successful reproductive tactic. On the other hand, a strong negative relationship existed between population size and the proportion of young birds that bred in their first year, and non-territorial birds of both sexes quickly filled territory vacancies created by experimental adult removals. Limited breeding habitat and territorial behavior of older birds thus appear to be the main causes of delayed breeding in kingbirds.

The frequency of delayed breeding in most species is unknown but of potential significance because failure to incorporate accurate estimates of age at first reproduction in population models may lead to flawed population projections.
breeding. Although the available data are meager, the few long-term studies that exist suggest that individuals from populations with pronounced variation in habitat quality can enhance lifetime reproductive success through delayed breeding (reviewed in Zack and Stutchbury 1992).

A fourth possibility is that breeding is not truly delayed, but instead, non-territory holders (‘floaters’) pursue an alternative mating strategy in which territoriality and breeding are disengaged. Floaters may avoid some of the costs associated with breeding (e.g., defense of territory or mate, feeding young, and nest defense against predators), yet achieve reproductive success through extra-pair fertilizations (Weatherhead and Boag 1995, Ewen et al. 1999, Kempenaers et al. 2001, Berg 2005) or intraspecific brood parasitism (Lyon 1993, Sandell and Diemer 1999, Saitou 2001). However, these strategies appear to be relatively uncommon and likely represent individuals that are making the best of a bad situation (but see Ewen et al. 1999, Kempenaers et al. 2001).

The eastern kingbird *Tyrannus tyrannus* is a socially monogamous, long-distance migrant with moderately high adult survival and high breeding site fidelity (Murphy 1996, Redmond et al. 2009). Since 2002, we have studied a population of eastern kingbirds (hereafter kingbirds) at Malheur National Wildlife Refuge (MNWR) in southeastern Oregon. Presumably because MNWR is an ecological island (Methods), natal return rate is extremely high. However, a large proportion of the nestlings that returned failed to breed in their first potential breeding season. Some of these birds were seen repeatedly in the study area, and although a few temporarily settled on the main study site, most were not seen consistently throughout the season and therefore did not hold territories. In addition, the few territory holding adults (of both sexes) that have died during the breeding season have all been quickly replaced by birds that did not previously occupy territories at our site. These observations suggest that a floater population exists at MNWR, and that most floaters are SY birds. Our goals in this report are therefore to (a) quantify age at first reproduction for kingbirds at MNWR, (b) determine whether floaters gain reproductive success through extra-pair mating behavior (brood parasitism or extra-pair copulations), and (c) test the hypothesis that non-breeding by SY birds is driven by limited habitat availability and the territorial behavior of older birds. We accomplished the latter through analyses of the relationship between population size and the proportion of breeding SY birds, and by experimental removal of territory holding males and females to test for the presence of replacement breeders.

**Methods**

**Study site, population and field methods**

MNWR is a 75 700 ha refuge located at an elevation of ~1300 m in Harney County, southeastern Oregon, near the town of Frenchglen (42°49′N, 118°54′W). The long (~64 km) and narrow refuge is located at the northern end of the Great Basin Desert. Most precipitation falls in winter (November through March: 56% of annual total of 26.8 cm), and relatively little falls from summer to early fall (May through September: 29% of annual total). The Donner und Blitzen River flows through the center of the refuge and supplies water year-round to support productive marshes and a 5 to 20 m wide riparian strip of willow *Salix* spp. trees in which nearly all kingbirds nest. Additional breeding habitat is available at the eastern boundary of the refuge along East Canal. Eastern kingbirds only rarely breed away from the river in the large tracts of high desert habitat dominated by juniper *Juniperus occidentalis*, sagebrush *Artemisia* spp., and greasewood *Sarcobatus vermiculatus* that completely surround the refuge, and as a consequence, MNWR is an ecological island for kingbirds.

The main study site is located at the southern end of the refuge, and extends from Paige Springs campground to a point 2 km north of the bridge to Krumbo Reservoir (20 km). The river is channelized throughout most of our study site and direct access is available from the Center Patrol Road (CPR). A secondary road provides similar access to East Canal. Complete population counts and intensive banding of the main population began in 2002, and 60% of the adult population was banded at the main site by the end of that year. This figure has risen to 70–90% since 2003, and as a consequence of relatively high adult survival rates (65%, Redmond et al. unpubl.) and high site fidelity (between years, nest sites of 60% of males and 35% of females are separated by less than 150 m; Redmond et al. 2009), a large proportion of birds are banded and seen at the start of the breeding season. A secondary study site (Mosquito Coast) is located about 10 km north of the main population. The CPR likewise closely parallels the river for its first 8 km at the Mosquito Coast, but for the remaining 4 km the river retains its natural course and access was only available by foot or canoe. The Mosquito Coast population has been studied less intensively and significant banding of adults began only in 2007.

