IN REFERENCE REFER TO:
03E19000-2018-F-1412 Rusty Patched Bumble Bee Recovery Permits

Memorandum

To: Assistant Regional Director, Ecological Services, Midwest Region (Region 3), Bloomington, Minnesota (Attn.: Carlita Payne, Recovery Permits Coordinator)

Assistant Regional Director, Ecological Services, Southeast Region (Region 4), Atlanta, Georgia (Attn: Karen Marlowe, Recovery Permits Coordinator)
Assistant Regional Director, Ecological Services, Northeast Region (Region 5), Hadley Massachusetts (Attn.: Abby Gelb, Recovery Permits Coordinator)
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From: Field Supervisor, Twin Cities Ecological Services Field Office, Bloomington, MN 3/1/2019

Subject: Intra-Service Programmatic Section 7 Consultation on Region 3's, Region 4's, Region 5's, and Region 6's Section 10(a)(1)(A) Permitting Program for the Endangered Rusty Patched Bumblebee

This document is the U.S. Fish and Wildlife Service's (Service) biological opinion per section 7 of the Endangered Species Act (Act) of 1973, as amended (16 U.S.C. 1531 et. seq.) U.S. (ESA) regarding our review of issuance of Section 10 (a)(1)(A) recovery permits to persons conducting surveys, research, and other recovery efforts for the endangered rusty patched bumble bee (*Bombus affinis*).

The action being considered is the issuance of section 10(a)(1)(A) recovery permits by the Regional Offices. This activity will improve our scientific knowledge of the species and promote its conservation.

This biological opinion evaluates the impacts of authorizing take for purposes of recovering the rusty patched bumble bee. Although these actions may result in short-term adverse effects to the species, the purpose of these actions is to facilitate its long-term recovery. For reasons discussed within, it is our biological opinion that the proposed actions carried out pursuant to section 10(a)(1)(A) are not likely to jeopardize the continued existence of the rusty patched bumblebee. No critical habitat has been designated; therefore, none will be affected.

cc: FWS R3, ES, Carlita Payne
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BIOLOGICAL OPINION
Section 10(a)(1)(A) Recovery Permits for the
Rusty Patched Bumble Bee
February 15, 2019

Species for Which Activity is Sought and Status: Endangered rusty patched bumble bee (*Bombus affinis*)

Proposed Action: The proposed action is issuance of 10(a)(1)(A) recovery permits to cover actions including capturing the species with aerial hand nets or vials, handling, temporarily holding (*e.g*, in nets, vials, baggies and/or coolers) and taking photographs of captured individuals; and capturing the species (using lethal methods) as an "accidental" species while conducting surveys for other bees or other insects within certain parts of the historical range of the rusty patched bumble bee (see map at https://www.fws.gov/midwest/Endangered/insects/rpbb/rpbbmap.html). These actions are for the purpose of supporting recovery efforts by conducting population surveys; identifying new locations; evaluating potential impacts; monitoring populations and estimating population and relative abundances; documenting population health; and determining population viability. Approved methodologies for proposed activities are online at: https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html under "Survey Protocols for the Rusty Patched Bumble Bee (*Bombus affinis*)" (hereafter referred to as "Survey Protocol", USFWS 2018a). These methodologies may be updated annually.

In addition to population surveys, proposed actions covered under recovery permits may include non-lethal research activities to support actions, such as DNA extraction, pollen collection, pathogen sampling, tagging and tracking activities, and the use of dogs for detection (*e.g.*, nest detection). These actions are for the purpose of supporting research to better understand population health, estimate population size, determine habitat and floral resource needs and foraging or dispersal distances, determine possible causes of decline, examine genetic structure and diversity, estimate population viability, and to address other research questions. Handling will follow Survey Protocol guidance and each permit will include the following measures (additional conditions may be included in permits and/or are discussed in detail below):

1. Sampling of newly emerged queens is not authorized to avoid harm to newly forming colonies.
2. Surveys (if handling rusty patched bumble bees) must be conducted between early June and mid-August (June 1st - August 22nd), for the highest detection probability and to reduce potential impacts to rusty patched bumble bee queens.
3. When possible to fit the confines of the study, sampling (DNA, pollen, and pathogen) and tagging will occur at or near full bloom, when forager abundance is at its peak (*e.g.*. see Pyke et al. 2011), for rusty patched bumble bees, this is typically mid-late summer.

4. No more than 15 individual workers or males may be tagged from one location (within any circle 0.5 km in radius) within a 15-day time period. Individual bees will be fitted with tags or transmitters on the dorsal anterior abdomen using minute amounts of adhesive.
5. No more than 30 individual workers or males may be sampled for DNA, pollen, or pathogens from one location (within any circle 0.5km in radius) within a 15-day time period.
6. No more than 15 individual workers or males may be used for training detection dogs from one location (within any circle 0.5km in radius) within a 15-day time period.
7. Permits will indicate that the permittee will need to stop activities if a mortality occurs and confer with the Service prior to resuming activities.

Regulatory Issuance Criteria

Under the authority provided by section 10(a)(1)(A) of the Endangered Species Act the Service may issue permits for scientific purposes or for the enhancement of propagation or survival of endangered species. This includes permits issued in support of Safe Harbor Agreements; Candidate Conservation Agreements with Assurances; and recovery permits. This biological addresses only the latter - recovery permits. When deciding whether or not it should issue a recovery permit, the Service shall consider the following factors (50 CFR 50.22(a)(2):

> (i) Whether the purpose for which the permit is required is adequate to justify removing from the wild or otherwise changing the status of the wildlife sought to be covered by the permit;
> (ii) The probable direct and indirect effect which issuing the permit would have on the wild populations of the wildlife sought to be covered by the permit;
> (iii) Whether the permit, if issued, would in any way, directly or indirectly, conflict with any known program intended to enhance the survival probabilities of the population from which the wildlife sought to be covered by the permit was or would be removed;
> (iv) Whether the purpose for which the permit is required would be likely to reduce the threat of extinction facing the species of wildlife sought to be covered by the permit;
> (v) The opinions or views of scientists or other persons or organizations having expertise concerning the wildlife or other matters germane to the application; and
> (vi) Whether the expertise, facilities, or other resources available to the applicant appear adequate to successfully accomplish the objectives stated in the application.

In addition to the issuance criteria described above, the Service also considers the general permit issuance criteria at 50 CFR 13.21(b). The Service will not issue a permit if:

1) The applicant has been assessed a civil penalty or convicted of any criminal provision of any statute or regulation relating to the activity for which the application is filed, if such assessment or conviction evidences a lack of responsibility.

2) The applicant has failed to disclose material information required, or has made false statements as to any material fact, in connection with his application;
3) The applicant has failed to demonstrate a valid justification for the permit and a showing of responsibility;
4) The authorization requested potentially threatens a wildlife or plant population, or
5) The Director finds through further inquiry or investigation, or otherwise, that the applicant is not qualified.

To ensure Federal recovery permit holders have the experience and qualifications necessary the Service will review applicant qualifications, listed below and available online (https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html).

In order to be qualified to survey for rusty patched bumble bee, an individual must meet the following criteria:

1. Receipt of a four-year university or college degree in a natural science (noted in resume).
2. Demonstrated ability to complete species surveys and resulting technical reports.
3. Demonstrated ability to use aerial nets, or vials to capture bees. Detail experience with aerial nets or vials, including (close approximation is fine) how many surveys and how many years you have used these techniques.
4. *Bombus* identification skills.
5. Experience with specific requested activities (*e.g.*, experience conducting non-lethal DNA sampling of *Bombus* or other bees, experience tagging *Bombus* or other bees).

To document that s/he meets these criteria, we ask that the individual submit the following supporting documents to accompany application materials submitted. Application forms and instructions on how to apply for recovery permits can be found here https://www.fws.gov/endangered/permits/how-to-apply.html.

