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A comparison of methods for the long-term harness-based attachment of radio-transmitters to juvenile Japanese quail (*Coturnix japonica*)

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Abstract

Background: While the period from fledging through first breeding for waterbird species such as terns (e.g., genus Sterna, Sternula) is of great interest to researchers and conservationists, this period remains understudied due in large part to the difficulty of marking growing juveniles with radio transmitters that remain attached for extended periods.

Methods: In an effort to facilitate such research, we examined the impact of various combinations of harness types (backpack, leg-loop, and 3D-printed harnesses), harness materials (Automotive ribbon, Elastic cord, and PFTE ribbon), and transmitter types (center-weighted and rear-weighted) on a surrogate for juvenile terns, 28-day-old Japanese quail (*Coturnix japonica; selected due to similarities in adult mass and downy feathering of juveniles*), in a 30-day experiment. We monitored for abrasion at points of contact and tag gap issues via daily exams while also recording mass and wing cord as indices of growth. This study was designed to serve as an initial examination of the impacts of marking on the growth and development of young birds and does not account for any impacts of tags on movement or behavior.

Results: While we found that treatment (the specific combination of the transmitter type, harness type, and harness material) had no impact on bird growth relative to unmarked control birds ($P \geq 0.05$), we did observe differences in abrasion and tag gap between treatments ($P \leq 0.05$). Our results suggest that leg-loop harnesses constructed from elastic cord and backpack harnesses from PFTE ribbon are suitable options for long-term attachment to growing juveniles. Conversely, we found that automotive ribbon led to extensive abrasion with these small-bodied birds, and that elastic cord induced blisters when used to make a backpack harness.

Conclusions: While these results indicate that long-term tagging of juvenile birds is possible with limited impacts on growth, this work does not preclude the need for small-scale studies with individual species. Instead, we hope this provides an informed starting point for further exploration of this topic.

Keywords: Backpack harness, Common tern, Japanese quail, Leg-loop harness, Radio telemetry, Radio transmitter, Tag attachment

Background

Radio telemetry has been successfully used for over 40 years to advance our understanding of almost every aspect of avian life-cycles [24, 43]. The application of these methods has increased as advancements in technology have allowed for smaller transmitters, longer battery life, and finer spatial resolution [11, 44]. However, one portion of the avian life history that has

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been relatively understudied until recently has been the period from fledging until first breeding [34], often referred to as the post-fledging period [2]. While the post-fledging dependency period is of special interest in many passeriform and charadriiform species [21, 23, 39] and has begun to receive significant attention (e.g., [34], there is still room for improvement in pre-fledging tag deployment. Most work focused on early age classes utilizes small tags designed for shorter-term deployments [21, 27]. With the advent of small, solar-powered transmitters, longer-term tagging projects on smaller bodied juveniles have become feasible. This presents intriguing opportunities for work with precocial or semi-precocial migratory waterbird species such as terns (Sternidae), which often spend their first summer away from breeding locations [19]. Gaining insight into the behaviors expressed and threats faced during the later portions of this post-fledging stage could provide critical insight for the management of these species [1, 32].

The primary reason for the paucity of information regarding movement and behavior of Sternidae and many other precocial species from pre-fledging through post-fledging, aside from recently ameliorated tag size limitations, is the need to attach transmitters in such a way that they are retained by the individual without causing physical harm or negative impacts on future fitness. One common method for attaching transmitters to young chicks is to glue the tag directly to skin or feathers (e.g., [20, 30, 50]) or via one of several external suture methods (e.g., [13, 21, 48]). Juvenile terns have been successfully tagged and subsequently tracked using an external suture approach [1, 21] but suture and implant methods are much more common in studies focused on young Galliformes [20, 22] and Anseriformes [3, 5]. Unfortunately, because solar panels cannot be used with implants (tag is inside the bird), the life span of the tag is limited by battery size which must be kept minimal to limit tag mass. Thus, implants on smaller species are usually limited to a matter of weeks. Conversely, suture methods do allow for solar-powered transmitters improving tag life, and retention is markedly better than observed with glue-based attachments [16, 21]. Suture methods have been found to reliably allow for data collection over a period of 3–4 months though retention can be negatively impacted by the vegetation of the study site and durability of the suture material [48]. Thus, while implant and suture methods present viable attachment methods that should be fully considered for studies focused on single season data or even transitions from pre-fledge to post-fledging periods, they may not be suitable when data across the entire post-fledging period or full migration cycle are desired.

While glue and direct attachment methods have relatively short retention periods, body harnesses present an opportunity for long-term transmitter attachment. There are numerous variations of harness types designed for individual species, with the most common being leg-loop harnesses [29] and backpack harnesses [49]. Harnesses have been used to track the movements of numerous tern species [7, 18, 29, 47], but work has been focused on adult birds due to the difficulty of ensuring a proper fit when tagging growing juveniles so as to avoid impeding development. Although harness attachments have not been used on juvenile terns, they have been successfully applied to juvenile passerines [8, 25]. Even if the attachment does not negatively impact the growth of the tagged chick, long-term attachment presents a unique set of challenges: long-term attachments have the greatest risk of significantly impacting lifetime fitness [6] and should only be used when study objectives necessitate such data. Furthermore, harnesses may influence various behaviors of marked individuals [9] though the level of impact varies by avian guild and attachment method [17].

One interesting solution to the concern of harnesses constricting growth in tagged juveniles is to design a harness intended to allow for growth. Fortunately, the development of elastic harness materials, paired with the recent development of extremely light weight transmitters, presents an opportunity for tagging chicks with the goal of obtaining long-term data. For instance, elastic materials have already been used for harnesses on adult and juvenile passerines, with considerable success [23, 39, 46, 51]. However, the use of elastic harness materials has yet to target long-term attachment on growing juveniles and has yet to be tested on terns of any age. It is likely not feasible to create a harness that allows for growth from shortly after hatching through full adult size, especially for slower developing species. However, if a harness can be designed that allows for tagging the individual shortly prior to fledging without the need for replacement later in life while retaining reliable long-term retention ability, a door would be opened for researchers to explore a variety of previously unanswered questions.

In this study, we aimed to determine point of contact impacts of tagging on morphological characteristics and body condition during the late juvenile growth phase. We tested combinations of two types of radio transmitters, three types of harnesses, and three different attachment materials on Japanese quail (*Coturnix japonica*), a readily available surrogate for common terns (*Sterna hirundo*), selected due to similarities in adult mass and juvenile traits (see "Methods"). By examining possible attachment methods on a readily available surrogate species potential negative impacts can be identified and avoided prior to use on species of conservation concern like the common tern. Although this study did not quantify behavioral effects, which are expected to be species and context-specific, it serves as an important building block for establishing attachment methods that can be used for wild bird trials. Although limited in ability to extrapolate our results to field applications due to the captive nature of the study, daily handling for evaluation and measurement purposes was possible.

Results

The study began with 68 individual birds marked with various combinations of transmitter types, harness types, and harness material (with each unique combination referred to hereafter as a 'treatment') along with an additional 23 unmarked individuals that served as controls (Table 1, Fig. 1). However, two birds (1 Rear-weighted/Leg-loop/PFTE ribbon; 1 Rear-weighted/Leg-loop/Automotive ribbon) were re-tagged one day after initial tagging due to harness failure (harness came off of bird) that resulted from improper harness construction (faulty stitching and belly loop sized too loosely, respectively). Additionally, three birds (2 Center-weighted/Backpack/Automotive ribbon; 1 Rear-weighted/3D) were replaced with new birds 2 days after initial tagging due to injury resulting from harnesses that were fit with bottom loops too loose allowing birds to work one leg out of the loop. Three birds were removed from the study and euthanized due to non-harness related injuries: one control bird on day 13 and one bird marked with Center-weighted/Backpack/PFTE ribbon on day 14 due to injuries sustained from brooder pen (i.e., leg stuck in frame of the rack), and a second control bird on day 25 after becoming severely egg bound. The three euthanized birds were replaced with fresh birds from the same original cohort to maintain rack density, but these replacement birds were excluded from all analyses due to late entry. The replacement for the Center-weighted/Backpack/PFTE Ribbon bird was not retagged since the death occurred midway in the experiment. Thus, our final analyses included 71 and 23 marked and control birds, respectively.

