ASSESSING NEW METHODS FOR PSITTACINE CONSERVATION AT THE CAPTIVE-WILD INTERFACE

A Dissertation

by

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ABSTRACT

This dissertation overviews novel methods related to breed and release of parrots for conservation. Broadly, the document discusses research about the maintenance of captive breeding parrots, and the preparation of their offspring for release into the wild. A conclusion chapter identifies conflicts common to this type of conservation work and discusses ways to create research projects that avoid them. The maintenance chapter, focuses on reducing the fear and chronic stress in captivity through the development and use of novel automated, computer tablet-based technology. The release preparation chapter, overviews a method training parrots to fly safely in wild areas that is derived from the parrot free-flight hobby community, using systematic exposure to outdoor environments. The conclusion is a reflection of lessons learned. The interdisciplinary nature of this work, bridging captivity, wild, and human dimensions, creates complications for the researcher who must bridge these disparate worlds.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor Donald J. Brightsmith of the Department of Veterinary pathobiology, Professor Walter E. Cook of the Department of Veterinary Pathobiology, Professor Rosemary L. Walzem of the Department of Poultry Science, and Professor Sharman Hoppes of the Veterinary Medical Teaching Hospital.

The data collection and editing in Appendix A included Amelia Min-Venditti, who is a published co-authors on the published version of the manuscript. The data collection in Chapter 2 included Jane Varkey.

All other work conducted for the thesis (or) dissertation was completed by the student independently.

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1. INTRODUCTION: DEFINING THE CAPTIVE-WILD INTERFACE

1.1. Reintroduction is an important tool

As species becomes extinct due to human activities, tools to preserve and restore biodiversity are increasingly important. These tools can be passive where the ecosystem if left alone, such as preservation of intact habitats and stopping human of impacted habitats so species can re-populate. These tools can also be active, where the ecosystem is intensively managed for recovery, such as replanting schemes, or the addition of animals. Reintroduction is the reinforcement of waning populations or total re-creation of extirpated populations of animals (Seddon 2010; Ewen, Armstrong, Parker, & Seddon, 2012.) When the total number of animals are low, there are not enough wild individuals available for reintroduction activities, where wild animals are translocated. In such a situation, reintroduction is only possible from captive bred stocks.

1.2. Many species are in need of captive breeding and release

Established in 1964 The International Union for Conservation of Natures Red List of Threatened Species (IUCN, 2020) has become the most complete database of species' conservation needs. While only a partial list, as many species are data-deficient, this database identifies species that need f captive breeding for conservation. Currently, the list identifies 13,843 species in need of captive breeding. Of these, 2,652 are animals, 256 are birds, and 49 are parrots. Within the parrots, 28% face extinction based on a study of 398 species (Olah et al, 2016), with the neotropical parrots bearing a larger proportion of extinction risk, 38% of 95 species (Berkunsky et al, 2017).

1.3. Reintroduction is a complex, multidisciplinary science

The tools to artificially repopulate diminished, or extinct populations are reintroduction and translocation (Seddon, 2010). Projects where humans release animals are often unsuccessful. In some species, survival rates when humans release wild animals can be as low at 3%, reflecting a lack of adequate technique and knowledge (Teixeira, De Azevedo, Mendl, Cipreste, & Young, 2007). Related to parrot reintroductions, multiple domains of knowledge have been identified as poorly explored and needing research (White et al, 2021). Participants in reintroduction projects can include scientists from multiple relevant backgrounds, land managers, government officials, commercial animal breeders, and veterinarians. Increasingly, academia is recognizing that the stakeholders of successful reintroduction projects and research may include non-scientific actors (Lebov et al, 2017).

1.4. Captive breeding and reintroduction is a young science

Those concerned about imperiled species have taken action to save the species they care for prior to the 1960s. Early projects were not part of a formalized science. For example, the captive Goodnight bison herd (Bos bison) in the 1800s was a major contributor to the recovery of the species. However, early intentional hybridization with domestic cattle has left lingering cattle genetics in conservation bison herds (Hedrick, 2009). Multiple early attempts to reproduce the NeNe goose in the first half of the 1900s failed (Smith, 1952). In contrast to early programs, formalized modern conservation activities involved more rigorous research into best practices and long-term plans for diversity and species survival. These modern programs began in the 1960s. The NeNe goose (*Branta sandvicensis*) releases began in 1960 (Kear & Berger, 2010), the first peregrine falcon (*Falco peregrinus*) conference occurred in 1965 leading to subsequent breed and release efforts (Cade, 1988). The first whooping crane (*Grus americana*) captive hatch occurred in 1967 (Erickson & Derrickson, 1981). The newness of reintroduction as a science, combined with the wide variety of species that require immediate action to conserve, causes researchers to struggle with drawing conclusions from limited data (Seiler, Angelstam, & Bergmann, 2000; White et al., 2012). New data and techniques are needed to improve reintroduction and captive breeding.

1.5. Reintroduction process

The captive breeding and release cycle can be thought of as having four steps (Figure1.1). Adults are maintained in captivity, then bred to produce genetically diverse and disease-free offspring. The offspring are placed in the correct conditions to prepare them for release or to prepare them for continued captive care. Rearing strategies are important, as behavior of young captive birds can be considered “plastic” and their end behavioral outcomes depend on early life experiences (Mason et al 2013). The fates of the young animals diverge, some going on to release and others creating a sustainable captive population.