1. A resume documenting education and work/volunteer experience. Please include the following information on your resume or as an attachment: Citations of technical reports or published studies that contains an example of the individual's previous survey work, in particular, citations of reports that document your work with *Bombus* or other bees. List any survey work (including dates, location, relevant techniques used, and supervisor) in which you surveyed for *Bombus*, other bees, or listed species. Note if you independently identified *Bombus* species.
2. Documentation of *Bombus* identification skills (*e.g.*, details of a bee identification workshop that was attended or taught).

3. Documentation of successfully implementing non-lethal tagging, non-lethal DNA sampling, non-lethal pathogen sampling, non-lethal pollen sampling, training dogs to conduct nest detection or other techniques with negligible injury on *Bombus* species, if any of these techniques are desired activities.
4. If applicable, list any Federal Permit numbers and names of Permit holders that you worked under, even if for other (non-*Bombus*) taxa.
5. Two letters of recommendation from agency or academic staff pertaining directly to the taxa for which the individual wishes to be considered.

Location: Permits may be issued for activities in the following locations within Region 3 of the Service: Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio, and Wisconsin; Region 4: Alabama, Georgia, Kentucky, North Carolina, South Carolina, and Tennessee; Region 5: Connecticut, Delaware, District of Columbia, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia and West Virginia, and Region 6: North Dakota and South Dakota.

Proximity and distribution of the permitted activities: The purpose of the permitted activities is to conduct surveys and other recovery activities for this species, so the activities will occur within habitats occupied by the species as well as locations within the historical range where occupancy is uncertain or unlikely.

Methodologies used in the permitted activities:

For all of the methods described below, we recommend following the "General Guidelines and Best Practices for Surveys" in the "Survey Protocol for Rusty Patched Bumble Bee (*Bombus affinis*)" (hereafter referred to as "Survey Protocol"; https://www.fws.gov/midwest/Endangered/insects/rpbb/surveys.html). Special attention should be paid to the "Adhere to the Endangered Species Act…", "Handling", "Release", "Reporting", and "Salvage" sections of the document. Further instructions may be provided in 10(a)(1)(A) permits conditions.

Non-lethal Catch and Release Surveys:
Detailed information on approved survey and capturing protocols are located in the "Survey Protocol." Any surveys conducted under the authority of section 10(a)(1)(A) permits must adhere to these protocols. Surveys (if handling rusty patched bumble bees) must be conducted between early June and mid-August (June 1st - August 22nd), for the highest detection probability and to reduce potential impacts to queens.

In addition to catch and release for the sole purpose of population surveys, the activities mentioned below all include some level of capture, handling and release.

Tagging and Tracking:
Methods commonly used for marking insects include, but are not limited to, the use of ink, wire bands, micro-dots, and wing trimming (De Souza et al. 2018, p. 1). Even with recent advances in image-processing-based systems that reduce labor, limitations in physical marking persist. Tagging and tracking insects continue to advance with improvements to electronic tagging, passive harmonic tagging, radio tagging and other techniques. For example, electronic tagging based on RFID (radio frequency identification) technology is starting to become more prevalent, due to the small tag size and the ability to code individual bees with unique identifiers. Tagging is being used to help estimate bee population size (*e.g.*, mark-recapture studies), track foraging and dispersal distances, and find nesting sites, among other uses.

After capture by net or vial, rusty patched bumble bees are transferred to vials or tubes and temporarily held following the handling guidelines in the "Survey Protocol." Individual worker female or male rusty patched bumble bees (not queens) will be fitted with tags or transmitters following established protocols (*i.e.*, Hagen et al. 2011). Prior to tagging, captured bees may be temporarily chilled on ice (*e.g.*, Holehouse et al. 2003 pp. 278-279) (not dry ice, which is lethal, J. Strange, USDA, pers. comm. 2018). To facilitate tag attachment rusty patched bumble bees may be placed in a small vial or tube (one end covered with gauze or similar "breathable" material and the other end covered with foam or similar material). The dorsal parts of the bumble bee can be more easily accessed by partly opening the gauze with scissors. Individual bees will be fitted with tags or transmitters on the dorsal anterior abdomen using minute amounts of adhesive (*e.g.*, combination of eyelash adhesive and superglue) and released immediately after tagging. Transmitter or tag weight should be kept to the minimum, but sufficient to accommodate the study purpose and length (*e.g.*, battery size and life to accommodate the number of days needed for the study). Transmitter or tag size should not exceed approximately 50% of the average life weight of rusty patched bumble bees workers or males (*e.g.*, Hagen et al. 2011 used transmitters that were 66-100% of the life weight of *B. terrestris* workers, 44-66% of the life weight of *B. hortorum* queens and 50-74% of the life weight of *B. ruderatus* workers).

The Service will encourage researchers to incorporate the following into their methods:

1. When possible to fit the confines of the study, tagging rusty patched bumble bee tagging should occur between early June and mid-August, to reduce potential impacts to queens. Tagging will preferably occur at or near full bloom, when forager abundance is at its peak, for rusty patched bumble bees, this is typically mid-late summer.
2. Tagging is not allowed for newly emerging queens or late-summer/fall gynes (new queens).

3. No more than 15 individual workers or males may be tagged from one location (within any circle 0.5 km in radius) within a 15-day time frame. No more than a total of 45 bees can be tagged from a location. Each permittee can tag bees at up to 3 locations.
4. Individual bees shall be fitted with tags or transmitters on the dorsal anterior abdomen using minute amounts of adhesive. No tags should be applied to the thorax.
5. Transmitter or tag size should not exceed approximately 50% of the average life weight of rusty patched bumble bees workers (if tagging workers) or males (if tagging males).

Non-lethal DNA Sampling:
DNA sampling is used to study conservation genetics and behavior, for example to estimate nest abundance and foraging distance (*e.g.*, Geib et al 2015), to estimate population data and look for possible causes of decline (*e.g.*, Kent et al. 2018), and to estimate dispersal (*e.g.*, Lepais et al. 2010) to examine patterns of genetic structure and diversity (*e.g.*, Koch et al. 2017), among other things.

After capture by net or vial, individual worker female or male rusty patched bumble bees will be transferred to vials or tubes and temporarily held following the handling guidelines in the "Survey Protocol". Captured bees may be temporarily chilled on ice (not dry ice). One partial leg fragment (*i.e.*, terminal part of the tarsus approximately 2 mm in length) may be removed (*e.g.*, using a clean scalpel) from individual worker female or male rusty patched bumble bees (*i.e.*, Holehouse et al. 2003). Bees will be released immediately after removal of one leg fragment per bee. DNA will be extracted from the tissue sample and stored at proper conditions (*e.g.*, Holehouse et al. 2003, Kent et al. 2018).

The Service will encourage researchers to incorporate the following into their methods:
1. When possible to fit the confines of the study, rusty patched bumble bee DNA sampling should occur between early June and mid-August, to reduce potential impacts to queens. DNA sampling will preferably occur at or near full bloom, when forager abundance is at its peak, for rusty patched bumble bees, this is typically mid-late summer.
2. DNA sampling is not allowed for newly emerging queens or late-summer/fall queens.
3. No more than 30 individual workers or males may be tagged from one location (within any circle 0.5 km in radius) within a 15-day time frame, to avoid duplicate sampling and reduce effects to individual bees.

Non-lethal Pathogen Sampling:
The pathogen sampling technique employs feces collection using capture by net or vial and induce bees to defecate. After capture by net or vial, individual worker female or male rusty patched bumble bees will be transferred to vials or tubes and temporarily held following the handling guidelines in the "Survey Protocol". Captured bees may be temporarily chilled on ice

(not dry ice). Each vial may be agitated, using a gentle shaking motion for a few seconds to induce defecation. Feces are collected from bees that are temporarily held in vials. Individual bees should be released immediately after feces collection. Bee feces are analyzed (*e.g.*, microscopic and PCR detection) for pathogens such as *Nosema, Crithidia*, and *Apicystis*.