Bird growth

Growth curves of mass, generated solely from control birds, show that males and females averaged 82.32 g (SE = 2.72) and 82.68 g (SE = 2.75), respectively, on day 1 (when treatment birds were tagged; Additional file 1). These weights corresponded to approximately 73% and 61% of the maximum mass obtained by males and females, respectively (from birth through 115 days old, see "Methods"). By day 30 of the experiment, when the transmitters were removed, both sexes appear to have been near their final adult weights, though mass of females continued to fluctuate during the 1 and 2 months post-tag removal, presumably from egg production. When examining the factors impacting the mass of birds in this study, we found no detectable effect of treatment ($X^2{}_{14} = 0.56$, $P = 0.90$), and removed the effect of treatment from the model. Moreover, for models containing the effect of treatment, the control group obtained masses comparable to the average of the other treatments, indicating no trend of mass being negatively impacted by the attachment of the transmitters (Additional file 2). There were significant effects of sex, day, and their interaction (Table 2), with male birds obtaining a lighter mass and growing slower (Fig. 2A). Similar results were observed when examining wing cord, as we found no effect of treatment ($X^2{}_{14} = 0.70$, $P = 0.78$), and the control group again fell out near the center of the

Table 1 The distribution of juvenile Japanese quail (*Coturnix japonica*) by treatment

Rack	Rear-weighted transmitter		Center-weighted transmitter		Control
	Harness type/harness material	***n***	**Harness type/harness material**	***n***	***n***
1	3D	4^{+}	3D	4	5^{E}
2	Leg-loop/Elastic cord	5	Leg-loop/Elastic cord	5	3^{E}
3	Leg-loop/Automotive ribbon	5*	Leg-loop/Automotive ribbon	5	3
4	Leg-loop/PFTE ribbon	5*	Leg-loop/PFTE ribbon	5	3
5	Backpack/Automotive ribbon	5	Backpack/Automotive ribbon	5$^{\#}$	3
6	Backpack/PFTE ribbon	5	Backpack/PFTE ribbon	5^{E}	3
7	Backpack/Elastic cord	5	Backpack/Elastic cord	5	3

$^{+}$ One bird substituted for another individual due to injury

$^{\#}$ Two birds substituted for other individuals due to injury

*A single bird retagged due to harness detachment

^{E} An individual was euthanized due to an injury unrelated to tag attachments and replaced with an unmarked bird

The distribution of juvenile Japanese quail across brooder racks based upon the method by which a mock-up of a small radio transmitter was attached to the individuals within the racks (i.e., transmitter type, harness type, and harness material)

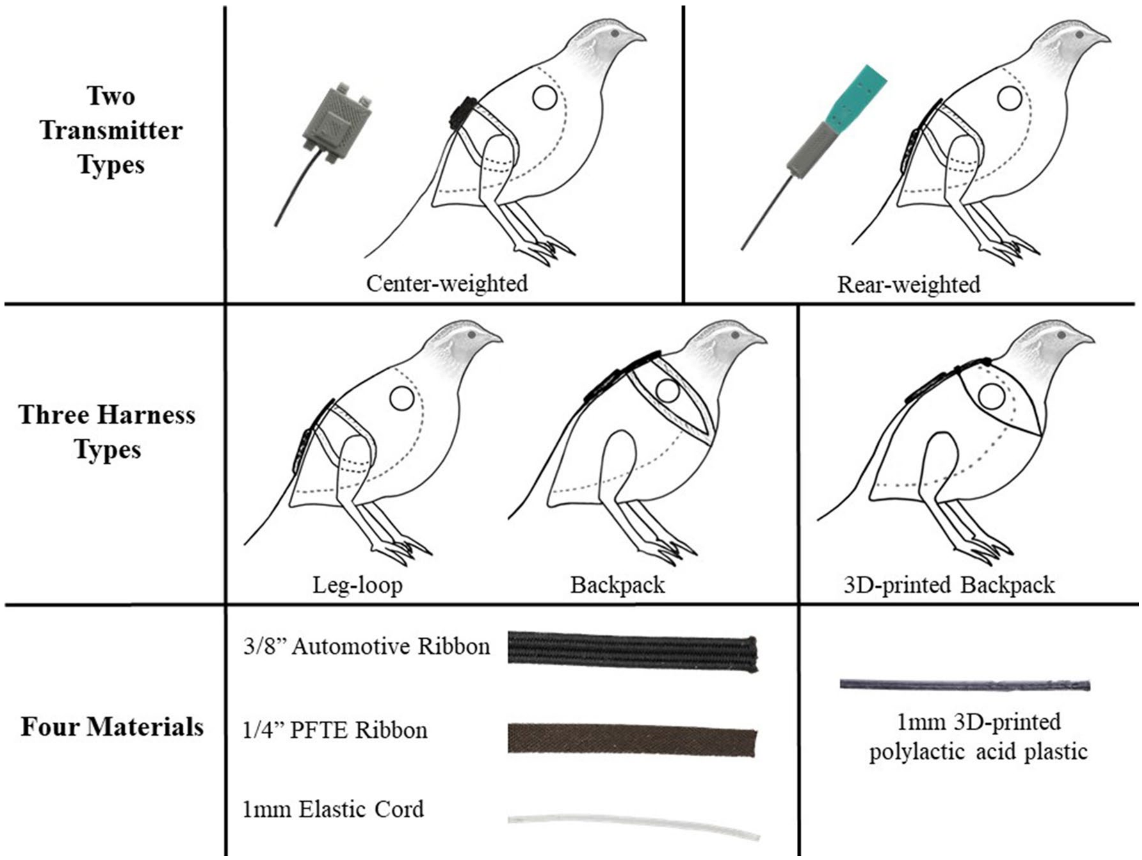

Fig. 1 A visual depiction of treatment components: transmitter type, harness type, and harness material. A visual depiction of treatment components used to attach small radio-transmitters to juvenile Japanese quail (*Coturnix japonica*): transmitter type, harness type, and harness material. While each harness type was used in combination with each transmitter type, leg-loop and backpack harnesses were constructed solely from Automotive ribbon, PFTE ribbon, or Elastic cord, while 3-D printed backpack harnesses used only the polylactic acid plastic. Thus, we tested 14 total combinations or treatments

other treatments (Additional file 3). Thus, treatment was removed from the model as was the interaction between sex and day, which was also not significant. However, there were significant effects of sex and day (Table 2), with female birds having marginally longer wing chords (Fig. 2B). Qualitative observations of mobility showed that all treatments were able to move about freely and had full range of movement in their wings (demonstrated by frequent flapping upon capture and handling).

Tag gap

For leg-loop methods, all variables and interactions showed significant effects on tag gap (amount of space between the harness and the skin, see "Methods") and were retained, except for the fixed effect of sex, which was retained because of its significant interaction with day (Table 2). Differences in treatment were driven by Center-weighted/Leg-loop/PFTE ribbon showing a decrease in tag gap (Fig. 3), with many tags becoming tight, while other treatments showed a slight but unexpected increase in tag gap over time. For backpack methods, all variables and interactions showed significant effects on tag gap and were retained, with the exception of the fixed effect of sex, which was retained because of its significant interaction with day (Table 2). Differences in treatment were driven by both Elastic cord treatments showing a decrease in tag gap (Fig. 4), with many tags becoming tight, while other treatments showed only a slight decrease. While not directly related to tag gap it should be noted that as birds grew, Center-weighted/3D tags rose up higher on the body in comparison with other treatments. This shift in placement allowed the tag gap of Center-weighted/3D to remain similar throughout the experiment.