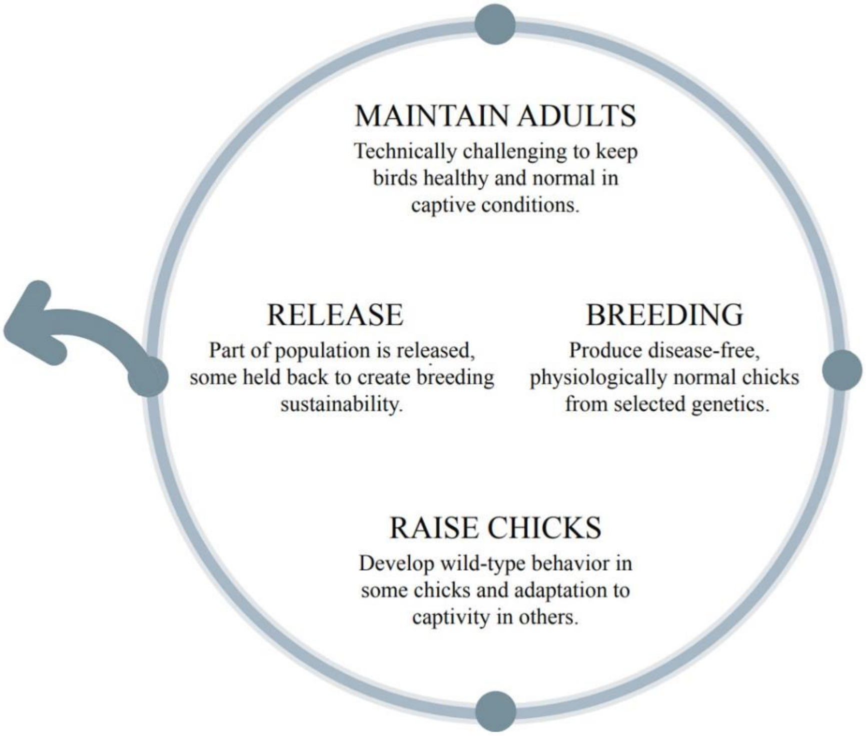

Figure 1.1 A captive breeding cycle relevant to birds, consisting of four steps. Captive birds are bred in a sustainable manner to ensure a viable captive population. Differential preparation of offspring prepares them to stay captive or to be wild. Appropriately reared birds are released and others are held back.

This dissertation will focus on two of these four steps. First, the maintenance of adults in captivity. The research asks if automated training can cause birds to be calmer in the presence of new staff. The work is related to Applied Animal Behavior and Animal Welfare. Due to the need for human labor in the care of animals, there are also consideration of economics. Conservation funding does not keep pace with growing needs (Echols, Front & Cummins, 2019). The broader implications of the experiment examine how much cost-savings could be associated with automated animal behavior modification. The results suggest an effect of the automation technology on one of four target behaviors, as well as a 97% reduction in costs.

The second chapter focuses on preparation of individual birds to create wildland survival behaviors, examining a method that has previously entered the literature. This study is in the area of Conservation Biology. The method is utilized by a subset of pet parrot enthusiasts who fly their birds in wildlands, using a particular method to increase survival of their birds when faced with predators. The paper examines the outcomes of 37 parrots under the training of Mr. Chris Biro, who created the studied method. The outcomes appear relevant to parrot reintroduction, based on areas identified as needing improvement (White et al, 2021).

The final chapter examines the difficulties and conflict inherent in my research which crosses multiple disciplines while working with non-scientists and commercial groups. The concluding chapter examines lessons learned and identifies solutions to conflict and ways to move forward. The appendix matter reflects my broader work including a paper on the issues of sustaining ecotourism in a system that protects reintroduced birds, as well a short report on validation of the automated training system.

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2. AUTOMATED TRAINING AS A MECHANISM TO REDUCE NEOPHOBIC RESPONSES IN CAPTIVE ANIMALS

2.1. Introduction

2.1.1. Neophobia causes chronic stress in captive animals

Neophobia is fear caused by new experiences (Mettke-Hofmann, Winkler, & Leisler, 2002). Neophobia in birds can be induced through human proximity (Seferta, Guay, Marzinotto, & Lefebvre, 2001). High staff turnover, seasonal workers, and semester internships at animal facilities can cause animals to repeatedly contend with unfamiliar humans. Due to their neophobic nature, birds will be wary of unfamiliar people, leading to undesirable behavioral change (Seferta, Guay, Marzinotto, & Lefebvre, 2001).

Animal fear and predator escape have long been the model for studying stress in captive animals, with a focus on models of sustained psychological stress, a chronic stress similar to post-traumatic stress disorder in humans (Clinchy, Sheriff, & Zanette, 2013). The long-term negative effects of stress on animals, and risk of stress biasing experiments, is well-understood. Animal stress can cause disease pathologies (Moberg, 2000) such as immune system dysfunction, (Maxwell, 1993; Martin, 2009) or disrupted brain and behavioral development, (Mason, 2010), even in short-term handling and restraint of birds (Berzins, Tilman-Schindel & Burnes, 2008). Within animal laboratories, husbandry related stress has consequences on research quality, creating confounded data and contradictions across study results (Balcombe, Barnard, & Sandusky, 2004; Reinhardt, 2004; Strekalova, Spanagel, Dolgov, & Bartsch, 2005).

If an unfamiliar human is a cause of stress, a negative first-time experience for an animal can permanently create fear and avoidance (Grandin, 2000). Due to neophobia, it is unlikely that a neophobic has an experience other than a fear response to a new person. If the human regularly interacts with the animal, then the animal will be subjected to chronic stress through repeated need to actively fight, flee, or passively flee and hide (Gray & McNaughton, 2000; Rupia, Binning, Roche, & Lu, 2016) in response to the human stressor. The problem of chronic stress caused by neophobia is compounded as fight, flight, or hiding is not possible when the animal is in a cage.

2.1.2. How caged animals cope with fear

Fear interrupts animals' behavior. When animals chronically cannot express their natural behaviors, undesirable changes in behavior often emerge as coping mechanisms. Animals that have their internal drives activated and thwarted (Bastock, Morris, & Moynihan, 1953) are believed to express behaviors that are undesirable in captivity for multiple reasons: (1) to reduce stress and allow the animal to better function, (2) to express an aggressive state more safely, (3) express the aggression when the object of aggression in unobtainable, as summarized by Waas, Innes, & Morgan (2007).