The Service will encourage researchers to incorporate the following into their methods:

1. When possible to fit the confines of the study, pathogen sampling of rusty patched bumble bees should occur between early June and mid-August, to reduce potential impacts to queens.
2. In field non-lethal pathogen sampling is not allowed for newly emerging queens or late-summer/fall queens.
3. No more than 30 individual workers or males may be used for non-lethal pathogen sampling from one location (within any circle 0.5 km in radius) within a 15-day time frame, to avoid duplicate sampling and reduce effects to individual bees.

Non-lethal Pollen Sampling:
Pollen is sampled to analyze floral preferences of bees (*e.g.*, Smart et al. 2017), but can also be used to study chemical loads *(e.g.*, Kriesell et al. 2016) and protein, lipid, and sugar concentration of the pollen (*e.g.*, Vaudo et al. 2018 or other studies).

After capture by net or vial, individual worker female or male rusty patched bumble bees will be transferred to vials or tubes and temporarily held following the handling guidelines in the "Survey Protocol". Captured bees may be temporarily chilled on ice (not dry ice). Small forceps will then be used to remove a pollen load from one of the pollen baskets. Only one pollen load will be affected to minimize the adverse impact due to loss of foraging output (E. Evans, University of MN, pers. comm. 2018). Bumble bees typically carry two pollen loads - one on each of two foraging baskets. Individual bees shall be released immediately after pollen collection.

The Service will encourage researchers to incorporate the following into their methods:

1. When possible to fit the confines of the study, pollen sampling should occur between early June and mid-August, to reduce potential impacts to queens.
2. In field non-lethal pollen sampling is not allowed for newly emerging queens or late-summer/fall queens.
3. No more than 30 individual workers or males may be used for non-lethal pollen sampling from one location (within any circle 0.5 km in radius) within a 15-day time frame, to avoid duplicate sampling and reduce effects to individual bees.
4. Only one pollen load should be collected per rusty patched bumble bee per day.

Detection Using Dogs:

Trained detection dogs are used to locate bumble bee colony nests and estimate nest density (*e.g.*, Waters et al. 2010) and this method is being tested to see if may work to detect species-specific nests (S. Colla, York U., pers. comm. 2018).

This technique typically employs the use of bumble bee nest material to train the dogs. Alternatively, training may involve capture of individual bees and exposure of captive bees to dogs (instead of using nest material). The Service may consider permitting captive colonies in future years, in which case, the use of rusty patched bumble bee nest material for dog detections may be considered at a later date. Here, we will only consider training dogs with individual bees, not nesting material.

Individual bees may be temporarily captured and used to train dogs. After capture by net or vial, individual worker female or male rusty patched bumble bees will be transferred to vials or tubes and temporarily held following the handling guidelines in the "Survey Protocol". Captured bees may be temporarily chilled on ice (not dry ice) before brief exposure to dogs (*e.g.*, dogs may sniff the bees while they are in vials). Individual bees should be released immediately after used for training.

The Service will encourage researchers to incorporate the following into their methods:

1. When possible to fit the confines of the study, capture and temporary holding of rusty patched bumble bee used for dog training should occur between early June and mid-August, to reduce potential impacts to queens. Training will preferably occur at or near full bloom, when forager abundance is at its peak, for rusty patched bumble bees, this is typically mid-late summer.
2. Dog training using newly emerging queens or late-summer/fall queens is not allowed.
3. No more than 15 individual workers or male bees may be used for training from one location (within any circle 0.5 km in radius).

Lethal Collection Methods:

The Service does not generally recommend that surveyors apply for permits to take the rusty patched bumble bee if they propose to work only in what we refer to as Unoccupied Zones (USFWS 2018a, p. 4). We believe the likelihood of finding the species in these areas is so low, that we do not recommend scientific recovery permits in these areas. If an insect researcher is surveying for bees in these areas and is concerned that they may accidentally collect a rusty patched bumble bee and would like to obtain a recovery permit, then they may apply for such a permit. The Service would authorize the use of lethal survey methods, until such time, if ever, that a rusty patched bumble bee is collected or otherwise detected in the survey area. At that time, the permit would specify that they switch to non-lethal methods within the occupied area. The extent of the area considered to be occupied will be specified at that time by the Service.

Effects of the Action

Nature of the effect:

All Activities:
Surveys and other non-lethal activities will take place at the time of the year when worker females are the most likely caste to be captured. It is unlikely that founder queens, new gynes (queens), or reproductive males will be flying from early June to mid-August. In some instances, males may also be captured to fit the confines of a particular study (*e.g.*, study of male dispersal). Temporary holding is not thought to appreciably reduce the amount of food that the individual worker bee is collecting or bringing back to the founder queen (*e.g.*, pollen). Colony sizes of rusty patched bumble bees are considered large compared to other bumble bees, and healthy colonies may consist of up to 1000 individual workers in a season (Macfarlane *et al.* 1994, pp. 3-4). In any given survey day, it is anticipated that surveyors would only encounter and net a small subset of the total number of individuals that comprise a colony (E. Evans, University of MN, pers. comm. 2017). Furthermore, we are not authorizing capture of queens.

Non-Lethal Capture and Release Surveys:
Methods outlined in the Survey Protocol have been drafted and reviewed by bumble bee experts to minimize any chance of causing physical harm to rusty patched bumble bees. Mortality of individual bees as a result of handling during surveys, capturing with aerial nets or vials, or temporarily holding and taking photographs is unlikely (S. Droege, USGS, pers. comm. 2017, R. Jean, ESI, pers. comm. 2017). While survey practices for rusty patched bumble bee result in temporary capture and handling of individuals, the practices are standard and mortality is unlikely. However, accidental capture of rusty patched bumble bees using lethal methods targeted at other species (explained in "Proposed Action", above) will result in mortality.

No fatalities have been reported as a result of non-lethal surveys by permitted entities, based on two seasons of data (2017-2018) from federal permit holders using the Survey Protocol guidance (USFWS unpublished data). We are aware of two fatalities during surveys conducted by non-permitted individuals. One of the fatalities occurred when two bees, one of which was a rusty patched bumble bee, were netted simultaneously resulting in a fatal fight between the two bees (C. Gratton, University of WI, pers.comm. 2018). As such, we recommend netting only one individual bee at a time (and one bee per vial), if possible. The other reported fatality occurred in a previously unoccupied zone and the bee was netted and killed prior to identification (S. Droege, pers. Comm. 2018). At this time, we are unaware of other rusty patched bumble bees that were taken during non-lethal surveys. In addition to catch and release for the sole purpose of population surveys, the activities mentioned below all include some level of capture, handling and release. Based on 2017 and 2018 data, we think it is reasonable to anticipate some low level of mortality - no more than five accidental fatalities per year from capture, handling, and release activity, associated with non-lethal surveys.

Tagging and Tracking:

Methods to affix tags or transmitters have been tested with bumble bee experts to minimize the chance of physical harm to bumble bees. The attachment of radio transmitters may constrain insect behaviour and incur significant energetic costs, but few studies have addressed this in detail (Kissling et al. 2013, p. 523). Tagging may affect the bees during attachment (*i.e.*, effects of handling), as well as affect the bee behavior post tagging. The effects of handling is anticipated to be minimized by using the guidance following the Survey Protocol. The behavioral effects may depend on the type and size of tag (*e.g.*, see Table 1) and are discussed below.