Table 2 The impact of covariates on the top models for mass, wing chord, tag gap, and abrasion as measured from juvenile Japanese quail (*Coturnix japonica*)

Response	Variable	df	X^2	*P*-value
Mass	Sex	1	26.97	< 0.001
	s(Day)	7.7	201	< 0.001
	s(Day,Male)	6.8	11.04	< 0.001
Wing chord	Sex	1	4.27	0.039
	s(Day)	8.6	53.66	< 0.001
Tag gap: leg-loop	Sex	1	0.17	0.680
	Treatment	5	14.46	< 0.001
	s(Day)	3.6	11.91	< 0.001
	s(Day):Male	2.7	3.91	0.027
	s(Day):Rear-weighted/Leg-loop/Elastic	3	4.58	0.004
	s(Day):Rear-weighted /Leg-loop/PFTE	1	5.43	0.020
	s(Day):Center-weighted/Leg-loop/Automotive	4.5	10.05	< 0.001
	s(Day):Center-weighted/Leg-loop/Elastic	2	19.52	< 0.001
	s(Day):Center-weighted/Leg-loop/PFTE	5.5	27.71	< 0.001
Tag gap: backpack	Sex	1	2.52	0.110
	Treatment	5	4.2	0.001
	s(Day)	3.3	3.25	0.021
	s(Day):Male	1	10.03	0.002
	s(Day):Rear-weighted/Backpack/Elastic	4.3	17.51	< 0.001
	s(Day):Rear-weighted/Backpack/PFTE	1	0.78	0.380
	s(Day):Center-weighted/Backpack/Automotive	1	2.6	0.110
	s(Day):Center-weighted/Backpack/Elastic	1.8	31.59	< 0.001
	s(Day):Center-weighted/Backpack/PFTE	3.3	3.23	0.025
Abrasion: leg-loop	Sex	1	6.11	0.014
	Treatment	5	82.99	< 0.001
	s(Day)	4.3	11.55	< 0.001
	s(Day):Male	2.8	5.76	0.001
	s(Day):Rear-weighted/Leg-loop/Elastic	1	5.84	0.016
	s(Day):Rear-weighted/Leg-loop/PFTE	1	0.003	0.950
	s(Day):Center-weighted/Leg-loop/Automotive	1	8.06	0.005
	s(Day):Center-weighted/Leg-loop/Elastic	1	3.01	0.084
	s(Day):Center-weighted/Leg-loop/PFTE	2.9	4.91	0.006
Abrasion: backpack	Treatment	7	52.65	< 0.001
	s(Day)	6	16.5	< 0.001
	s(Day):Rear-weighted/Backpack/PFTE	1	0.123	0.730
	s(Day):Rear-weighted/Backpack/Automotive	1.8	0.542	0.460
	s(Day):Rear-weighted/Backpack/Elastic	1	0.374	0.543
	s(Day):Center-weighted/3D	1	4.26	0.040
	s(Day):Center-weighted/Backpack/Automotive	1	1.15	0.280
	s(Day):Center-weighted/Backpack/Elastic	3	3.12	0.030
	s(Day):Center-weighted/Backpack/PFTE	1	3.99	0.046

The impact of covariates on the top models for mass, wing chord, tag gap, and abrasion as determined via ANOVA. Smooth terms are indicated by s(). Male indicates the effect of being male, with Female as the reference category

Abrasion

For Thigh Abrasion (impact of treatment on the skin, see "Methods"), the random effect of rack did not improve model fit, and was removed to prevent problems with singular fits, while all other variables were retained with significant effects (Table 2). There were strong differences among treatments, with both Leg-loop/Automotive ribbon treatments rapidly obtaining and retaining high rates

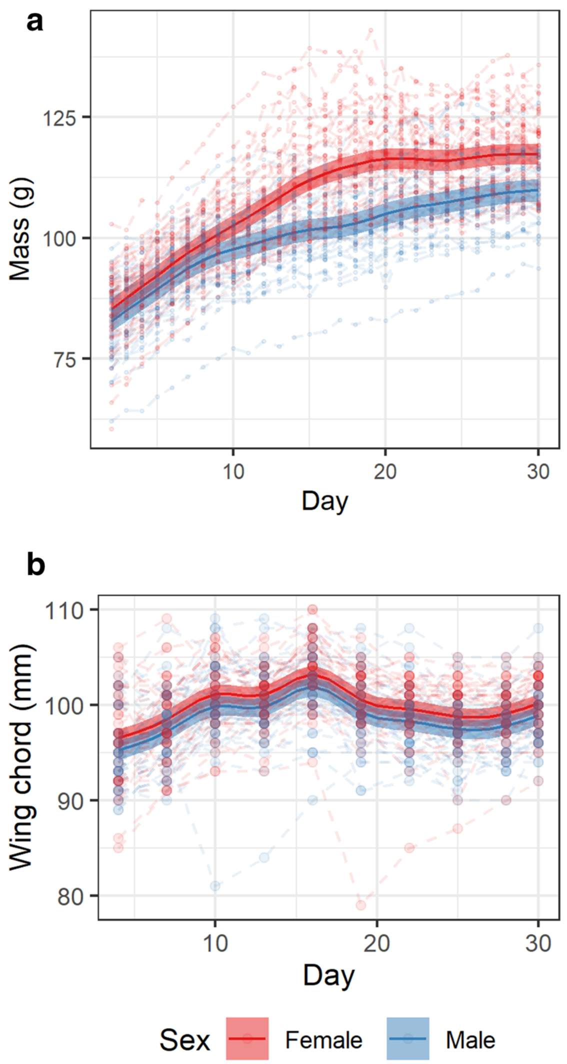

Fig. 2 Fitted model values (± SE) of top model predicting bird mass **a** and wing chord **b** of juvenile Japanese quail (*Coturnix japonica*). Fitted model values (± SE) of top model predicting bird mass **a** and wing chord **b** of juvenile Japanese quail. Points represent masses of individual birds connected by dashed lines. Birds differ by sex in both cases with males being smaller. The sudden drops in wing cord observed in two individuals are believed to be from beating wings against the rack walls. Impacted individuals showed no other signs of injury

of abrasion (Fig. 5). This was especially pronounced for the Center-weighted/Leg-loop/Automotive ribbon treatment where almost all severe cases (Abrasion scores above 2) occurred. The abrasion observed in individuals marked with Center-weighted/Leg-loop/Automotive ribbon were the result of the thick Automotive ribbon laying perpendicular to the body cavity allowing the ribbon edge to dig into a bird's side resulting in sores which, in some instances, developed into open wounds. In a few cases, sores scabbed over and began to heal after the ribbon shifted away from the irritated body region as birds grew. The other treatments performed comparably to each other, all showing levels of abrasion peaking around day 15, followed by a decrease in abrasion scores as birds recovered.

For Posterior Wing Abrasion, we dropped the random effect of rack, as well as the effect of sex and its interaction with day (Table 2). There were strong differences between treatments, with both Backpack/Elastic cord treatments rapidly obtaining and retaining high rates of abrasion (Fig. 6). These treatments are also where almost all severe cases occurred, with blisters on the posterior edge (metapatagium) of the wing where it joins the thoracic section, ranging from 1 to 5 mm in length and width. Depending on the individual bird, blisters were generally asymmetrical due to off-center harness centerpieces although some birds with symmetrical harnesses had symmetrical sores. The two Backpack/PFTE ribbon methods were less likely to show abrasion, with the Backpack/Automotive ribbon methods performing intermediately and with variable outcomes for the two 3D treatments.