Beginning by mid-May (2002–2005) or the second week of May (2006–2007), we slowly drove the CPR at least once per day to establish return dates of individuals, determine locations of territory holding adults, and find and monitor nests. Kingbirds are extremely conspicuous and breeding habitat is limited so we were able to census essentially the entire population each day. We also surveyed the small amount of suitable habitat away from the road or river on foot at least twice yearly to find pairs that may have settled away from the main breeding habitat. Most kingbird nests are placed on tree limbs that overhang water and therefore we also completely surveyed the main study site from the river by canoe multiple times per year. We found nests of most pairs during nest building (~80%) and checked nests every 2–4 days to establish egg-laying dates, clutch size, and hatching and fledging success (see Redmond et al. 2007 for a complete description of field methods). Replacement nests were located for pairs whose nests failed and identical data were collected to determine seasonal reproductive success. Similar procedures were followed at the Mosquito Coast except that in most years nests were monitored less frequently.

Adult females were captured with mist nets during the nestling period whereas adult males were captured throughout the nest cycle. Each bird was fitted with a unique combination of one aluminum US Fish and Wildlife Service band and three colored plastic bands. Sex was
Sex determination and parentage analyses

We determined the sex and parentage of nestlings to evaluate the reproductive success of non-territory holding adults. Methods are described in detail in Dolan et al. (2007), but briefly, we extracted DNA from 100 μl of blood for whole-nucleated blood. The DNA was then amplified in PCR reactions using GE Healthcare PuReTaq ready-to-go PCR beads. We used the 2550F/2718R primers to amplify the CHD1W and CHD1Z introns from the blood drawn from the young to determine sex. The two introns are found in different lengths on the Z and W chromosomes (Fridolfsson and Ellegren 1999). Females are the heterogametic sex (ZW) in birds and therefore nestlings in which one and two bands were expressed were classified as male and female, respectively. Tests of 20 adults of known sex confirmed the accuracy of the test.

To establish parentage, we first compared nestling genotypes to the presumptive parents at seven microsatellite loci (Dolan et al. 2007). Primers were labeled with fluorescent tags and microsatellites were visualized and interpreted using fluorescent detection. Maternity was confirmed by direct comparison of mother and offspring genotypes, while paternity was assessed by direct exclusionary analysis using the nestling’s non-maternal genotype. The 7 loci yielded 81 alleles, resulting in a total exclusionary power of 0.998. To be assigned parentage, a nestling had to match its parent at all seven loci. If offspring and parent were mismatched, we first compared the offspring genotype to all proper sex adults known to hold territories on the breeding grounds, and if no match was found we then compared the offspring genotype to individuals of the appropriate sex that were fledged in previous years, regardless of whether or not we had observed them on the breeding grounds. The latter enabled us to evaluate whether at least some individuals within the pool of floaters gained reproductive success. Microsatellite data were available from 2002 through 2004, and therefore we were able to test for the reproductive success of floaters only in 2003 and 2004.

Removal experiment

We removed breeding, territory-holding adults of both sexes in 2007, and to avoid interference with ongoing research on the main population, all removals were conducted at the Mosquito Coast. Removals were performed between 24 June and 10 July, after both the end of migration (Cooper unpubl.) and the initiation of breeding by nearly the entire population. Hence, a bird that filled a vacated territory was unlikely to be a late arriving bird or a migrant passing through to breed elsewhere (Newton 1992, Marra and Holmes 1997).

We selected territories randomly from a subset of all territories in which mist nets could be placed in a position that was likely to result in the capture of the female. Nests were known for nearly all pairs by the start of the removals, and repeated observations prior to removal allowed us to determine the approximate boundaries of all breeding territories. All territories in which removals were performed were at least 3–4 territories apart (300–400 m). Just prior to the start of the experiments, 60% of the main breeding population and 22% of the Mosquito Coast population was banded.