One type of response to thwarting includes displaced aggression (Miller & Pollock, 2007), a concept originating in human psychology. The animals' physiological systems that lead to fighting are aroused but the target is out of reach, so the nearest available animal is attacked instead. Another hypothesis is redirected aggression (e.g., Chapman & Voith 1990), a veterinary medicine concept where a drive unrelated to attacking is aroused but due to an inability to act on the drive the animal expresses a behavior seemingly not appropriate to its internal state. In the redirected aggression case, if the cage set up does not allow the animal to successfully escape from the approaching human, the bird may switch to mate attacking through redirected aggression.

For captive wild birds, intraspecific mate aggression is common. Historically, captive cagemate killing and mate aggression is a welfare issue (Fox, 1923; Evans, 1953). Some birds respond poorly to the presence of caregivers, attacking mates, killing young, and destroying eggs (AZA, 2010).

2.1.3. Captive care techniques and neophobia

A veterinary recommendation to reduce the undesirable fear-associated behaviors in parrots is to minimize the environmental stressors, such as minimizing caregiver presence (Frey, 1998), which may not be possible during required minimum care. When it is not possible to remove the caregiver, it may be possible to train the birds to accept specific individuals. Animals respond differently to unfamiliar verses familiar human beings. Captive apes will modify their behavior based on how familiar they are with a given human being (Pedersen, Sorensen, lupo & Marx, 2019). Similarly, birds can discriminate between familiar and unfamiliar people (Belguermi et al., 2011; Cibulski, Wascher, Weiß, & Kotrschal, 2014; Cornell, Marzluff, & Pecoraro, 2012) and can identify if someone is familiar or unfamiliar when looking at a digital image on a computer screen (Stephan, Wilkinson, & Huber, 2012). In a flock setting, an individual's behavior is changed by observing group members responses. For example, crows used "horizontal social learning" to gain information about fear of humans from their social group (Cornell, Marzluff & Pecoraro., 2012). Familiarity may be a component in reducing the high intensity responses of the fight or flight system. Captive storks discriminated between humans and acted aggressively in the presence of an unfamiliar person (Shannon, 1987).

Multiple behavior modification strategies exist to transform a fear or aggression response to a neutral response, or even modify the animal's behavior such that the animal desires the previously fear-inducing stimulus (Ramirez, 1999, pp 137). Counter-conditioning is commonly used to reduce fear responses by activating a conflicting appetitive response that eventually replaces the fear response to the presented stimuli (Keller, Hennings, Dunsmoor, 2020). Unfortunately, the time involved in husbandry may be too intensive to regularly carry out, requiring unaffordable hours of labor. For example, Grandin's medical husbandry training for a voluntary antelope blood draw took 118 days (Grandin, 2000).

The parrot species make up 50 of the 250 bird species in need of captive breeding (IUCN 2020). Studying stress reduction in parrots has utility for creating captive conservation tools. Self-harm associated with fear and stress is common in the taxon (Costa et al, 2016). Parrots' displaced aggression in response to a human is frequently encountered (Welle & Luescher, 2006; Romagnano, 2006). For example, the Philippine cockatoo is critically endangered and subject to *ex situ* captive conservation breeding (BirdLife International, 2017), it has been identified as a species especially prone to mate killing in captivity (Romagnano, 2006; Frey, 1998).

We used a digital enrichment system that paired video with a reward, to associate the image on the video with a desired experience. We hypothesized that using video training to become familiar with a face prior to interacting with a new person would lead to reduced neophobic responses.

2.2. Methods

2.2.1. Study species

Our study included 66 Rose-ringed parakeets (*Psittacula krameri*), housed in 33 opposite-sex pairs. The parakeets were mature individuals two years old or older, displaying a variety of color mutations. Birds with red-eyed albinism were not utilized because albino birds often have vision issues that may confound results (Balkema & Dräger, 1991).

2.2.2. Justification for animal model

Within human-animal relationship research, there is a need for more research on non-domesticated animals due to research bias toward domesticated animals (Hosey, 2008). Rose-ringed parakeets are common, allowing for experimental access and replication. They are both popular pets worldwide and have become cosmopolitan through naturalized populations in 51 countries and islands (Desmet et al, 2020).

Aggression across Rose-ringed parakeets' sexes are more comparable than in other species. Uncommonly for parrots, both the male and female of the species will similarly engage in mate aggression in captivity (Romagnano, 2006) reducing the need to study male and female responses separately.

Rose-ringed parakeets behave similarly to several closely related endangered species, making them a useful model species for both pet and conservation breeding programs. Two congeners of Rose-ringed parakeets are threatened with extinction (echo parakeet, (*Psittacula eques*), and long-tailed parakeet, (*Psittacula longicauda*) and six other congeners are near-threatened. Several members of the genus are subject to *ex situ* conservation breeding (BirdLife International 2018, BirdLife International 2019). Information derived from rose-ringed parakeets can likely be directly applied to captive conservation management of its near relatives.

2.2.3. Study site and husbandry

The study was conducted from June 1, 2016 to July 28, 2016 at a commercial bird breeding aviary located outside of Austin, TX. The facility breeds birds as part of commercial and conservation activities. All birds were property of the aviary. The 66 experimental birds were part of a larger colony of ~500 birds held in a wire-mesh sided pole-barn building. The male-female pairs of rose-ringed parakeets were in a total of 33 flight cages arranged among six aisles. Each pair's cage was 0.6m wide by 1.2m tall by 1.2 meters deep. Birds in the experiment were randomly selected and scattered throughout the barn. To ensure that the birds in experimental cages could not see other birds in the experiment, experimental cages were either > 6 cages apart or separated by a corrugated, opaque plastic divider, hand cut to extend more than 15 centimeters beyond the cage wire on each side.

Birds were fed daily a "chop" diet with a sprouted seed, diced vegetables, and commercial pellet base. The diet was provided in a deep dish to encourage digging and searching as environmental enrichment. Birds were given clean water daily. Birds received novel items in the diet as a form of edible enrichment, i.e., fruit slices, or celery leaves. The birds had access to nest boxes that they used for breeding earlier in the year. JV and CJW began daily care of birds one month prior to the experiment so researcher presence would not affect the animals.