Discussion

Our data indicate that marking juvenile Japanese quail with lightweight radio transmitters prior to fledging but after the majority of skeletal development is complete does not impact growth. This lack of impact on development suggests that efforts to tag and track juvenile terns across a full annual cycle are at least theoretically feasible and helps to assuage fears of additional negative impacts of research on monitored individuals outside of acknowledged inherent risks [6]. When marking small-bodied juvenile birds, such as tern chicks, our data suggest the use of Elastic cord or PFTE ribbon for leg-loop and backpack harnesses, respectively, regardless of transmitter type. However, similar to previous studies, our data also indicate that both harness type and harness material have demonstrable impacts on the long-term fit and the likelihood of attachments causing skin abrasion or other negative effects [10, 26, 35]. Thus, a thorough examination of the factors influencing the varied impacts of transmitter type, harness type, and harness material on marked individuals observed in this study is needed to understand the best tagging options for specific studies.

One critical element of the interplay between transmitter type, harness type, and harness material is the impact of these variables on long-term fit. For instance, across tag types and attachment materials, tag gap generally increased in leg-loop attachments while experiencing minor declines in backpack attachments. We think that this is the result of tags settling into the thigh region

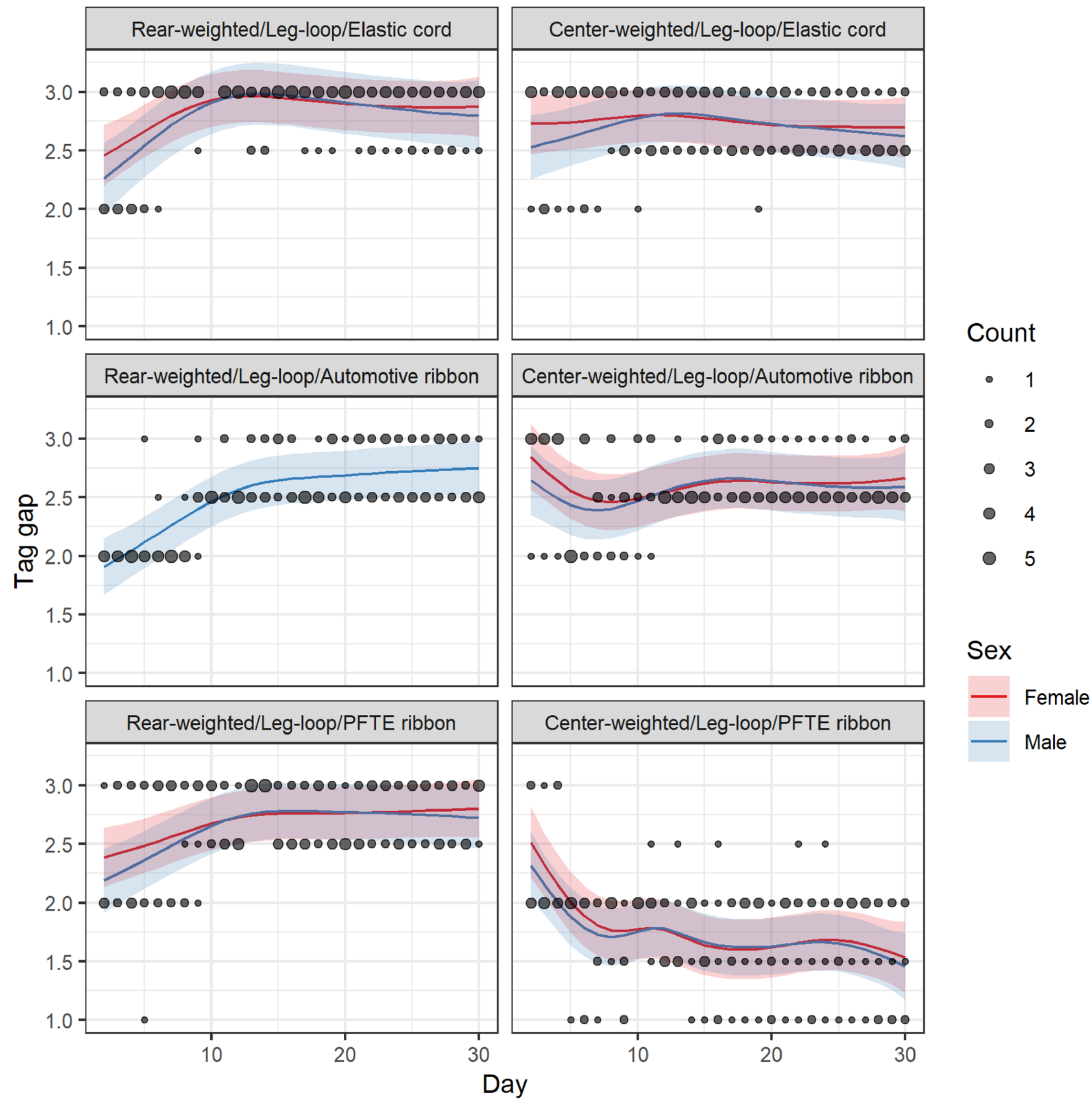

Fig. 3 Effect of leg-loop treatments on tag gap. Fitted curves (± SE) for effect of leg-loop treatments on tag gap in juvenile Japanese quail (*Coturnix japonica*). Tag gap, or the amount of space between the harness and the skin, was scored on a Likert scale with zero being no gap and three being a large gap (equates to a very loose harness)

following attachment and the fact that the thigh region appeared to be mostly developed at the time of tagging. Conversely, backpack harnesses were impacted by the dramatic growth of the breast region and relatively little settling of the attachment material into the underside of the wing (likely due to fewer feathers directly under the material when tags were attached). Thus, when tagging juvenile birds of any species, potential for future growth and mass fluctuation in the breast or thigh regions should be considered and harness type selected accordingly [36, 41, 42]. Fortunately, Japanese quail experience more dramatic expansion of the breast muscle than would be expected in terns, suggesting that tightness observed in this study is more extreme than what would be reasonably expected from deployment on juvenile terns.

While the differences between backpack and leg-loop harnesses helps to explain some broad level trends in our tag gap data, some results were much more specific to individual treatments, including the type of transmitter used. For instance, Center-weighted tags have small attachment tubes included on the end of the tag that allow the tag to be attached to PFTE ribbon or Elastic