Table 1: A few examples of tags that have been used on *Bombus.*

Description	**Mass (g)**	**Dimensions (mm)**	**Ratio (%) tag mass to ave. *B. affinis* worker mass (approximate)**	**Citation**
RFID	0.0054	2.5 x 2.5 x 0.4	2.6	DeSouza 2018
Radio transmitter ATS	0.2	12 x 5 x 1.5; 3cm antenna	100.0	Hagen et al. 2011
Harmonic radar	0.0008	16mm dipole	0.4	Lihoreau et al. 2012
Wireless sensors	0.102	6.1 × 6.4	49.3	Iyer et al. 2018

Some behavioral changes have been documented as a result of radio tagging. Hagen et al. (2011, p. 4) found that *Bombus* with radio transmitters took off within one minute of transmitter attachment, flew to a nearby bush or tree and cleaned themselves for one-half to two hours before flying to foraging areas. This behavior is unusual for bumble bees and was likely related to handling and transmitter attachment (Hagen et al. 2011, p. 6). One radio-tagged garden bumble bee (*B. hortorum*) individual rested for long periods of time (>45 minutes) during flights, suggesting that transmitter weight may incur significant energetic costs (Hagen et al. 2011, p. 4). Natural rest to flight ratios have not been quantified (Hagen et al. 2011, p. 6). Thus, the degree to which transmitters may affect resting versus active behavior may not be estimated precisely. Individuals fitted with transmitters were able to feed successfully (Hagen et al. p.5), but transmitters affected foraging behavior of buff-tailed bumble bee (*B. terrestris*) – affected individuals had significantly lower flower visitation rates and spent significantly more time foraging than individuals without transmitters. Hagen was unable to determine if foraging efficiency (nectar consumption per time) was reduced, but stated that a bee carrying a transmitter

of 66-100% of its body mass affects flight performance. Bumblebees are known to carry loads of pollen up to 100% of their body weight (*e.g.*, Heinrich 1979), but the attachment of transmitters could have additive and long term effects (Hagen et al. 2011, p. 7).

In addition to effects to foraging behavior, the addition of tags to rusty patched bumble bees may also affect dispersal of reproductive individuals - new queens and males. Reduced dispersal distance of tagged bees may result in fewer successful matings. Hagen et al. (2011, p. 8) compared observed flight distance with predicted homing distance and found that predicted distances were larger than empirically measured flight distances of tagged bees.

Bumblebees with transmitters attached to the dorsal thorax showed unbalanced flight behavior (Hagen et al. 2011, p. 2). This is the basis for our recommendation to researchers that they attach transmitters to the dorsal anterior abdomen. We expect that all permitted researches will follow this recommendation to avoid or minimize adverse effects caused by their placement on the thorax.

Challenges of radio-tracking insects in the field include the short battery life (7–21 days) of small transmitters, limited tracking range on the ground (100–500 m), and transmitter weight (Kissling et al. 2013, p. 511). Other tagging alternatives (*i.e.*, passive harmonic radar, RFID, wireless sensors), however, are available for various applications (*e.g.*, Nunes-Silvia, Iyer et al. 2018, Kissling et al. 2014, pp. 518-519) and this smaller and lighter tag technology (*e.g.*, see Table 1) may alleviate some of the negative behavioral issues. For example, DeSouza (2018, p. 2) used RFID tags measuring 2.5 mm × 2.5 mm × 0.4 mm in size, with a weight of 5.4 mg. RFID tags are increasingly being used in bumble bee research (see review - Nunes-Silva et al. 2018). Harmonic radar has been used to determine *Bombus* foraging distance (*e.g.*, Lihoreau et al. 2012, Riley et al. 1999, Osborne et al. 1999) and these lightweight tags are thought not to affect flight behavior (Osborne et al. 1999, p. 527) (although the sample size in this study was too small to test statistically). Lightweight wireless sensors are also being tested for use on bumble bees (*e.g.*, Iyer et al. 2018). It is reasonable to assume that these lighter tags would have some behavioral effects, such as reduced foraging, but we think it is reasonable to also assume that behavioral effects will decline with tag weight.

McFarlane (1974, p. 56) estimated that the average weights of field-captured pollen collector and nectar collector rusty patched bumble bee worker females were 207 mg (standard error =12 mg) and 215 mg (standard error =11 mg), respectively. Average weight of field-captured rusty patched bumble bee males was 131 mg (standard error =4 mg) (Macfarlane 1974, p. 56). Tag technology continues to improve resulting in smaller, more effective tags (*e.g.*, Hitachi Chemicals RFID tags Table 1), which would equate to approximately 2.6% of the average female pollen collector weight, 2.5% the average nectar collector weight and 4.1% of the average male weight.

In addition to behavior, tag or transmitter weight may affect the energy budget and metabolism (Kissling et al. 2014, p. 522-523), for example, the energistic costs of flight and movement. Although bees may carry pollen loads up to 100% of their body weight, heavy tags are likely to significantly affect normal energy expenditure if carried beyond a single foraging trip (Kissling et al. 2014, p. 523). Hagen et al. (2011, p. 7) used transmitters that ranged from 44-100% of body mass and found that those transmitters may increase energetic costs, reduce the amount of pollen and nectar harvested by the bee, and have additive long term effects. It is reasonable to assume that lighter tags would have less energetic costs.

Tags placed on individual bees may affect behavior and reduce foraging for individual bees, however, by limiting the number of individuals researchers can tag it will reduce the number of individuals in a colony likely to be affected. Furthermore, we think it is reasonable to assume that lighter tags will have less effects on the bee behaviors, such as those that result in less foraging.

To guard against adverse impacts to any population of the RPBB we will limit the number of individuals that may be tagged at each study locale. Colony sizes of rusty patched bumble bees are considered large compared to other bumble bees, and healthy colonies may consist of up to 1000 individual workers in a season (Macfarlane *et al.* 1994, pp. 3-4). The size of current colonies is not known, but based on other *Bombus* colonies (*sensu stricto*) and recent field observations, it is more likely that colonies may now range in size from roughly 50 to 500 individuals (one expert expressed with more confidence a second, wider range, from roughly 20 to over 1000) (S. Carpenter, University of WI, pers. comm. 2018; E. Evans, University of WI, pers comm. 2018; R. Jean, ESI, pers. comm. 2018; J. Strange, USDA, pers. comm. 2018). Individuals concurrently visiting a site are often from different colonies (J. Strange, USDA, pers. comm. 2016), and limited information suggests populations are densely aggregated (L. Richardson, University of Vermont, pers. comm. 2016), so it is likely that individuals captured at one location originate from more than one colony. In any given day, it is anticipated that surveyors would only encounter and net a small subset of the total number of individuals that comprise a colony (E. Evans, University of MN, pers. comm. 2017). If we use a mid-range estimate of roughly 300 individuals during the summer season, then tagging 15 individuals, if from the same colony (which is unlikely), it would represent roughly 5% of that colony. It is unlikely that all 15 would be from one colony, but this may be a reasonable worst case scenario

Our requirement that researchers allow at least 15 days between tagging efforts is also likely to reduce the chances that any individual colony will be disproportionately affected. The longevity of adult workers is variable among *Bombus* species and new individuals are added to the population as others die. Mean worker life-span for temperate species ranges from 13.2 days for yellow-banded bumble bee (*B. terricola*) (Rodd et al. 1980, p. 1719) (a closely related species) to

34.1 days for the yellow bumble bee (*B. fervidus*) (Goldblatt and Fell 1987, p. 2352). Given the mean worker longevity of the closely related species, *B. terricola*, we believe it is reasonable to assume that a 15 day time span between sampling would minimize chances of capturing the same bee twice in one location and would help to ensure that less than 5% of the adults in any single colony will be tagged at once. Populations consist of multiple interacting colonies (USFWS 2016, p.17). Therefore, this will help to ensure that less than 5% of any population is tagged.