Prior to a removal, the mate of the individual to be removed was captured, banded, and released at the capture site. We captured females between days 4 and 6 of incubation (incubation length = 15 days) and between 07:00 and 11:00 h in nets that surrounded her nest. Males were caught just prior to dawn (04:00–05:30 h) by attracting them to a single net using conspecific song playback and a taxidermic mount of a kingbird. Ultimately, three females and five males were captured, banded, and immediately driven at least 60 km away to be released in suitable foraging habitat. Two of the males that we removed and released 60 km away returned in less than 24 h to re-establish their original territories with their original mates before a floater filled the vacated territory and thus these two removals were uninformative for our purposes. The two males removed after this were driven 150 km away and they and the third male released 60 km from the study site were not seen again. Clutches were collected after female removals, but we allowed females to continue incubation after males were removed to avoid desertion. After each removal, we surveyed the entire study site to determine not only if the experimental vacancy had been filled, but also to confirm the location of all other banded and unbanded birds.

Data analysis

Given that MNWR is an ecological island, we assume that few (if any) birds bred in habitats surrounding the refuge. Repeated failure to detect all but a few pairs away from the river support our assumption, and therefore we quantified age at first reproduction through direct observation of age at first known nesting attempt. We then used least squares linear regression to test the hypothesis that delayed breeding was driven by a negative density-dependent effect of population size on the proportion of SY birds that bred. Because we have studied the main breeding population more intensively than the Mosquito Coast population, we restricted this analysis to the main breeding population. As SY and after second year (ASY; birds in at least their second potential breeding season) kingbirds cannot be distinguished on the basis of plumage or morphology, we limited our analyses of age at first reproduction to birds that were banded as nestlings and therefore of known age. We excluded all nestlings that were seen in their first year of life but were never later observed breeding because we could not determine whether these birds may have passed through
the study site to breed elsewhere or remained as non-breeders on the study site but died before returning in the next year. This conservative approach may slightly underestimate the frequency of delayed breeding, but analyses that included these birds resulted in identical conclusions (results not shown). We also excluded two individuals who may have delayed breeding for two consecutive years, because they were first observed breeding in a small area that was not regularly surveyed until 2006 and therefore we could not be certain of their age at first reproduction. Finally, we limited analyses to young banded before 2006 so that each nestling was given two years to appear as a breeder. Means are reported ± SE, all statistical analyses were conducted using SPSS (ver. 13.0.0), and specific tests are described in the results.

**Results**

**Age at first reproduction**

Of the 240 nestlings that were banded and fledged on the main study site between 2002 and 2005, 50 (21%) were seen again on the main study site, while six more (2.5%) were seen at Mosquito coast. Of the birds seen on the main study site, 35 (70%) eventually bred there, and across all years, 18 (12 males and 6 females) bred as SY birds, while 17 (7 males and 10 females) did not breed until their second potential breeding season. Males tended to be more likely to breed in their first potential breeding season, but the difference was not significant (Fisher’s exact test, p = 0.181). The average age of first reproduction varied with cohort and ranged from a high of 1.8 ± 0.51 years (n = 35), but mean age at first reproduction varied with cohort and ranged from a high of 0.46 years for the 2005 cohort. The main breeding population decreased from 60 pairs in 2003 to 35 pairs in 2006 and as predicted by the hypothesis that habitat availability and territorial behavior limited breeding opportunities, the proportion of SY birds to breed declined as population size increased (proportion to breed = 1.442 – 0.020 [population size]; F^2^ = 3521.06, p < 0.001, r^2^ = 0.99; Fig. 1).

**Reproductive success of non-territorial birds**

An average of 2.8 ± 0.10 young fledged from successful nests (n = 95) between 2002 and 2004, and all of the 264 genotyped young (2002 through 2004) matched the putative mother at all seven loci, and therefore no female gained reproductive success through intraspecific brood parasitism (Dolan et al. 2007). In 2003 and 2004, extra-pair paternity accounted for 53.7% of all young, and of the extra-pair young, 63.7% were assigned to paired, territorial males (Table 1). None of the young of unassigned parentage in 2003 matched the genotype of males fledged in 2002, but in 2004 two unpaired, non-territorial males from the 2002 cohort, sired three nestlings. Thus, based on direct estimates of sighted SY birds and known adult floaters, 2 of 17 (11.8%) floater males sired young between 2003 and 2004. However, direct observation almost certainly underestimated the true number of floaters, due to the difficulty of sighting non-territorial birds, and thus our estimate of extra-pair success by floater males is likely an overestimate. Using juvenile survival probabilities obtained from program MARK (L. J. Redmond unpubl.), we estimated that 22 of the 98 male young fledged in 2002 and 2003 for whom we had DNA samples, were present in the population but not territorial in 2003 and 2004. An additional eight adult males were known to be alive but without territories. Based on this more realistic estimate of the number of fledged males present, only 2 of 30 male floaters (6.7%) sired young. Additionally the percentage of young for whom paternity could not be assigned (24% and 14% in 2003 and 2004, respectively) was similar to the percentage of territory holding males for whom we failed to obtain a DNA sample (38% and 12% in 2003 and 2004, respectively; Table 1), suggesting that most, if not all, of the sires of the unidentified young were known, but unsampled, territorial males and not floaters.