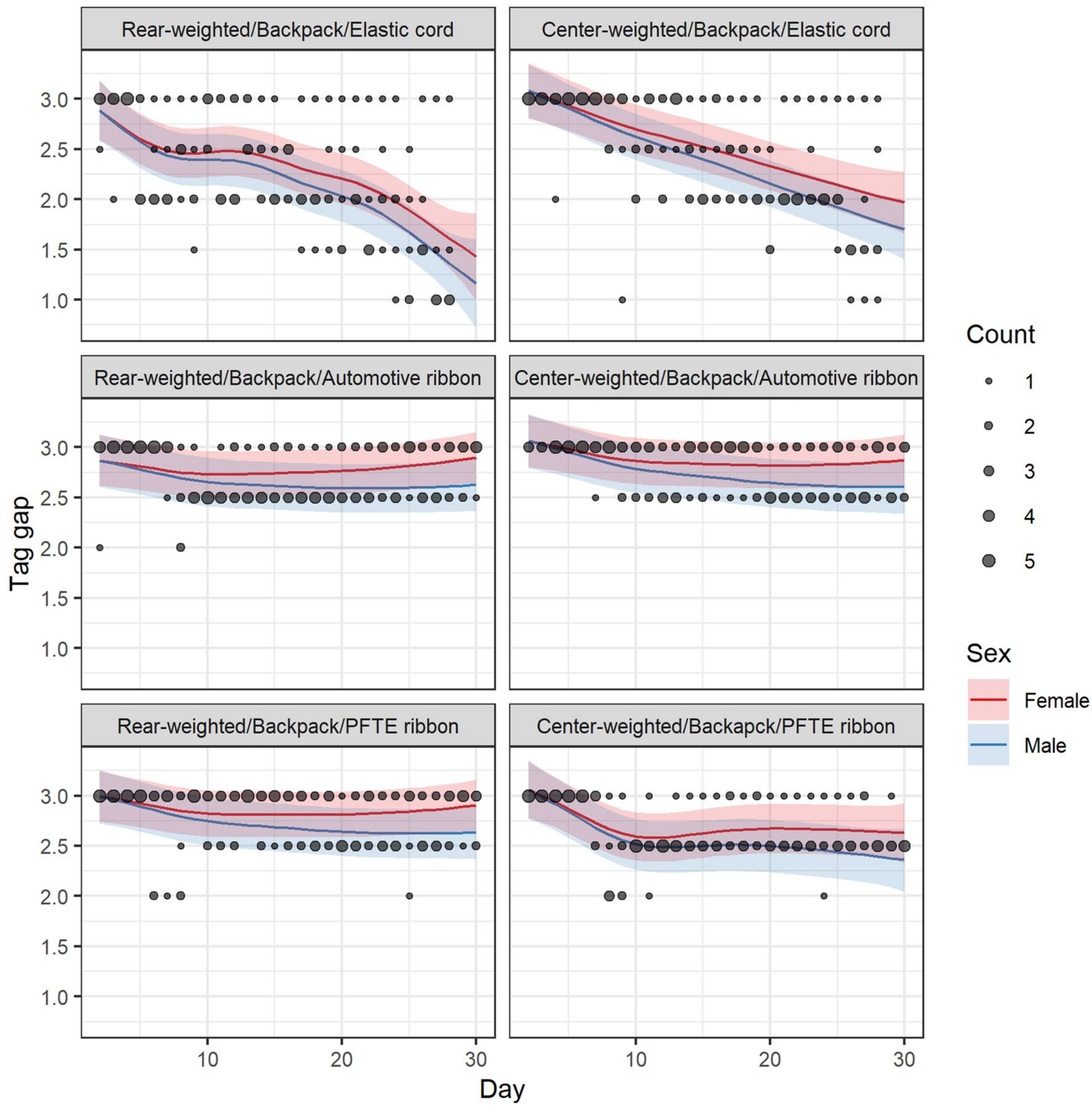

Fig. 4 Effect of backpack treatments on tag gap. Fitted curves (± SE) for effect of backpack treatments on tag gap in juvenile Japanese quail (*Coturnix japonica*). Tag gap, or the amount of space between the harness and the skin, was scored on a Likert scale with zero being no gap and three being a large gap (equates to a very loose harness)

cord securely without the need for sewing (Automotive ribbon is too wide for these tubes and must be sewn). Conversely, Rear-weighted transmitters must be sewn onto all harness materials. As seen in the failure of one harness due to improper stitching, any additional components increase the risk for harness failure and subsequent tag loss. Additionally, noticeable stretching occurred in PFTE ribbon where the thread passed through the attachment points. Thus, while the securely attached Center-weighted/PFTE ribbon (no sewing) combinations experienced reduced tag gap as birds grew and the material could not expand, the sewn on Rear-weighted/PFTE ribbon combinations expressed increased or relatively stable tag gaps due to stretching at the hole where the thread passed through the Teflon or expansion of the stitches themselves. While neither of these created much additional gap individually, the cumulative effect was meaningful. This demonstrates the primary advantage of Elastic cord and Automotive ribbon which have greater plasticity in the harness material and allow for bird growth without straining the harness or the bird. However, the elasticity of these materials does not mean that

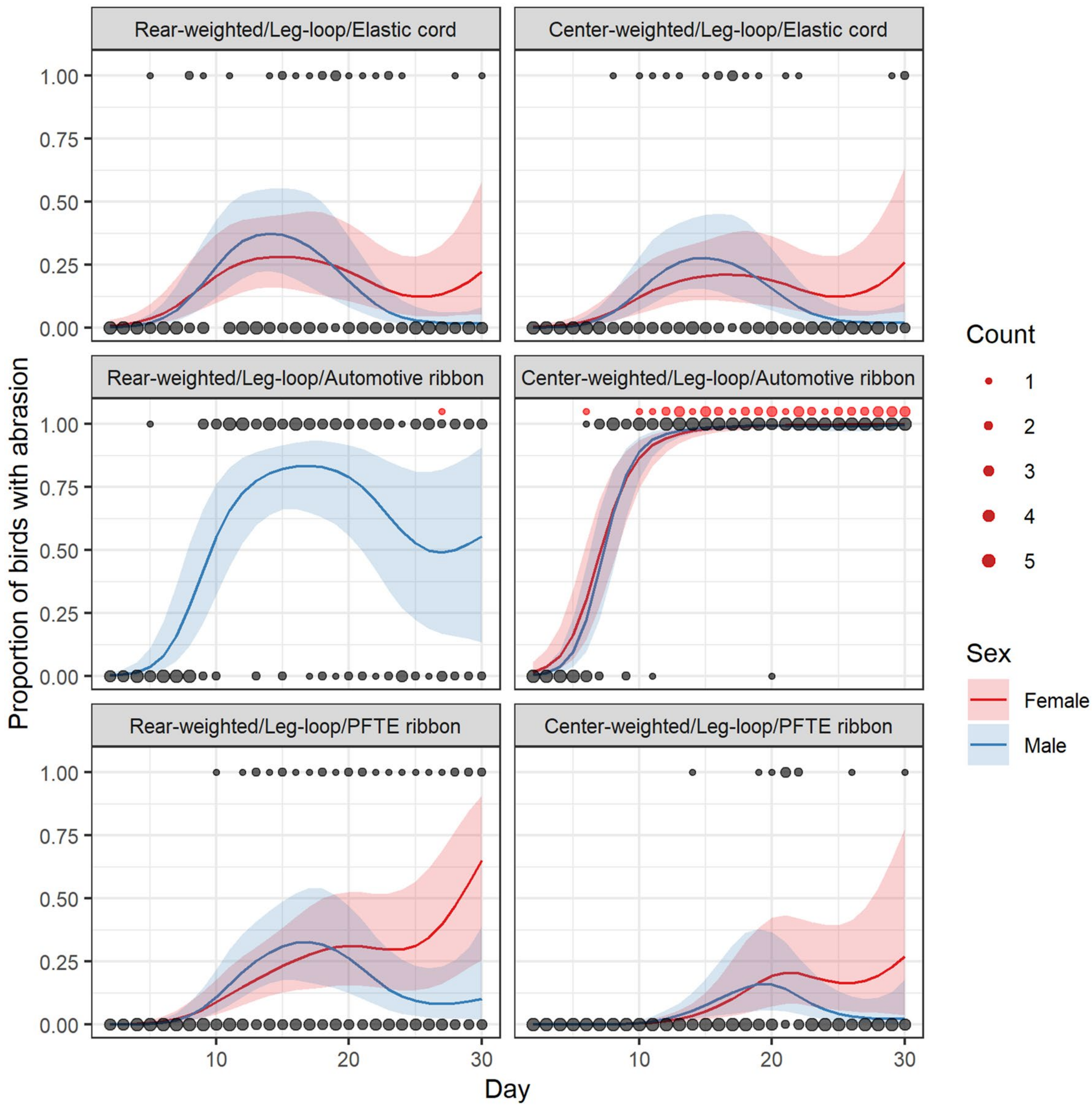

Fig. 5 Effect of leg-loop treatments on thigh abrasion. Fitted curves (± SE) for effect of leg-loop treatments on thigh abrasion. Abrasion was scored on a Likert scale with one being no abrasion and higher values indicating increasingly severe abrasion. Grey dots are weighted by number of birds. Red dots, also weighted by number of birds represent severe cases (Abrasion scores of 3 or 4). There is no fitted line for females in the Rear-weighted/Leg-loop/Automotive ribbon treatment because there were no females randomly assigned to this treatment group

tightness cannot occur. Elastic cord backpack harnesses were often unknowingly fit with the central X off-center, causing one side to be tighter than the other and the harness to function improperly (see below for more details).