2.2.4. Training Technology

The technology consisted of a tablet, connected to a dispenser, both running on batteries (figure 2.1). The tablet was mounted in bird-proof housing inside the cage while the dispenser was mounted outside the cage. Chopped peanuts reached the birds through a clear plastic funnel, hung from the dispenser. The tablet software was set to play a video and dispense items at set times. Each cage was assigned to one of three treatment groups, containing 11 cages each.. Treatments were a one-minute portrait video of novel person “A” waving and moving, novel person “B” waving and moving in the same sequence, or a camera pan and zoom around a novel pink plastic tray.

Figure 2.1 Tablet and delivery system equipment setup in caging.
Rose-ringed parakeets at a commercial aviary near Austin, Texas. Birds are visually separated with an opaque barrier between cages. The black tablets, mounted inside the cage in bird-proof boxes, connect to the maroon colored delivery device. A plastic funnel (pulled out for photograph, rightmost cage), delivers items from the tablet system into the food dish. The birds' nest boxes are visible on the front of the caging.

2.2.5. Experimental procedure

The procedure involved recording an initial mock inspection of each cage by A and B, followed by hardware installation and 21 days of video training treatment, and then follow up inspections by A and B.

Birds were randomly assigned to one of three treatments and given a dedicated tablet for their cage. Video assignment per cage was hidden from JV to facilitate a double-blind study. The tablets were removed and charged during daily feeding for use the next morning. Tablets were turned on by JV or CW at 9:00 a.m. The caretaker then left and did not return until 12:30pm. From 10:00 a.m. to 12:00 p.m., once every 15 minutes, the tablet system played the one-minute video and halfway through the video dispensed a small portion of chopped peanuts (~2 grams of peanuts). Eeach cage displayed fourteen video sessions per day.

This training was repeated daily for 21 days (study days 4 to 24). On day 24, tablets were removed after 12:00 p.m. The morning of day 25, person “B” repeated the one-minute cage visits to each cage following the protocol outlined above. On the morning of day 26 person “A” repeated the one-minute cage visits to each cage following the protocol outlined above.

2.2.5.1. Inspections

On day one, person “B” walked up and stood in front of each of the 33 experimental cages for one minute. During these cage visits the person was instructed to hold still, show a neutral facial expression, and look at the back of the cage simulating a cage inspection. A known caregiver (author JV) followed quietly behind person B and recorded video. On day two, person “A” conducted cage visits following the same procedure. Both sets of cage visits occurred during the mid-morning between 9am and

11:30am. Both person A and B wore similar clothing to each other in their videos and during both walkthroughs. JV wore the same clothes each day. No notable events, such as weather change, grounds maintenance, or differences in care occurred during these two days that would lead to a behavior change.

2.2.5.2. Initial installation

On day three, the technology was attached to all 33 cages by JV and CJW. To check if equipment presence affected birds, a peanut was placed in the normal food bowl under the tablet holder immediately after technology installation. After two hours, all peanuts had been consumed, indicating that birds were willing to visit their feeding stations. During the duration of the study, the birds were carefully observed during their daily care by JV to check that the experimental birds and the birds not included in the study showed similar behaviors.

2.2.6. Video analysis

Videos of the two pre-treatment cage visits and two post-visit cage visits by person A and B were scored for behavior (Table 2.1) by JV, AR and a student assistant, playing the videos at half-speed using the VLC player (videolan.org). Scorers were blind to the treatment assignments of the cages.

Table 2.1 Ethogram for behaviors recorded in this study

Behavior	Description
Alarm call	A single shrill vocalization, counted in events.
Flight	Both feet leave perch or wire while wings are flapping, counted in events.
Mate attack	Open-mouthed lunge that contacts the cagemate, counted in events.
Wire cling	Still, both feet grasping the cage wire, counted in seconds.

To check for accuracy of scoring, CJW re-scored 14% of all data, which included all the mate aggression data. To determine agreement levels between scorers, we calculated the intraclass correlation coefficient (Shrout & Fleiss, 1971), using the IRR function in the Pysch package for R (Revelle, 2019). The ICC3 test type, two-way mixed-effects model, compared pooled student rater scores (n=72) compared to CJW scores (n=72) across four categories. ICC scores for each behavior category of 18 samples were mate attack=1; flights=1; alarm calls= 0.99; wire cling=0.71. Alarm call, flights, and mate attack scores showed excellent agreement (ICC >0.90) while wire cling showed moderate agreement (ICC between 0.50 and 0.75, scoring criteria from Koo & Mi 2016).

2.2.7. Statistical analysis

2.2.7.1. Software

In order to understand what data were comparable and whether effects of the treatment could be detected, analyses were conducted in R, version 3.6.10 (R Core Team, 2020). For examining treatment effects, we utilized mixed linear models, the lmer function from the LME4 package (Bates et al., 2013). Behavior correlations used the Spearman method of the rcorr function of the Hmisc package, version 4.4-0 (Harrell, 2020). Spearman's method is appropriate for non-normal data.

2.2.7.2. Effects of social caging

To determine if birds in social housing could be considered independent of each other, or needed to be grouped by cage, we combined the random effects of cage and bird ID into one model. Whichever random effect came first explained all the variance between cages or birds. There was no effect of cage over and above the effect of the bird's individual ID. While it would be expected that cage would have some kind of effect, with only two birds per cage, if effects were present they were too small to detect. Based on this, birds were treated as independent individuals. Any time a treatment effect was significant we re-ran the data at the cage level as a biologically and statistically conservative approach as it lowered the degrees of freedom, even though the explorations of the data suggested that there was no additional variability at the level of the cage.