We anticipate that several permittees will be conducting research using tagging methods each year. Each of those researchers may be using tagging at one to several locations, once to several times per year. Furthermore, the number of individual bees tagged at each location (0.5 km radius circle) within a 15 day time period may vary from one to 15 bees.

Because of this variability it is impossible to accurately enumerate the number of bees that may be tagged per year. We can, however, estimate the number based on some reasonable assumptions. No more than 15 bees are allowed to be tagged in one location, and tagging is limited to 15-day periods at any location within the season (June 1- August 22nd), each of which must be separated by a 15-day period with no tagging. Therefore, a maximum of 6 sampling events could occur at a single location, resulting in no more than 90 tagged bees (n= 90 =15 bees at 6 sites). We are however, limiting the number of total bees that could be tagged at any one location to 45 (*e.g.*, 3 sampling events of 15 bees each or more sampling events with less tagged bees not exceeding 45 total) and further limiting the number of locations in which each permittee can tag bees per year to 3 locations. If we estimate that a maximum of 45 bees will be tagged at 15 locations per year (based on our best estimate of 5 permittees engaging in this activity), we can estimate the total number of tagged bees to equal approximately 675 bees annually - a conservatively high estimate.

Non-lethal DNA Sampling:
Holehouse et al. (2003, pp. 281-283) found that, to facilitate genetics analyses of bumble bees, microsatellite DNA could be reliably amplified from a tarsal tip of a worker bumble bee and that tarsal sampling did not significantly reduce the survivorship of confined workers relative to controls. Holehouse et al. (2003, pp. 281-283) also found that tarsal sampling had no significant effects on survivorship, body mass of foragers, the frequency or duration of foraging trips, mass of pollen collected or mass of nectar collected of worker bumble bees allowed to forage in the external environment. Further, they detected no significant differences between the effects of sampling a mid-leg and those of sampling a hind-leg. Similarly, they found no significant difference between sampling the left or right hind-leg (Holehouse et al. 2003, p.281-283).

Another tested method, hemolymph sampling, significantly reduced the survivorship of confined workers compared to controls (Holehouse et al. 2003, p. 280) and was less efficient for DNA

extraction and amplification. Partial wing removal has also been attempted. Due to impairment of flight ability and overall performance after employing this technique, however, Holehouse et al. (2003, p. 284) recommends partial tarsal sampling of one leg as described in the previous paragraph. We do not anticipate any fatalities or any injuries to the bees as a result of DNA sampling in and of itself.

Non-lethal Pathogen Sampling:
This method involves using a gentle shaking motion for a few seconds to induce defecation. Feces are collected from the vials in which the bees were temporarily held. Bees are released immediately following processing. Although handling and gentle agitation of bees while in temporary captivity (*e.g.*, vials) may cause some temporary disturbance, we do not anticipate harm to the bees as long as researchers are following the handling guidance as outlined in the Survey Protocol (*i.e.*, limiting holding time, avoiding overheating, each bee held in individual vials). We do not anticipate any fatalities or any injuries to the bees as a result of this activity in and of itself.

Non-lethal Pollen Sampling:
This method involves collection of pollen from one pollen basket of captured bees. Only one pollen load will be affected to minimize the adverse impact due to loss of foraging output. Bumble bees typically carry two pollen loads - one on each of two foraging baskets. This method has been tested and no evidence of negative effects to the individual bee were observed (bees simply flew away) (E. Evans, University of MN, pers. comm. 2018; J. Strange, USDA, pers. comm. 2018). Although handling of bees while in temporary captivity (*e.g.*, vials) may cause some temporary disturbance, we do not anticipate harm to the bees as long as researchers are following the guidance as outlined in the Survey Protocol (*i.e.*, limiting holding time, avoiding overheating, each bee held in individual vials). We do not anticipate any fatalities or any injuries to the bees as a result of this activity in and of itself.

Collecting pollen from individual bees may reduce the amount of pollen that is brought back to the colony, however, by limiting the number of individuals researchers can collect pollen from and limiting collections to one basket, per bee it will reduce the number of individuals in a colony likely to be affected and minimize the reduction in pollen delivered to the colony.. Bumble bee workers can have several foraging bouts per day. For example a recent study of *B. impatiens* showed that workers made a mean of 5.9 (+/- 0.4 SE) foraging trips per day (Minahan and Brunet 2018, p. 6). If we assume rusty patched bumble bees average five foraging trips per day, then taking one pollen basket would amount to approximately one tenth of that bee's pollen gathered for the day. This will amount to a reduction in pollen for the colony, but will depend on the size of the colony. For example if one pollen basket is collected from 30 of 300 bees in a colony, this would equate to an approximate five percent reduction of collected pollen for the

day for that colony. We find it unlikely that all 30 captured bees would originate from one single colony, however.

Detection Using Dogs:
Dogs may be trained using individual bees captured using the guidance described above. Dogs trained to detect any species of *Bombus* (or another *Bombus* species, other than rusty patched bumble bee) nests may still encounter rusty patched bumble bee nests. As long as dogs do not disturb the nests, we do not anticipate that trained dogs will have an effect on rusty patched bumble bees in their nests. Although handling of individual bees while in temporary captivity (*e.g.*, vials) may cause some temporary handling disturbance, we do not anticipate significant harm as long as researchers are following the guidance as outlined in the Survey Protocol (*i.e.*, limiting holding time, avoiding overheating, each bee held in individual vials). Temporarily captive bees used to train dogs (*e.g.*, dogs sniffing bees while they remain in vials) are not anticipated to sustain any injuries. We do not anticipate any fatalities or any injuries to the bees as a result of this activity in and of itself.

Handling effects of non-lethal tagging, DNA sampling, pathogen sampling, pollen sampling, and detection dog training

Each of the above activities involve capture, handling, and release. We anticipate a small number of rusty patched bumble bees to die as a result of capture and handling for these activities.Based on 2017-2018 non-lethal survey data and by implementing Survey Protocol handling guidance, we think it is reasonable to anticipate no more than ten accidental fatalities per year from capture, handling, and release activity, associated with non-lethal tagging, DNA sampling, pathogen sampling, pollen sampling, and detection dog training activities, combined.

<u>Disturbance frequency, intensity and severity:</u>

Disturbance associated with surveying and capture may occur multiple times if an individual is recaptured, although recapture of an individual during a survey is unlikely (E. Evans, University of MN, pers. comm. 2017). In most cases, captured rusty patched bumble bees are to be released after processing (*e.g.,* after photographs are taken, tarsal clipping, pollen sampling, and/or fecal collection) and handling will be minimized to the maximum extent practicable, while still meeting research objectives. The intensity of the disturbance for individual rusty patched bumble bees will be minimal and will follow standard practices. In the case of surveys (using lethal methods) directed at other species, capture of rusty patched bumble bees will result in mortality. For more details relating to the capture, handling, and holding of rusty patched bumble bees please refer to the Survey Protocol.

Disturbance associated with non-lethal activities of DNA sampling, tagging, pollen sampling, pathogen sampling, and training detection dogs using rusty patched bumble bees will occur no more than once every 15 days from one location (within any circle 0.5 km in radius). The 15 day time-period and sample radius is based on the approximate life-span of worker bees and estimated foraging distance (less than 1 km from nest, USFWS 2016, p. 16). The longevity of adult workers is variable among *Bombus* species. Mean worker life-span for temperate species ranges from 13.2 days for yellow-banded bumble bee (*B. terricola*) (Rodd et al. 1980, p. 1719) (a closely related species) to 34.1 days for the yellow bumble bee (*B. fervidus*) (Goldblatt and Fell 1987, p. 2352). Given the mean worker longevity of the closely related species, *B. terricola*, we believe it is reasonable to assume that a 15 day time span between sampling would minimize chances of capturing the same bee twice in one location. This is particularly pertinent to relatively invasive activities, such as DNA sampling and tagging. Furthermore, these activities, if within the confines of the study, will only be authorized from early June and mid-August, eliminating impacts to spring queens and reducing the likelihood of impacts to new gynes.