Abrasion differences observed in this study appeared to be a function of harness material and harness type, with minimal distinction between transmitter types. For instance, PFTE ribbon performed well for both backpacks and leg-loops, whereas Automotive ribbon resulted in increased abrasion for both harness methods, likely the result of differences in the flexibility and thickness of these materials. Unlike PFTE ribbon, Automotive ribbon cannot bend across its width to contour to the body of the individual. Thus, while PFTE ribbon could lay flat the Automotive ribbon presented an abrupt edge that would rub against skin. These issues are exacerbated by the relatively small body cavities of Japanese quail, explaining why this method has been shown successful for larger species such as wild turkey and waterfowl [15, 26] but was problematic during this study. While we would have

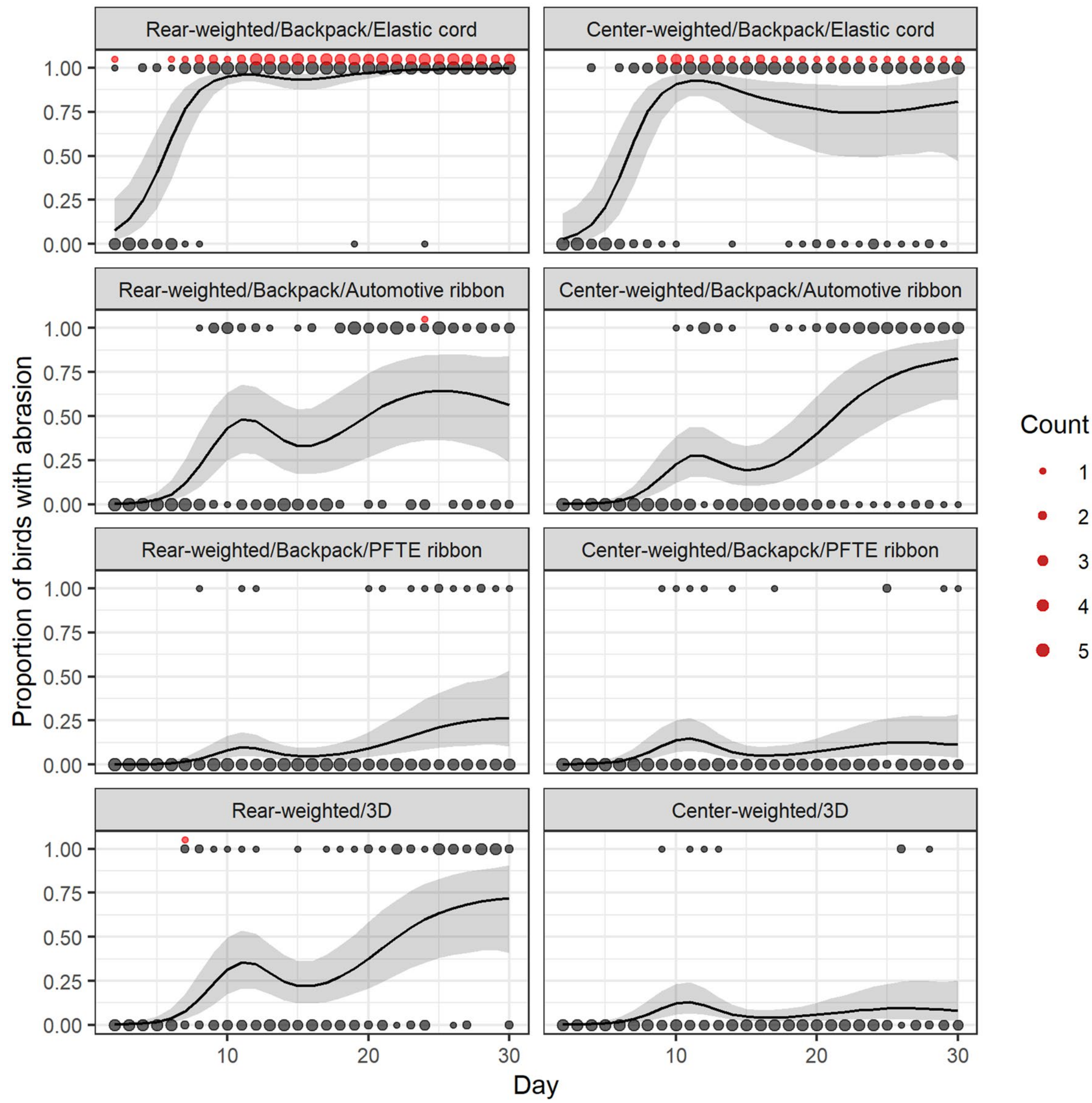

Fig. 6 Effect of backpack treatments on posterior wing abrasion. Fitted curves (± SE) for effect of backpack treatments on posterior wing abrasion. Abrasion was scored on a Likert scale with one being no abrasion and higher values indicating increasingly severe abrasion. Grey dots are weighted by number of birds. Red dots, also weighted by number of birds represent severe cases (Abrasion scores of 3 or 4). There was no effect of sex

preferred to test a lesser width of Automotive ribbon that may have minimized the abrasion seen in this study, this product is not manufactured in a smaller size.

Although harness type had some impact on abrasion levels observed in treatments where Automotive and PFTE ribbons were used, the general trends (meaningful abrasion observed or not) remained relatively consistent regardless of harness type. However, we observed dramatically different results between leg-loop and backpack harnesses attached with Elastic cord. Minimal abrasion was seen with the Leg-loop/Elastic cord treatments which, paired with the positive tag gap data, suggests this is a good approach for tagging juvenile birds. This conclusion is further supported by the safe and successful use of various elastic cords to mark adult and juvenile passerines with leg-loop harnesses [23, 39, 46, 51]. While this method resulted in tag retention and constriction issues when tested on 11-day-old juvenile quail by Terhune et al. [48], the authors suggested this may have been related to issues with age at attachment and technique used. Unfortunately, the positive results seen with Leg-loop/Elastic cord treatments were not repeated with Backpack/Elastic cord treatments. We believe the high levels of abrasion seen in these treatments was predominately caused by the misaligned centerpiece forming asymmetrical wing loops, as evidenced by cases of uneven abrasion between wings. However, some birds exhibited abrasion even when harnesses were properly aligned, likely due to the Elastic cord, which has a narrower diameter than our stretchable materials and can have a "tacky" texture when stretched, pulling against the skin as the bird grew and material expanded (functioning as designed but with the unintended consequence of abrasion). This suggests that this combination of harness type and material is problematic unless the Elastic cord can be run through a small tube that reduces contact between the material and the skin. While such an approach has been used successfully with American Woodcock [33], this would be problematic with species such as terns that regularly dive, as the tubes would fill with water increasing harness weight and resistance.