2.2.7.3. Comparability of data, sexes, and treatment groups

Visual review of histograms of the data showed non-normal distribution of behavior data. A count of zero behavior, made up approximately 30% of alarm data, 40% of cling data, 50% of flight data, and 90% of attack data. Percents of zeros were similar between time 1 and time 2, within 5 % for each category. When considering initial comparisons of means, the zeros in flight and attacks and flights made the Mann-Whitney U non-parametric t-test equivalent a less appropriate model as it is particularly sensitive to zero inflation (McElduff, Cortina-Borja, Chan, & Wade, 2010), and multiple samples per animal violated regression model assumptions. For simplicity, t-tests were utilized. For comparing bird responses between person A and B at time 1, paired t-test of initial behaviors was utilized (table 3.2). For comparisons between male and female birds, Welch’s t-test was utilized as it is more robust against unequal variances than student’s t-test (table 3.3). Time 1 behavior data was similar both between sexes and people.

Table 2.2, Paired t-test for differential response to humans A and B in pre-treatment behavior.
Behaviors recorded in n=66 rose-ringed parakeets in n=33 male-female caged pairs. Birds responded with two novel humans, each present for a one-minute duration. Mean (x̄) and standard deviation (σ) are described for each behavior. P-value is for the paired t-test, comparing behavior responses to two humans. Zeros in flight and mate attack data may have biased test results. Birds responded similarly to both person A and B.

	Flight	Alarm	Mate attacks	Cling
Person A x̄	2.83	21.76	0.46	22.12
Person A σ	4.94	4.38	1.46	25.90
Person B x̄	3.03	19.88	0.27	22.55
t-statistic	-0.33	0.58	1.22	-0.10
p-value	0.74	0.56	0.23	0.92

Table 2.3 Welch's t-test tests for sex-based differences in pre-treatment behavior. Descriptions of behaviors recorded in n=66 rose-ringed parakeets in n=33 male-female caged pairs checking if female and male behavior was comparable. Birds were challenged with two novel humans, for two minutes of behavior recording per bird. Mean (x̄) and standard deviation (σ) are described for each behavior.

	Flights	Alarm calls	Wire cling	Mate attacks
Behavior count	387	2748	2948	48
Female x̄	2.88	21.17	22.36	0.23
Female σ	5.246	38.723	25.183	0.86
Male x̄	2.985	20.788	22.303	0.50
Male σ	4.773	26.422	25.29	2.10
T- statistic	-0.12	0.07	0.014	0.16
P-value	0.90	0.95	0.99	0.33

2.2.7.4. Correlations of behavior

To understand if all four behaviors were equal in their intensity, the four categories of behaviors of each bird were correlated for time 1, time 2, and amount of change (time 2 minus time 1). The amount of relatedness of each behavior could show if the birds' have equal increase or decrease in the fear-associated behaviors.

2.2.7.5. Effects of treatment factor, face versus object

To understand if training on video of faces versus an object had an effect, mixed linear models to were used. Birds trained on a human face video (two treatment groups, n=44 birds in n=22 cages) and birds trained on an inanimate object video (one treatment group, n=22 birds in n=11 cages) were examined. This model ignored if the birds saw a familiar or unfamiliar person during the time two inspections. Models for this set were in the form of Time 2 behavior ~ face or object video + sex of bird + time 1 behavior + (bird ID). There are repeated measures of the same bird so the bird's ID is treated as a random effect. Histograms of residuals for all models were visually normal, suggesting the mixed linear model type was a good fit for the data.

2.2.7.6. Modelling familiarity as a predictor of behavior

To examine if any factor predicted change in behavior, a mixed linear model examined birds trained to be familiar with a person (n=44 birds in n=22 cages). Models were run for each of the four measured behaviors, in the form of time 1 behavior ~ time 2 behavior + sex of bird + familiarity + (bird ID). There are repeated measures of the same bird so the bird’s ID is treated as a random effect. Histograms of residuals for all mixed linear model models were visually normal, suggesting the mixed linear model type was a good fit for the data.

2.3. Results

2.3.1. Behavior outcomes

We recorded a total of 6,526 behavior events (flights, alarms, and mate attacks combined) and 5,690 seconds of birds clinging to the cage wires. Time 1 had 49% of events and 52% of seconds. Time 2 had 51% events and 48% of seconds. The behaviors displayed by individual birds was variable. Scores from cage inspections varied, individual bird’s score scores ranged from zeros in all categories, to a very active bird that alarm called 112 times, flew nine times, clung to the wire for 14 seconds, and attacked her mate 4 times. The vast majority of birds displayed fear-associated behaviors. During time 1 and 2, most walkthroughs featured more than one category of behavior (time 1, zero behaviors n=10, one behavior n=38, two behaviors n=44, three behaviors n=38, four behaviors n=2; time 2, zero behaviors n=14, one behavior n=34, two behaviors n=51, three behaviors n=29, four behaviors n=4).

2.3.2. Treatment effects

Time 1 behavior and familiarity significantly influenced the model outcomes, while birds’ sex had no impact. For all four behavior categories, time 2 behaviors were significantly predicted by time 1 behavior (p>0.2), presented in Table 2.4. For three of the categories of behaviors (alarm, flight and cling) the treatment did not significantly influence the post treatment behavior of the birds.

The only behavior that showed statistically significant change related to the treatment was mate attack (p=0.015). In the model, mate attack decreased for those birds that became familiar through video training, with the person who inspected their cage. No other behavior was predicted by familiarity. Thirteen of 66 birds (20%) engaged in mate attacks and these attacks occurred in 8 of 33 cages (24%). Mate attacks occurred as reciprocal during 5 cage inspections and 6 cage inspections were one-way events. Of the birds who attacked their mates, 6 were female and 7 were male.

Table 2.4 Linear models to explain behavior change
All fear-associated behaviors during time 2 were strongly predicted by behavior during time 1. Familiarity with a face was predicted to reduce mate attack, the only significant treatment effect. (β) are the estimates of the fixed effects and (SE) is the standard error of mean. All models are controlling for individual as a random effect due to multiple samples per individual.