Up to 15 bees can be used for tagging and/or detection dog training from one location (within any circle 0.5 km in radius) and up to 30 individual bees may be used for non-lethal DNA sampling, pollen sampling, and pathogen sampling. These numbers are based on the numbers of workers or males sampled per colony relative to estimated colony size. Colony sizes of rusty patched bumble bees are considered large compared to other bumble bees, and healthy colonies may consist of up to 1000 individual workers in a season (Macfarlane et al. 1994, pp. 3-4). One captive rusty patched bumble bee nest studied in the 1970s, was found to have produced over 2,100 bees, which as the largest nest recorded for a North American species at the time (MacFarlane 1974, p. 151), but wild colonies are thought to be much smaller (COSEWIC 2010, p. 12). The number of rusty patched bumble bees per colony has not been quantified since the species witnessed rangewide declines in the early 2000s. Even where they are still found, the decline in relative abundance to other *Bombus* species, indicates that colony sizes may often be smaller now than they were historically. Relative abundance of rusty patched bumble bee ranged from approximately 7 to 10 percent between 1950 and mid-1990s and declined to approximately 1 percent between 2000 and 2015 (USFWS 2016, p. 29, 34). The size of current colonies is not known, but based on other *Bombus* colonies (*sensu stricto*) and recent field observations, it is more likely that colonies may now range in size from roughly 50 to 500 individuals (one expert expressed with more confidence a second, wider range, from roughly 20 to over 1000) (S. Carpenter, University of WI, pers. comm. 2018; E. Evans, University of WI, pers comm. 2018; R. Jean, ESI, pers. comm. 2018; J. Strange, USDA, pers. comm. 2018). Individuals concurrently visiting a site are often from different colonies (J. Strange, USDA, pers. comm. 2016), and limited information suggests populations are densely aggregated (L. Richardson, University of Vermont, pers. comm. 2016), so it is unlikely that all observed individuals in one location originate from one colony. If we use a mid-range estimate of roughly 300 individuals, then

sampling 30 individuals, if from the same colony (which is unlikely), it would represent roughly 10% of that colony. Similarly, 15 individuals would represent approximately 5% of a colony.

Following the descriptions above, the number of individuals that can be sampled per location within a 15-day time frame is limited to 15 for tagging and dog training; and 30 for DNA non-lethal surveys, pollen collection, and pathogen sampling as described above. The numbers of workers or males sampled per colony is likely to be small relative to colony size. Therefore, any negative impacts from sampling on individual colonies are also likely to be small (Holehouse et al. 2003, p. 283). Furthermore, in order to reduce or eliminate negative impacts to new colony formation, sampling of newly spring emerging queens and late summer/fall queens is not permitted at this time.

Summary of Effects

The proposed actions are expected to contribute to the recovery of the species, but some adverse effects in the form of harassment and mortality will result during the capturing, handling, or holding of the rusty patched bumble bee. Mortality is unlikely to occur using non-lethal survey methods and will occur as a result of lethal survey methods. Negative effects of the lethal survey activities will be minimized by only permitting them in areas without recent evidence of the species' presenced and including a permit condition that directs the surveyor to stop using lethal techniques if they catch a rusty patched bumble bee or if one is documented in their survey area by another person. Either of these will prompt the Service to update our bumble bee occupancy maps with the new location, which will ensure that anyone carrying out actions in the area will be alerted to the potential presence of the species.

Healthy rusty patched bumble bee colonies may consist typically of hundreds of individuals and we anticipate that tagging, and DNA, pollen, and pathogen sampling, and use of bees for detection dog training would usually affect only a small proportion of individuals in a colony. Pollen sampling may reduce foraging of individual bees, by reducing the amount of pollen available to the colony by one pollen basket on the day of sampling per colony member sampled. This is not expected to negatively affect any colony due to the low proportion of individual bees affected per colony. Similarly, DNA collection by partial tarsal sampling and pathogen sampling by agitation are not anticipated to cause any negative foraging or other behavioral changes and are not anticipated to negatively affect any colony due to the low proportion of its members that would be affected. Use of dogs to detect nests is not expected to cause any behavioral changes or disturb nesting and training of dogs would be limited to a set number of individual bees, representing a small proportion of any one colony.

Tagging may cause some behavioral changes and result in some energetic costs, that result in reduced foraging or dispersal distance of individual bees, but this is not anticipated to negatively affect any colony due to the low proportion of its members that would be affected. Tags or transmitters placed on individual bees may affect behavior and reduce foraging for individual

bees, however, by limiting the number of individuals researchers can tag it will reduce the number of individuals in a colony likely to be affected. Furthermore, we think it is reasonable to assume that lighter tags will have fewer effects on the bee behaviors, such as those that result in less foraging. By limiting the number of individuals that are tagged within a certain distance, it is reasonable to assume that colony level effects would also be low.

In summary, the proposed actions will result in purposeful take in the form of capture and incidental take in the form of behavioral changes (*i.e.*, increased resting behavior, reduced foraging, reduced dispersal) of captured individuals. The effects of the behavioral changes to individuals is not anticipated to result in significant negative effects at the colony or population (multiple interacting colonies) level. We expect these actions to have a net benefit to the recovery of the rusty patched bumble bee. While the potential for complications (or accidental mortality) resulting from stress of capture and handling are possible, this potential should be nullified by following the standard Survey Protocol guidance. Each permit will include a condition for the permittee to stop activities if a mortality happens and confer with the Service prior to resuming activities.

We anticipate some indirect effects that may occur due to tagging. Specifically, tagged males with reduced dispersal distance may result in fewer successful matings (*i.e.*, matings with unrelated individuals). Based on studies of a closely related species, *B. terrestris* (Kraus et al. 2009, p. 249; Lepais et al. 2010, pp. 826-827), the maximum dispersal distance of untagged rusty patched bumble bee males likely ranges from 1 to 10 km. Tags may reduce dispersal distance (*e.g.*, Hagen et al 2011, p. 8) but colony level effects have not been quantified and would have to consider the variability in normal dispersal distance, unpredictable direction of travel, and the location of unrelated females. By limiting the number of individuals that are tagged in one location, it is reasonable to assume that future colony level effects would also be low. Furthermore, it is reasonable to assume that lighter tags will have less negative effects on dispersal distances.

Effects of Interrelated and interdependent actions: No interrelated and interdependent actions are associated with the proposed action.

Any Incidental Effects to Other Listed Species or Critical Habitats: No incidental effects to other listed species or critical habitats are anticipated from the Service's issuance of recovery permits for work on the rusty patched bumble bee.

Cumulative Effects: Cumulative effects are the effects of non-federal actions that are reasonably certain to occur in the action area. For this biological opinion, the action includes the entire historical range of the rusty patched bumble bee. Anticipated cumulative effects are described as risk and beneficial factors in the SSA report (USFWS 2016, pp. 40-53). In brief, these effects

may include actions that incorporate pesticide use, introduction of pathogens, or cause habitat loss and degradation. Beneficial effects may include pollinator habitat improvement projects and research on other *Bombus* or bee species that may inform rusty patched bumble bee recovery efforts. For additional information regarding these stressors and measures to avoid or reduce relevant adverse effects, see the *Rusty Patched Bumble Bee Conservation Guidelines* (USFWS 2018b) and the Rusty Patched Bumble Bee Section 7 Guidance (USFWS 2019 draft).

Conclusion

After reviewing the current status of the rusty patched bumble bee, and the effects anticipated from implementing the proposed recovery actions described above, it is the Service's biological opinion that issuance of Section 10(a)(1)(A) recovery permits for these actions is not likely to jeopardize the continued existence of the species. The proposed actions are not likely to appreciably reduce the species' numbers, reproduction, and distribution. If the permitted activities result in the discovery of new populations or provide information that may be used to benefit one or more known populations, they are likely to affect the species beneficially.