While the impact of a selected tagging method and materials is crucial to the success of a project, ensuring harnesses are appropriately sized for the tagged individual is also important. It is our suggestion that harnesses deployed on juveniles be sized for a full-grown adult, even if tags are intended to break away as the individual grows. Such an approach eliminates the risk of injuries such as those reported by Hubbard et al. [22] when backpack harnesses caused severe wing swelling after harnesses failed to fall off as wild turkey poults grew. While ensuring adequate room for growth is important, juvenile birds must also be of sufficient size that a tag cannot fully or partially slide off, restraining or otherwise incapacitating the bird as seen with some failed attachments in this study. One major advantage of the Leg-loop/Elastic cord treatments was the ability to quickly make minor adjustments that allowed us to size the harness to the individual bird during attachment and avoid the attachment failures seen with some other treatments, a known advantage of leg-loop harnesses [46]. Unfortunately, it was much more difficult to make accurate adjustments on the Backpack/Elastic cord harnesses without causing a misalignment that was not noticeable until the material settled into the wing cavity. Similarly, methods which require sewing must be pre-sized, and any efforts to make real-time adjustments dramatically extend handling time and increase the risk of improper stitching. While the 3D-printed harnesses present an intriguing opportunity for a tag attachment method that can be rapidly placed on the bird, the design of these harnesses is such that they are made to rise and fall on the bodies of birds in response to body mass fluctuations through the annual cycle (pers. comm. David La Puma, Cellular Tracking Technologies, 2020). This is ideal for adults of species that experience significant weight fluctuations (i.e., *Calidris* sandpipers), but is problematic for juveniles that are still experiencing skeletal growth.

It is important to recognize that this study was only concerned with long-term attachment options, and thus only tested harness-based methods. However, other methods may be preferable if study objectives allow for short-term attachment. For instance, adhesive, implant, and suture-based attachments all have potential advantages with short-term studies (see Introduction), but are not viable options for long-term attachment of solar-powered transmitters [16, 21, 48, 50]. However, it should be noted that if research goals require data from hatching through fledging and subsequent migration, then a combination of glue or sutures being replaced with a harness as the bird grows should be seriously considered. While neck collars have been used with success for long-term attachment on adult gallinaceous birds [28], they are not practical for highly aerial birds such as terns. Additionally, despite the successful use of tracking devices affixed to tarsal bands on adult terns, the previously reported cases of egg breakage and leg abrasion forced us to preclude this method. While, it should be noted that this study did not examine the impact of weather or habitat on tag retention, the success of harnesses and these materials in other studies leads us to believe that retention issues should be unlikely.

Conclusions

Our data indicate that if proper consideration is given to the combination of transmitter type, harness type, and harness material, pre-fledge juveniles of a surrogate for medium-sized terns can be safely tagged with lightweight radio transmitters in an effort to collect long-term data. While our data support the use of Leg-loop/Elastic cord or Backpack/PFTE ribbon when marking small-bodied juvenile birds such as terns, it is also important to consider multiple factors including the physiology and behavior of the target species. It is our hope that this positive data will spur additional work in this area and create a useful starting point when considering the potential opportunities and challenges of such a study. However, this work was not intended to provide a protocol for tagging all species, but instead to lay the groundwork so researchers could proceed with increased confidence knowing selected methods had been thoroughly tested on a less sensitive proxy species. Thus, this study should not replace smaller scale trials where intended methodologies are tested on the desired target species prior to a large-scale marking effort to determine any potential negative impacts on flight ability, reproductive success, or general behavior.

Methods

Husbandry

For this study, we used captive-reared Japanese quail from the long-term research colony at the U.S. Geological Survey's Eastern Ecological Science Center. We chose Japanese quail as our proxy for juvenile common terns (*Sterna hirundo*). Japanese quail were selected due to the similarities in adult mass (~ 120 g [for common terns see [4], for Japanese quail see "Results"]) and because both species are fully covered in down at hatching [41]. Additionally, the slower development (relative to common tern chicks) of the strain of Japanese quail used in this study enables a careful examination of any complications as they emerge. Similarly, while body shape is admittedly different between the two species, the elevated levels of breast expansion seen in Japanese quail relative to common terns enable these results to account for exceptional scenarios and provide greater confidence that selected methods will be less likely to result in unanticipated impacts. While it would be ideal to test attachment methods directly on common tern chicks, this species is protected at the federal level by the Migratory Bird Treaty Act (16 US Code §§703-711) and provided varying degrees of special protections in multiple states along the Great Lakes and coastal regions of the United States [4], e.g., [31] making initial testing when potential injuries are unknown inadvisable (see [35, 45]). Fortunately, Japanese quail are an easily accessible domestic species without conservation concerns and can serve as a surrogate. While it should be noted that these species vary markedly in behavioral characteristics (i.e., terns dive for food and rely more regularly on flight), we believe that Japanese quail allow for a reliable initial examination of potential physiological and point of contact impacts of marking juveniles. This work is not intended to provide a final definitive answer for the best tagging method for use with juvenile terns, but instead to provide guidance on a safe place to begin such investigations and limit potential injuries to wild birds during method development.

Birds were hatched in incubators and transferred to multi-rack brooder towers when they reached 2 days of age. At 18 days old, the study birds were divided among 7 racks with 13 birds per rack. To promote timely development, light exposure varied throughout the study, beginning with constant light exposure (birth until 14 days old) and slowly transitioning towards a more darkness-oriented routine. At 41 days old, in an effort to curtail the development of aggressive behavior [14], the amount of light was reduced from 13 to 9 h, and the light source was switched from overhead to wall-mounted lights with one of two bulbs from each light fixture in the facility when birds reached 43 days old. Chicks were given food and water ad libitum throughout the study, initially being fed a diet of gamebird starter crumble, but transitioned to a lower-protein maintainer diet at 43 days old. Following the conclusion of the study, colony managers selected individuals to retain as breeders for colony purposes and moved these individuals to breeding towers.

Tag construction and attachment

We constructed transmitter packages consisting of three varying components: the transmitter type, the harness type, and the harness material with each unique combination serving as one treatment in this study (see Fig. 1 for complete breakdown of treatment components). In order to facilitate the examination of any differences in effect based on the type of transmitter attached to juvenile quail, we created mockups of two models of small transmitters suitable for birds of this size, the CTT LifeTag (mass = 0.8 g; Cellular Tracking Technologies, Rio Grande, NJ) and the Lotek NTS-1 solar NanoTag (mass = 1.4 g; Lotek Wireless, Newmarket, Ontario). While these tags are similar in size, they differ in the way the tags attach to harnesses. CTT LifeTags attach to harness material at connection points in a vinyl strip that extends from the main body of the tag, causing the tag to sit below the attachment points (hereafter CTT tags referred to as Rear-weighted; Fig. 1). Conversely, Lotek tags are centered between attachment points on both sides of the tag allowing them to be more centered within the harness (hereafter Lotek NanoTags are referred to as Center-weighted). Unfortunately, we could not use real transmitters in this study due to concerns of inadequate direct light resulting in the tags becoming nonoperational. Mock-ups were custom designed to match the dimensions of the actual transmitters and were 3D printed out of a polylactic acid plastic. Antennas, also made to replicate the features of those found in functional tags, were made of vinyl coated, 26 AWG Poly-STEALTH wire (Davis RF Co., North Haverhill, NH) and secured to the transmitter body by melting the surrounding plastic onto the antenna. In order to mimic the thin vinyl portion of a Rear-weighted tag, sections of vinyl folder were shaped and melted onto the transmitter body. Final mock-ups were within 0.1 g of their respective units (Rear-weighted mock-up = 0.9 g, Center-weighted mock-up = 1.5 g).