Response variable	Explanatory variable	β ± SE	t(df)	p
Time 2 alarm	Time 1 alarm	0.80 ± 0.047	16.99(95)	<0.0001
	Familiarity	0.23 ± 2.93	0.078(94)	0.94
	Sex	1.78 ± 3.19	0.56(63)	0.58
Time 2 flight	Time 1 flight	0.36 ± 0.043	8.25(128)	<0.0001
	Familiarity	-0.29 ± 0.46	-0.64 (128)	0.52
	Sex	0.40 ± 0.43	2.53(128)	0.35
Time 2 wire cling	Time 1 wire cling	0.42 ± 0.074	5.72 (123)	<0.0001
	Familiarity	-4.77 ± 3.63	-1.31 (82)	0.19
	Sex	0.33 ± 4.16	0.079(60)	0.94
Time 2 mate attacks	Time 1 mate attacks	0.38 ± 0.036	10.71(128)	<0.0001
	Familiarity	-0.30 ± 0.12	-2.46(128)	0.015
	Sex	-0.01 ± 0.11	-0.12(128)	0.90

2.3.3. Effects of face video training without familiarity

The effects of face-based video training versus object did not predict change in behavior (Table 2.5). The p-value for face versus object training explaining mate attack was close to one (p=0.76), suggesting that training on a human face, in general, did not have an affect upon fear-associated behavior. Similar to the previous analysis, time 1 behaviors were significant in influencing time 2, while sex of the bird had no significant effect on the model.

Table 2.5 Training type, face or object, as a predictor.
All behaviors during time 2 were strongly predicted by behavior during time 1. Training on either face video versus the object video did not influence the model. (β) are the estimates of the fixed effects and (SE) is the standard error of the mean. All models are controlling for the individual as a random effect due to multiple samples per individual.

Response variable	Explanatory variable	β ± SE	t(df)	p
Time 2 alarm	Time 1 alarm	0.80 ± 0.047	16.88(95)	<0.0001
	Training type	-0.34 ± 3.41	-0.099(62)	0.9213
	Sex	1.78 ± 3.21	0.554(62)	0.5818
Time 2 flight	Time 1 flights	0.36 ± 0.04	8.19(128)	<0.0001
	Training type	0.14 ± 0.46	0.31(128)	0.75
	Sex	0.40 ± 0.43	0.93(128)	0.35
Time 2 wire cling	Time 1 wire cling	0.40 ± 0.075	5.40 (120)	<0.0001
	Training type	7.49 ± 4.61	1.63(67)	0.11
	Sex	0.33 ± 4.16	0.079(60)	0.94
Time 2 mate attacks	Time 1 mate attacks	0.38 ± 0.037	8.19(128)	<0.0001
	Training type	0.038 ± 0.12	0.31(128)	0.76
.	Sex	-0.012 ± 0.12	0.93(128)	0.92

2.3.4. Familiarity as a predictor of mate attack

The treatment of becoming familiar with a person from watching a video was predicted to significantly reduce mate attack. Outcomes remained significant when data was combined at the cage level with the cage as the random effect (linear mixed model: t= -2.06 df=63 p=0.043). Mate attacks clustered in treatment group A, with fewer observations in other groups (Table 2.6), went from 24 to zero mate attacks after training. Birds from treatment group A that were not familiar with person B showed a similar frequency of attack and attacking individuals across time. Non-familiarity, having become familiar with a different person than that present, did not appear to increase mate attack. The time 2 cessation of mate attacks in treatment group A birds trained on person A, drove the decrease of mate attack in the linear model (Table 2.4).

Table 2.6 Mate attack totals
Each treatment group contained n=22 birds in n=11 cages. During time 1, each birdcage was inspected by person A and person B, and the birds' behavior recorded during the inspection. The birds were randomly split into three groups and given a video-based learning treatment for 21 days that featured person A, person B, or a plastic tray object, then inspected again by person A and person B. By chance, most mate attacks occurred in the treatment A grouping. Noted in bold is the cessation of all mate attacks in treatment A in response to person A. The mate attacks in response to person B, who was not on screen in the A video treatment group, remained similar between time 1 and 2.

		Pre		Post	
Treatment	Person	Mate attacks	Attackers	Mate attacks	Attackers
A video	A	**24**	6	**0**	0
	B	**16**	3	**15**	3
B video	A	2	1	0	0
	B	0	0	1	1
Object	A	4	1	0	0
	B	2	1	4	3

2.3.5. Correlation of behavior

Correlation of the four behavior categories revealed a significant positive correlation of alarm calls and flights per bird pre and post-treatment (Pre-treatment: r=0.30 p= 0.0004. Post-treatment r=0.33 p<0.0001). No other behavior correlated as strongly nor met the 0.05 p-value significance threshold. The change in behaviors, time 1 minus time 2, showed no significant correlations between categories.

2.3.6. Behavior similarity across the sexes

When preliminarily comparing male and female data at time 1, no behaviors showed a significant difference between the sexes (table 3.2), suggesting that male and female birds showed similar stress-associated behavior frequencies.

2.4. Discussion

2.4.1. Study outcomes

This study aimed to assess if video training featuring novel humans could reduce neophobic behaviors. The study examined the influence of video training upon birds that were visited by novel humans. The training system installation did not appear to increase any fear-associated behaviors and the birds' behavior was largely similar pre and post treatment. The strong prediction of time 1 behavior upon time 2 suggests that the birds displayed similar, non-random behavior across time, allowing for a meaningful analysis.

Seeing a human face during training, as opposed to a plastic tray, was not predictive of any change. However, seeing a specific human face, that of the human that would visit the bird, was associated with a significant decrease in mate attacking behavior. The other recorded behaviors of alarm calls, clinging to the cage wire, and flights, did not appear to be influenced by the treatment. The alarm calls and flights were significantly positively correlated, suggesting they are part of the same sequence of escape behaviors, while bar clinging and mate attack did not correlate with other behaviors. The study suggests that the video face training influenced the mate attack behavior but did not affect escape behavior or bar clinging behavior.