We reached this opinion based on the following factors:

1. The Survey Protocols to be used by those operating under a recovery permit were developed in coordination with the Service's lead recovery biologist for the species to ensure work is consistent with the species' recovery program and to ensure actions would benefit the rusty patched bumble bee and not reduce the viability of the affected populations.
2. Capturing, handling, and holding rusty patched bumble bees will cause temporary stress to individuals and may cause incidental take in the form of accidental mortality of captured individuals (using non-lethal survey methods). Methods in the approved Survey Protocol for areas that are likely inhabited by the species or have even a low likelihood of occupancy are unlikely to result in the mortality of any individuals and are unlikely to appreciably reduce growth and reproductive output compared to individuals not affected by permitted recovery actions.
3. Non-lethal tagging, DNA extraction, pollen sampling, pathogen sampling, and handling to train detection dogs will follow handling guidelines and best practices as described in the Survey Protocol and/or other established protocols that minimize harm to individuals.
4. When possible to fit the confines of the study, activities will occur at or near full bloom, when forager abundance is at its peak (*e.g.*, see Pyke et al. 2011), for rusty patched bumble bee this is typically mid-late summer.
5. Surveys and other non-lethal activities will take place at the time of the year when worker females are the most likely caste to be netted. It is highly unlikely that founder queens, new gynes (autumn queens), or reproductive males will be flying from early June to mid-

August. Temporary holding is not thought to appreciably reduce the amount of food that the individual worker bee is collecting or bringing back to the founder queen (*e.g.*, pollen).

6. Sampling of newly emerged queens is not being authorized and thereby eliminates harm to newly forming colonies.
7. Colony sizes of rusty patched bumble bees are considered large compared to other bumble bees, and healthy colonies may consist of up to 1000 individual workers in a season (Macfarlane *et al.* 1994, pp. 3-4). In any given survey day, it is anticipated that researchers would only encounter and net a small subset of the total number of individuals that comprise a colony (E. Evans, University of MN, pers. comm. 2017). The numbers of individuals sampled or tagged is likely to be low compared to the colony sizes.
8. Surveys using lethal methods are restricted to areas outside the presumed current range of rusty patched bumble bees. Any mortalities that result from lethal methods will occur in areas where we lack sufficient recent evidence of the species' occurrence to assume its presence. These collections will indicate that the species' distribution is broader than we had previously anticipated. Knowledge of these currently unknown populations will allow us to implement measures to protect and conserve them and will affect only a few individuals each year. Two lethal mortalities occurred due to the use of lethal collection methods in 2018 and we expect no more than five in any single calendar year.
9. No more than 15 individual workers or males may be tagged or used for dog detection training from one location (within any circle 0.5km in radius) within a 15-day time frame, to avoid duplicate sampling, reduce effects to individual bees, and to reduce colony level effects (*e.g.*, from loss in foraging). No more than a total of 45 bees may be tagged at an individual location (within any circle 0.5km in radius).
10. No more than 675 individual bees may be tagged annually.
11. No more than 30 individual workers or males will be sampled for DNA, pollen, and/or pathogens from one location (within any circle 0.5km in radius) within a 15-day time frame, to avoid duplicate sampling, reduce effects to individual bees, and to reduce colony level effects (*e.g.*, from loss in foraging).
12. Only one pollen basket will be taken per individual at a location to reduce negative impacts to the colony in terms of reduced food.
13. Individual bees will be fitted with tags or transmitters on the dorsal anterior abdomen using minute amounts of adhesive. Tagging is not allowed on the thorax.

No critical habitat has been designated; therefore, none will be affected.

INCIDENTAL TAKE STATEMENT

Section 9 of the Act and Federal regulations under section 4(d) of the Act prohibit the take of endangered and threatened species, respectively, without special exemption. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct. Harm is further defined by the Service to include significant habitat modification or degradation that results in death of listed species by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering. Harass is defined by the Service as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(a)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of this Incidental Take Statement.

The measures described below are non-discretionary, and must be undertaken by the Service so that they become binding conditions, as appropriate, of any authorization issued for the exemption of section 7(a)(2) to apply. The Service has a continuing duty to regulate the activity covered by the Incidental Take Statements (ITS). If the Service (1) fails to require permittees to adhere to the terms and conditions of the ITS through enforceable terms that are added to the permit, and/or (2) fails to retain oversight to ensure compliance with these terms and conditions, the protective coverage of Section 7(a)(2) may lapse. In order to monitor the impact of incidental take, the permittee must report the progress of the action and its impact on the species to the Service as specified in the ITS [50 CFR §402.14(1)(3)].

AMOUNT OR EXTENT OF TAKE ANTICIPATED

Take due to Accidents When Implementing Non-lethal Surveys and Studies
Incidental take may occur as a result of accidental deaths that occur as a result of survey methods that are intended to be non-lethal - i.e., capture and release methods. Although mortality is infrequent and typically not expected while using non-lethal survey methods, a few rusty patched bumble bees may die due to accidental injuries suffered before their release. We will include a permit condition for the permittee to stop activities if a mortality happens and confer with the Service prior to resuming activities to further minimize the likelihood of accidental injuries that lead to death. We anticipate no more than five accidental mortalities per year as a result of these surveys.

Similarly, we anticipate a low level of accidental mortalities will occur as a result of handling rusty patched bumble bees when implementing other activities that are intended to non-lethal. This includes DNA sampling; non-lethal pathogen sampling; pollen sampling; handling for dog

detection studies; and, handling during tagging. As with non-lethal surveys, harm or death of handled individuals is not typically expected to occur when implementing these techniques. A few individuals may die as a result of handling, however, and we conservatively estimate that no more than ten accidental mortalities per year will occur due to handling for purposes that are intended to be non-lethal.

In total, we anticipate no more than 15 individual mortalities per year will occur as a result of the issuance of 10(a)(1)(A) recovery permits.

Take due to Indirect Effects of Tagging

We will use the number of rusty patched bumble bees that are tagged and released as a surrogate measure of the number of tagged individuals that are incidentally taken as a result of this practice. We anticipated that some rusty patched bumble bees that are tagged (electronic tagging, passive harmonic tagging, radio tagging and other techniques) will die or be injured as a result of the effects of the tags on their behavior that occur after they are released. This will occur to only a subset of the tagged individuals - not to all of them. A significant proportion, if not all, of the post-release deaths or injuries of tagged rusty patched bumble bees will not be detected. Therefore, we must use a surrogate to monitor the extent of this incidental take.

It is appropriate to use surrogates to describe the extent of incidental in an incidental take statement (ITS) as long as 1) the ITS describes the causal link between the surrogate and the take of the listed species; 2) the ITS describes why it is not practical to express the amount or extent of anticipated take or to monitor take-related impacts in terms of individuals of the listed species; and, 3) the ITS sets a clear standard for determining when the level of anticipated take of the listed species has been exceeded.

In this case there is a causal link between the surrogate for post-release death or injury of tagged rusty patched bumble bees - number of rusty patched bumble bees captured - and the take. The number tagged and released will represent the maximum number that will be taken incidentally due to post-release death or injury. We anticipate that each year no more than 675 rusty patched bumble bees will be tagged. Although we will know that the surrogate represents the maximum, actual take will likely be only a proportion of the surrogate measure. The bees that die or are injured as a result of tagging may be found in some cases, but researchers will likely be unable to find most of the dead or injured. Finally, researchers will be able to monitor and report to the Service the number of rusty patched bumble bees that are tagged and released. This will allow us to determine when the level of anticipated take has been exceeded.