<table>
<thead>
<tr>
<th>Year</th>
<th>Genotyped young</th>
<th>Sire of nestlings</th>
<th>% sires known</th>
<th>% territorial males sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WP male</td>
<td>Paired EP male</td>
<td>Unpaired EP male</td>
<td>Unknown</td>
</tr>
<tr>
<td>2003</td>
<td>74</td>
<td>35</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>116</td>
<td>53</td>
<td>44</td>
<td>3^a</td>
</tr>
</tbody>
</table>

^a two of the three young were sired by one male and therefore only two unpaired, males fledged in previous years sired young.
Removal experiment

New birds filled all three experimentally created female vacancies within 48 hours. One replacement was a banded SY female that had not been seen previously that year. She began building a new nest within three days and laid eggs, but the nest was later depredated and another was not built. The two other female replacements were unbanded and therefore of unknown age. One re-used the original pair’s nest, laid eggs after approximately 10 days, but then lost the clutch to predators. The second unbanded replacement female completed a nest nine days after replacement, but it was either depredated early during egg-laying or she failed to lay eggs.

New birds also replaced all three removed males. A banded SY male that had not been seen previously that year replaced the first removed male within five hours. He actively defended the nest and/or female, but the original male came back two days after being removed, displaced the replacement and regained his territory and mate. The replacement male remained on the territory for several more days but was actively attacked by the original male. This same replacement male later filled the second experimentally created vacancy. He actively defended the nest and/or female but the nest was depredated two weeks later. An unbanded male replaced the third removed male in approximately eight hours. The original female on this territory deserted later that same day, presumably because her nest had failed the previous day (unbeknownst to us), but she was almost immediately (<1 day) replaced by one of the banded females removed during the female removal experiments. This entire new pair was sighted on the territory several times, once with nesting material, but eventually deserted the territory one week after removal (July 12). Hence, all experimentally removed individuals of both sexes were replaced.

Discussion

Few studies of passerine birds have thoroughly documented age at first reproduction (but see Arcese and Smith 1985, Sternberg 1989). This is likely because of the low natal philopatry that is typical of temperate-zone breeding passerines (Weatherhead and Forbes 1994), an inability to easily distinguish age classes in many species, and the difficulties of detecting non-territorial birds. In contrast, natal philopatry is unusually high in the MNWR eastern kingbird population due to the highly insular nature of the system, and banding efforts allow for age identification of most birds. Direct estimates suggest that nearly a quarter of the fledged young returned to the refuge, and of the fledged young that later returned to the main breeding population, roughly half did not breed until their second potential breeding season, and a few may have delayed even further. Delayed breeding in kingbirds cannot be explained by mate limitation because we document, through observational and experimental means, that some SY kingbirds of both sexes did not breed until after their first potential breeding season. This delay in breeding also cannot be accounted for by alternative mating strategies as we found no evidence of intraspecific brood parasitism, and only limited evidence that floater males produced young through extra-pair fertilizations. Pursuit of extra-pair fertilizations without holding a territory might at first seem like a successful strategy for kingbirds because of the very high rates of extra-pair paternity in this population (60% of nests in all years between 2002 and 2004; Dolan et al. 2007), but only a small percentage of floater males sired young. Overall, avoidance of the cost of reproduction associated with nesting does not provide an alternative route to reproductive success for female kingbirds, and the reproductive success achieved by a few floater males is best interpreted as individuals making the best of a bad situation.