The training only involved the appearance of a small version of a face low in the cage. This experience was very different than training to accept a whole person standing above them. It may be that familiarity with a face was not similar enough to training for extended, close approach to influence the predator escape sequence behaviors of alarm calling and flying. Potentially, mate attack is a defensive response where the birds stand their ground and displace aggression upon one another, while flying and alarm calling are a predator escape sequence. Familiarity with a face may have a greater effect on defensive behavior though both would appear to be motivated by neophobia.

Being trained on any human face was not enough to predict mate attack reduction compared to being trained on a video of an object. It appears that identity learning, not just exposure to human faces, is important to reducing mate attacks. Identity of the face from training matching the human present appeared important. This finding is in keeping with the literature about birds' abilities in recognizing individual humans (Belguermi et al., 2011; Cibulski, Wascher, Weiß, & Kotrschal, 2014; Cornell, Marzluff, & Pecoraro, 2012).

An unexpected result was that mate attacks appeared different from other fear behaviors as that they did not correlate with the flight and alarm call behaviors nor the wire clinging behavior. Aligned with the fight, flight, freeze concept of response to fear (Gray & McNaughton, 2000, pp. 40), mate attack could be considered as a displacement of fight, alarm calling and flying as flight, and wire clinging as passive freezing or hiding. It may be that this training was most effective on the fight response. Further study might focus on fight responses, such as testing if the birds lunge at a human finger.

Alternately, the training may have been faulty and had an overall low affect. If the birds did not associate their food reward with the image of the human on the video, such as not retrieving dispensed food items during the video presentation, the repeated viewing of the video could cause habituation to the face of the novel people, a more neutral response training that is not as effective as actual counter-conditioning (Keller, Hennings, Dunsmoor, 2020). Future work should track when the birds consume the reward.

2.4.2. Limitations

The study had several issues. Due to the layout of the building, it was not possible to randomly introduce the birds to A or B first, as a new person walking up and down the rows repeatedly caused an uproar of alarm calls in the birds at the start of a row that were subjected to repeated walkthroughs. Controlling for time and order of seeing a novel person could improve the study design, such as splitting the study birds between two buildings to create a block design.

The small total number of mate attacks makes it the poorest behavior for analysis. Future work will require inclusion of only those birds that demonstrate mate attack to better detect treatment effects versus random change. Due to random assignment of birds into treatment groups, there was a clustering of the majority of mate attack events in treatment group A. This cluster meant that treatment groups B and C had little to offer a predictive model. Respective to mate attacks, effectively these models represent a single treatment group. A benefit of having two groups with low total mate attack rates was showing that mate attack was not increased by the presence of the training system for those groups with already low mate attack rates. For future study inclusion only of animals that mate attack could offer a stronger case.

The moderate inter-rater agreement for bar clinging might make data based on wire clinging less useful. Raters' started their stopwatches at different moments, such as when the bird landed on the wire versus when the bird became fully still, which could create several seconds' discrepancy over the course of an observation period. Future work will require more precisely defining when clinging begins and stops.

2.4.3. Correlation of behaviors

The positive correlation between flights and alarm has a small p-value. This indicates that these may be related behaviors that are part of the same behavioral sequence. It appears that alarm calls and fleeing flights are related, even when escape is thwarted. Alarm calls and escape flights are co-occurring behaviors for wild flocking birds that are fleeing predation (Fallow, Pitcher, Magrath, 2013). Gray & McNaughton (2000) note that some fear behavior models make freezing and hiding an alternative tactic to escape, with freezing and hiding occurring when escape is not possible. A correlation of wire clinging when thwarted escape flights occurred was not observed. In this case, wire clinging does clearly not fit as with the thwarted escape model. Hyperactivity related to anxiety has confounded other experiments (Strekalova, Spanagel, Dolgov & Bartsch, 2005) and the captive situation may interrupt normal patterns of escape and hiding. The comparisons between male and female parakeet behavior under stress (table 3.2) support the subjective interpretation of similar cagemate aggression between male and female rose-ringed parakeet (Romagnano, 2006). It appears that the other fear-associated behaviors of alarm calling, flight, and wire clinging are also similar between the sexes.

2.4.4. Conclusions

A tablet-based automated video training system did not increase fear-associated behaviors in rose-ringed parakeets, a neophobic species. Familiarity with a person from video training was associated with a significant decrease in mate attack behaviors. Identity of the face used in training, not just any human face, mattered for mate attack decrease. Male and female birds showed similar fear-associated behaviors in the presence of novel humans.

2.4.5. Implications for practice

Labor costs for automated training versus human habituation are strikingly different. Assuming animal staff costs of $9.50.hr USD, automating would cost $366 in labor, while a traditional program would cost $8,681.

Due to the lack of electricity in the pole barn location, without access to a road, this study required a laborious set up to charge and hang up the tablets. The automation took 110 minutes a day on average to charge, hand carry, and hang up tablets, refill dispensers, and take down tablets. The location was not optimal for automation, creating 38.5 hours of work over 21 days.

To habituate or counter-condition birds to a worker, that worker has to be paid to be present during the training, and a professional will need to visit the site and teach the workers what to do. At 14 minutes a day of habituation per cage, for 33 cages, for 21 days, that is 808.5 hours of work. There is an additional labor cost of bringing in a behavioral specialist to a remote site to teach the staff how to habituate and counter-condition animals, estimated at $1,000.

There are three areas of practice where this technology may have application. First, commercial facilities such as breeders, zoos, and research facilities that have high staff turnover and seasonal staff. Second, conservation breeding where any loss of an animal or failure of a pair bond affects critically needed reproduction. Third, pet owners who want to habituate their pet to a veterinarian, pet sitter, or another caregiver, where repeated in-person habituation is unfeasible.