EFFECT OF THE TAKE

In the accompanying biological opinion, the Service determined that the level of anticipated take is not likely to jeopardize the continued existence of the rusty patched bumble bee.

REASONABLE AND PRUDENT MEASURES

The Service believes the following reasonable and prudent measures (RPMs) are necessary and appropriate to minimize impacts of incidental take of the rusty patched bumble bee:

1. The Service will minimize stress and risk of mortality while meeting research objectives needed to advance the recovery of the rusty patched bumble bee by reviewing survey and handling protocols and revising them annually, as needed.

2. The Service will ensure the number of rusty patched bumble bees accidentally killed by Federal recovery permit holders does not exceed the number evaluated in this Biological Opinion. In addition, any rusty patched bumble bees found dead or killed will be salvaged to retain scientific information that may be obtained from the specimen.

TERMS AND CONDITIONS

In order to be exempt from the prohibition of section 9 of the Act, the Service must comply with the following terms and conditions, which implement the RPMs described above and outline required reporting/monitoring requirements. These terms and conditions are non-discretionary.

1. Terms and Conditions to implement RPM 1:

   The Region 3 lead recovery biologist for the rusty patched bumble bee will update the Survey Protocol on the website annually by February 28th of each year (if needed). The lead biologist will notify the recovery permits coordinator for each Service region that overlaps with the species' historical range when the updated Survey Protocol are posted to the Internet. If no updates to the Survey Protocol are needed in any year because the existing protocols are sufficient to minimize the likelihood of adverse effects and incidental take, the lead biologist will notify the regional permits coordinator that the Survey Protocol will remain unchanged for that year.

2. Terms and Conditions to implement RPM 2:

   A. The lead recovery biologist for the rusty patched bumble bee will ensure research objectives for the recovery of the species are being met by ensuring all permittees file an annual report with the Service by January 31. These reports will detail activities carried out under the section 10(a)(1)(A) permit during the previous calendar year. The annual reports shall include:

a. A description of locations (date, time, geographic locations (state, county, and geographic coordinates using latitude and longitude in decimal degrees) of areas surveyed, even if no rusty patched bumble bees were encountered.
b. The date, time, geographic locations (state, county, and geographic coordinates using latitude and longitude in decimal degrees) of any rusty patched bumble bees encountered.
c. A description of the habitat conditions where the surveys took place (*e.g.*, floral diversity and abundance, management practices and potential stressors).
d. A description of field procedures, data collection methods, and survey effort.
e. Photographs of the identifying characteristics for each individual federally-listed species.
f. A complete description of injuries or mortalities to listed species while in a surveyor's possession, the dates of occurrence, any circumstances surrounding the incidents, and a description of any steps taken to reduce the likelihood that such injuries and/or mortalities will occur in the future.
g. Any other data a surveyor/researcher may have collected for individual rusty patched bumble bees, such as evidence of damage or injury, mortalities, locations where salvaged specimens are being kept, results of DNA analysis, results of pollen analysis, results of tagging studies, results of pathogen studies, results of nest studies, etc.
h. Copies of any separate reports and/or publications resulting from work conducted under the authority of a recovery permit.
i. Copies of all site specific authorization letters.

B. The Service will ensure the number of post-release harm and mortality due to tagging activities does not exceed the number evaluated as a surrogate to mortalities in this BO, by ensuring all permittees periodically submit the number of individual bees tagged and released to the Service. Permittees should submit numbers of tagged rusty patched bumble bees (by location and tagging date) by June 30th, July 31st, and August 31st of each year. Permittees shall report the numbers to Tamara_Smith@fws.gov, including point locations, tagging date, tag type, unique tag ID (if applicable), and an enumeration of the number of tagged bees. Spreadsheet format is preferred.

C. The Region 3 recovery permits coordinator will require federal recovery permit holders to contact the Service within 48 hours in the event that their activities result in the mortality of a rusty patched bumble bee. Following the mortality of more than one rusty patched bumble bee permittees may not resume activities authorized by their recovery permit without written permission of the U.S. Fish and Wildlife Service, Regional office in Bloomington, Minnesota.

D. The Service will monitor the mortality of rusty patched bumble bees (as events are reported) in conjunction with the issuance of section 10(a)(1)(A) permits and will track Section 10(a)(1)(A) permit accidental take with the Service's Permits Issuance Tracking

System (SPITS) and compare this amount to the level of accidental take evaluated in this biological opinion (BO).

E. The Service will ensure the number of accidental mortalities does not exceed the number evaluated in this BO by conferring with permittees who report bumble bee mortalities and comparing total mortality levels reported to date for the year with the level of accidental take evaluated in the BO before approving re-initiation of activities by surveyors reporting mortalities. To ensure that incidental take levels are not exceeded, the Service may deny the permittee's request to resume activities, or consider modifying survey protocols and amend all permits that have been issued, or revoke permits if necessary.

E. To retain scientific information that may be obtained from dead specimens, the Region 3 recovery permits coordinator will require Federal recovery permit holders to salvage any rusty patched bumble bees that are found dead or killed accidentally. Permit holders will preserve and ship the specimen(s) to the Service's Minnesota/Wisconsin Ecological Services Field Office according to the procedures outlined in the Survey Protocol or according to procedures outlined within permit conditions. Specifically,

All dead specimens shall be preserved in 70-95% ethanol (EtOH: not isopropyl alcohol) and placed in a small vial upon collection (dried specimens preserved using standard museum practices are also acceptable) and sent to the USDA ARS Bee Laboratory in Logan, Utah, for analysis. Double bag the vial and ship in a strong outer container with enough absorbent material to soak up any ethanol that might accidentally leak. Include a copy of permit(s) under which the sample(s) was collected when shipping vials. Label the outer shipping box "Samples. This package conforms to 49 CFR 173.4". Please notify the USDA Bee Lab prior to shipping specimens. All specimens should include proper identification and indices [include permit number, date, complete scientific and common names, and geographic location (township, range, section, and UTM) where salvaged]. When agreed upon in writing by the Minnesota/Wisconsin Ecological Services Field Office, specimens may be sent out of state to an alternate specialized educational facility or museum within the United States.

The reasonable and prudent measures, with their implementing terms and conditions, and the associated Survey Protocol are designed to minimize the impact of incidental take that might otherwise result from the proposed action. If, during the course of the action, or with substantive updates to the Survey Protocol, the levels of incidental take described in this biological opinion/conference opinion are exceeded, such incidental take represents new information requiring reinitiation of consultation and review of the reasonable and prudent measures

provided. The Service must immediately provide an explanation of the causes of the taking and review the need for possible modification of the reasonable and prudent measures.

Conservation Recommendations

Section 7(a)(l) of the Act directs Federal agencies to utilize their authorities to further the purposes of the Act by carrying out conservation programs for the benefit of endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help implement recovery plans, or to develop information.

We have not identified actions the Service could take, on a programmatic basis, to address section 7(a)(l) that are not already a part of its normally mandated mission. The Service will, however, evaluate individual permit proposals to identify opportunities to facilitate recovery of affected species.

REINITIATION-CLOSING

This concludes formal consultation on the action outlined in the opening paragraph. As provided in 50 CFR 402.16, reinitiation of formal consultation is required where discretionary Federal agency involvement or control over the action has been retained (or is authorized by law) and if: (1) the amount or extent of the incidental take levels described in this biological opinion is exceeded; (2) new information reveals effects of the Service's action that may affect listed species or critical habitat in a manner or to an extent not considered in this opinion; (3) the Service's action is subsequently modified in a manner that causes an effect to the listed species or critical habitat not considered in this opinion; or (4) a new species is listed or critical habitat designated that may be affected by the action. In instances where the amount or extent of incidental take is exceeded, any operations causing such take must cease pending reinitiation.

If you have any questions, please contact Pete Fasbender or his staff at: (952) 252-0092.

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