On the other hand, we found that the proportion of fledged young that bred in their first year declined with increasing population size (Fig. 1). Thus, successful acquisition of a territory by SY birds of both sexes was related in a negative density-dependent manner to population size, suggesting that older, earlier arriving (Cooper et al. in press), and presumably dominant adults prevented some SY birds from obtaining a territory when population size was high. This conclusion is strongly supported by our removal experiment: both male and female birds settled on territories when vacancies were experimentally created through removal of territorial adults. All existing pairs (banded and unbanded) were accounted for after each removal, and thus we are certain that the replacement birds were floaters and not neighbors that switched or expanded their territories. While we were only able to determine the age of two of the five replacement birds, both were SY birds. Adults rarely skipped a year of breeding (7 of 151 individuals from 2002–2004), yet nearly half of the nestlings recruited into the population did not breed in their first year, and thus we suspect that most, if not all, floaters are SY birds. We conclude that the most parsimonious explanation of delayed breeding in kingbirds is that territorial behavior, in combination with a limited amount of breeding habitat, prevents some SY birds from breeding. None of the nesting attempts of replacement birds were successful, but nest failure rate in 2007, when the removals were performed, was unusually high (~75%). The apparent lack of any benefit for the replacements may have therefore simply have been a consequence of the generally high nest failure rate. However, the possibility remains that replacement birds may reap benefits in the future, because they may be more likely to acquire a territory in the following season as a result of the benefits of prior territory ownership (Maynard Smith and Parker 1976, Stamps 1987, Morrison et al. 2008).

Do SY birds ‘choose’ to delay breeding?

Our observational and experimental evidence indicate that many SY birds were unable to acquire space to breed due to the territorial behavior of older individuals. Nonetheless, even in years of low kingbird breeding density (i.e. 2004–2005), when formerly used habitat was available, some SY birds failed to breed. In addition, large sections (up to several km) of riparian habitat, that to our eyes were identical to habitats used regularly, remained unoccupied in all years. As previous analyses of kingbirds at MNWR have failed to show any relationship between nesting success and

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variation in either food or nest site availability (Redmond et al. 2009), we cannot directly measure the suitability of these areas. However, we saw no obvious change in conditions (e.g. no loss of tree cover or foraging habitat except for one area that burned in 2004), and thus have no reason to believe that the formerly used areas had become unsuitable for breeding. Adult kingbirds generally exhibit high site fidelity (Murphy 1996, Redmond et al. 2009), but when dispersal occurs, ASY birds virtually always move to sites that were more successful than the site that they occupied in the previous year (Redmond et al. 2009). Given this, the fact that several formerly occupied areas went unused in 2004 and 2005 suggests that perhaps these areas were of inherently low quality. If this was the case, our results suggest that either some SY birds were not physiologically capable of breeding on low-quality territories (Newton and Marquiss 1991) or that some birds may have made a strategic choice to forgo breeding on low quality territories (Newton 1992, Zack and Stutchbury 1992). Given the high site fidelity of adults, and the fact that SY birds of both sexes arrive later on the breeding grounds than ASY birds (5 d for SY males, 7 d for SY females; Cooper et al. unpubl.), returning ASY individuals, especially males, often preemptively occupy the best sites. In an absolute sense, therefore, habitat may not be limited, but rather, high-quality sites may simply be unavailable to SY birds. This interpretation is consistent with results from our removal experiment. Large areas of unused habitat were available at Mosquito Coast, but the floaters only attempted to breed once space was made available by removing a resident bird. We cannot exclude the possibility that some SY birds were physiologically incapable of breeding on low quality sites, but this seems far less likely than the possibility that they ‘chose’ to forego settling on sites that were unlikely to be productive in order to secure access to high quality sites in the future. However, under either scenario, it is ultimately the availability of habitat and the territorial behavior of adults that drove some SY kingbirds to delay breeding.

**Conservation implications**

Age at first reproduction is a critical component of age-structured population growth models. Most models assume that breeding begins shortly after reaching sexual maturity but, as we have shown, this assumption may often be suspect. Failure to include accurate data regarding age at first reproduction may lead to flawed predictions because growth rates and population projections generated by these models are sensitive to age at first reproduction (Caswell 2001, Morris and Doak 2002). All other things held constant, falsely assuming that all birds breed in their first year will lead to an overestimate of population growth rates and persistence. Conversely, growth rate and persistence may be underestimated if age at first reproduction is assumed to be constant between years. For example, Ferrer et al. (2004) also found a negative density-dependent relationship between population size and age at first reproduction in imperial eagles *Aquila adalberti*, and concluded that population viability would have been underestimated had this relationship not been incorporated into their model. Clearly, the importance of conducting long-term studies on marked populations of animals cannot be overemphasized. Delayed breeding is difficult to document and may occur in many breeding populations, especially those in which fragmentation has resulted in habitat loss and/or degradation. As this is the typical situation for many threatened or endangered species, greater effort should be directed towards establishing better estimates of age at first reproduction to improve management prescriptions for wildlife populations.

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