In addition to the two transmitter types we used three different materials to make harnesses: ¼" tubular Teflon ribbon made from PFTE (Bally Ribbon Mills, Bally, PA) hereafter "PFTE ribbon"; 3/16″ Conrad-Jarvis automotive ribbon (the smallest width available for this product; Conrad-Jarvis Corp. Pawtucket, RI), hereafter "Automotive ribbon"; and 1 mm Stretch Magic elastic cord (Soft Flex Company, Sonoma, CA), hereafter "Elastic cord"). The PFTE material was relatively thin with a smooth surface and very pliable across both length and width but was non-elastic. Conversely, Automotive ribbon was very elastic and pliable along its length but not width, while being somewhat textured along the surface and presenting a thicker profile. Finally, the Elastic cord was pliable along both length and width while being elastic only along its length. Elastic cord was also smooth along its surface but had a "sticky" texture when stretched. Images of these materials can be found in Fig. 1. These materials were selected based upon communications with researchers with extensive experience tagging tern species based upon what materials they felt had the ability to operate properly based on the physiology and habitat of common terns (pers. comm. E. Craig, P. Loring, D. Lyons, J. Spendelow, and L. Welch).

We used these materials to construct both backpack and leg-loop harnesses. Backpack harnesses were roughly modeled after Thaxter et al. [49] "wing harness" method (selected due to lower number of sewing points and better ergonomic fit for common terns versus the "body harness" method reported by the same authors) while leg-loop harnesses were a modified version of the Rappole–Tipton Harness [38]. Harnesses made of Automotive ribbon were sewn to the tags using upholstery thread, whereas the Elastic cord harnesses were threaded through tubes or eyelets added to the tags during manufacturing and secured with 2 mm sterling silver crimp beads. The Elastic cord backpack harnesses were then secured in the center, across the breast, using a small section of 2 mm heat shrink tubing. Finally, harnesses made from PFTE ribbon were either sewn to the tag (Rear-weighted) or threaded through eyelets and secured via a knot (Center-weighted) depending on tag type. We also used a 3D-printed harness (Cellular Tracking Technologies, Rio Grande, NJ) with each tag type (material was non-elastic and somewhat stiff but presented a smooth surface). They were secured to tags using small sections of 2 mm heat shrink tubing and ethyl cyanoacrylate glue (Krazy Glue®). Images of all fully assembled treatments can be found in Additional file 4. Only tagging methods suitable for long-term attachment were tested in this study (see "Discussion" for more details).

We attached tags to the quail at 28 days old (hereafter, day 1). This date was chosen to represent the approximate point in the development of an 11-day old common tern chick (slightly earlier than any anticipated tagging of terns). Upon attachment, we recorded mass and wing chord for all birds in the study (both tagged and control birds). All harnesses except for those constructed out of Elastic cord were pre-sized based on the mass of birds, with the goal of similar amounts of tag gap across treatments and harness sizes. Elastic cord harnesses were sized on the bird prior to being locked to size with a crimp bead. Overall, this resulted in fourteen treatment groups (unique combinations of transmitter type, harness type, and harness material) divided among seven 13-bird racks, with two treatments per rack. Each treatment contained five birds, except for the two 3D-printed harness treatments with four birds; this rack had five control birds. A complete breakdown of racks, treatments, and sample sizes is available in Table 1. Control birds (generally n = 3) were included in each rack and were handled and treated in the same manner as treatment birds throughout the experiment.

Monitoring for effects

Following tag attachment, daily checks assessed tag gap, abrasion, or tag damage. Tag gap, or the amount of space between the harness and the bird's back or rump (for backpacks and leg-loop harnesses, respectively), was scored on qualitatively on a Likert scale with zero being no gap and three being a large gap (equates to a very loose harness, ~ 1 cm). While measuring tag gap, the transmitter was gently pulled up, away from the bird. Enough tension was applied as to take out any slack but not enough to cause noticeable stretching in the harness material. Abrasion was also scored on a Likert scale with one being no abrasion and higher values indicating increasingly severe abrasion. Photographic examples of each abrasion score can be found in Additional file 5. All birds, regardless of treatment, were also weighed each day and wing chord was taken every three days. These metrics were selected as they provide a look at potential adverse impacts of these tagging methods on wild birds. For instance, while a large tag gap could result in the bird snagging the harness on debris and becoming entangles, too small of a tag gap could result I constriction of blood flow. Similarly, abrasion would indicate potential for injury and possibly result in altered behavior. General qualitative observations of mobility were also made during handling. Tags were removed on day 30, at which time a final evaluation was conducted for each bird, and the complete suite of measurements was repeated. All birds selected to be retained as breeding pairs in the colony were also measured at 60 and 87 days after study initiation.

Analysis

We generated growth curves using control bird mass to visualize the percent of total growth completed at the age at which the birds were tagged. We tested for effects of treatment (each combination of transmitter type, harness type, and harness material) on growth metrics (bird mass and wing cord, independently), tag gap, and skin abrasion using generalized additive mixed models using the function "gamm()" from the package mgcv [52] in R version 4.0.2 [40]. All models presented only included the 30-day treatment period as no differences in growth were observed between tagged and untagged birds (see "Results").

Full growth models contained the effects of treatment and sex. To examine changes across time, a smooth effect of day was added with a cubic regression spline, as well as smooth interactions between day and treatment and day and sex. To control for correlated errors across time an Ar1 temporal autocorrelation structure was included [37], as well as the random effect of bird ID nested within rack to control for repeated measures and rack effect, respectively. Models were assessed using a Wald Chi-square test, and we removed non-significant terms through backwards model selection. Similar models were used for changes in tag gap, but with the leg-loop and backpack methods compared in separate models due to the inherent differences in these attachment types. While we acknowledge that our experimental design forces rack effect to be confounded with harness material, the lack of a significant rack effect in growth models (see "Results") suggests differences in tag gap or abrasion are unlikely due to any effect of rack and can likely be attributed to differences in treatments. The 3D harness treatments were not evaluated in this metric due to their dramatic difference in design and function.

Skin abrasion on the thighs and posterior of the wing/body juncture (metapatagium) were only monitored for the attachment types that could damage these respective areas and were thus analyzed separately. Due to a near complete lack of higher abrasion classes in most treatments, abrasion was treated as a Bernoulli variable, where abrasion values greater than 1 were coded as “Abrasion Present” and values of 1 coded as “No Abrasion.” Birds were then pooled by rack, sex, and treatment for binomial regression. The full models contained the same explanatory variables as those in the growth models, excluding the random effect of bird ID as birds were grouped for analysis. All data used in analyses are available at the USGS ScienceBase repository [12].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40317-021-00257-9.

Additional file 1. Growth curve of unmarked (control) juvenile Japanese quail showing average mass (± SE) by sex. Birds generally reached full size by end of 30 days (though females still fluctuate from eggs)

Additional file 2. Fitted model values (± SE) of top model containing the effect of treatment predicting mass of juvenile Japanese quail. Points represent masses of individual birds connected by dashed lines. Black dashed line indicates control group.

Additional file 3. Top model with effect of treatment for wing chord (± SE). Dashed line indicates control birds. Interestingly, Rear-weighted/Backpack/Automotive ribbon is the lowest, dragged down by the two birds that show sudden decreases.

Additional file 4. Assembled harnesses prior to attachment on juvenile Japanese quail by transmitter type, harness type, and harness material.

Additional file 5. Examples of the abrasion observed from Leg-loop and Backpack style harnesses (made with Automotive ribbon and Elastic cord, respectively) and their respective abrasion scores. Abrasion values were determined using the following criteria: open sore (4), blistering (3), red irritation of the skin (2), or no apparent impact (1).

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Authors’ contributions
EJB, JDS, and DJP conceived and designed the study; EJB, JDS, and DJP collected data; CMK and JMM analyzed the data; and EJB, JDS, CMK, JMM, and DJP wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets analyzed during the current study are available in the USGS ScienceBase repository: https://doi.org/10.5066/P9DQ6ITL [12].

Declarations

Ethics approval and consent to participate
All data reported in this study were collected in accordance with protocol approved by the Patuxent Wildlife Research Center Animal Care and Use Committee (2012-07).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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