2.5. Ethical considerations

This work described in this article was approved by the Texas A&M University Institutional Animal Care and Use Committee as well as the Clinical Research Review Committee. The procedures were non-invasive and the experimental treatments of video training were non-stressful. The one-minute inspections by novel people were brief and non-invasive.

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2.7. Authorship statement

The experiments were designed by Constance Woodman and Jane Varkey. The experiments were performed by Jane Varkey, Constance Woodman, and Ashley Ridlon. The data were analyzed by Constance Woodman and Ashley Ridlon. The paper was written by Constance Woodman, Donald Brightsmith, and Jane Varkey.

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3. PARROT FREE-FLIGHT TRAINING AS A CONSERVATION TOOL

3.1. Introduction

Inventive approaches to release or relocate animals can be a necessary conservation strategy in the face of rapid environmental change (Seddon, 2010). However, the successful release of captive-raised parrots has been limited due to predation, loss of fear of humans, inadequate foraging skills, and inappropriate socialization (Snyder et al., 1996; White et al., 2012). In terms of best practices, it is known that released parrots do better when added to established flocks (Seddon, 2010; Snyder et al., 1996; White et al., 2012). However, there are not always appropriate flocks available and creating a wild parrot flock de novo from captive-reared birds is a challenge.

A limited number of pre-release preparation regimes have been investigated (White Jr, Collazo, & Vilella, 2005; White et al., 2012), but many variables remain uninvestigated. Bird release projects struggle with identifying factors for success, doing their best to pull limited information from meta-studies (Seiler, Angelstam, & Bergmann, 2000; White et al., 2012). According to Griffin, “instinctive” skills needed by conservation-release animals are not automatic but emerge as a product of animals’ experiences during their development (Griffin, Blumstein, & Evans, 2000). The interaction of environment and animals over time creates a huge number of variables to test. Variables may be at different scales such as immediate versus long term effects and span a huge number of factors which continues to grow. More obvious immediate effects include hand-rearing causing a lack of human wariness (Valutis & Marzluff, 1999). Multi-generational effects can be hard to anticipate, such as a captive-reared ancestor that failed to develop a full set of survival skills producing multiple generations of skill-deficient wild animals (Stoinski & Beck, 2004). Newer considerations are emerging, such as levels of social functionality in released animals (Goldenberg et al., 2019). There are so many variables that fully comprehensive evaluations of methodologies are not possible.

During raptor conservation activities, these considerations were greatly reduced. Conservation releases utilized established practices of falconry (Bolton, 1997), including captive breeding, rearing, physical conditioning, and release methods, arguably speeding recovery success through the use of pre-developed, field-proven methods. For raptors, release success can be impressive. For example, captive-reared kestrels have shown equal long-term survival when compared to wild bred individuals, through falconry techniques and falconer staff participation (Nicoll, Jones, & Norris, 2004). Falconry methods applied to conservation have performed better than techniques (Kenward, 2009; Weaver & Cade, 1991).

Similar to falconry, there is a system of practice for flying parrots outdoors, called free-flight (Moser, 2004). Free-flight can consist of sport flying of pet parrots, outdoor educational bird shows, and parrot keeping where parrots fly in and out of a window similar to an indoor-outdoor pet door. Free-flight tends to utilize internet groups, classes, and in-person seminars to disseminate this practice (Biro, 2000; Moser, 2004).

Aspects of parrot free-flight involve intensive human-bird interaction. The free-flight practitioner will have a social bond with their birds. Through these bonds, the birds follow their trainers into selected landscapes and are trained to function and survive in the selected places. In the wild, behavioral flexibility allows wild parrots to adapt to human-altered environments (Renton, Salinas-Melgoza, De Labra-Hernández, & de la Parra-Martínez, Sylvia Margarita, 2015; Salinas-Melgoza, Salinas-Melgoza, & Wright, 2013). When carefully planned hand-rearing has the potential to magnify the ability of a parrot to adapt to its environment. When animals are raised by human caregivers, their behavioral repertoire may increase, through the introduction to novel food types, foraging behaviors, and habitats unused by their ancestors (Dinets, 2015).

Presented here is one version of free-flight training. To introduce this method to conservation scientists, a cohort of four sun parakeets was reared and documented under the instruction of Chris Biro, a professional bird trainer. The rearing process and short-term outcomes for the cohort are reported here. To understand the long-term outcomes of this method we present a summary of information gleaned from the raising and flying of 37 parrots, (7 species and two hybrids), over a total period of 17 years. A special focus has been given to early rearing in the description of the method. Early development is believed by the free-flight community to have an important affect on bird success. The period of behavioral development prior to outdoor flying is a major stage in the method Biro uses (Biro & Woodman, 2009).

3.2. Materials and methods

The training process used in this study begins with unweaned, pre-fledge birds and trains them in a series of more and more complicated physical and ecological systems. The guiding principle of this process is that when placed in the appropriate environments, the birds’ behaviors are shaped by interaction with the environment and other animals (Biro & Woodman, 2009). The method relies on the birds’ natural responses to wild environments during juvenile development.

The method relies on the birds’ natural responses to wild environments during juvenile development, as opposed to behaviors being shaped one at a time through interactions with a human trainer. Through this process, normal parrot survival skills develop by mimicking what happens in the natural rearing process of parrots raised by in the wild by their parents.

The birds learn in six distinct environment levels (heretofore referred to as training levels, Figure 1. As the birds' abilities improve, they progress from simple environments (level 0: indoors in a room) to highly complex environments (level 5: forests and landscapes with major elevation changes inhabited by dangerous avian and mammalian predators with potentially dangerous weather conditions). Dynamic factors of weather, wildlife presence, and seasonal landscape changes, were considered prior to and during each flying session to make sure the level did not possess temporary features of a higher level such as predators or weather.

This method follows trainer Chris Biro's approach. Biro was an early organizer of free-flight enthusiasts online through an interest group in 1999 (Biro, 2000). In 2020, Biro taught his 400th student as part of his free-flight classes (pers. comm. Chris Biro).

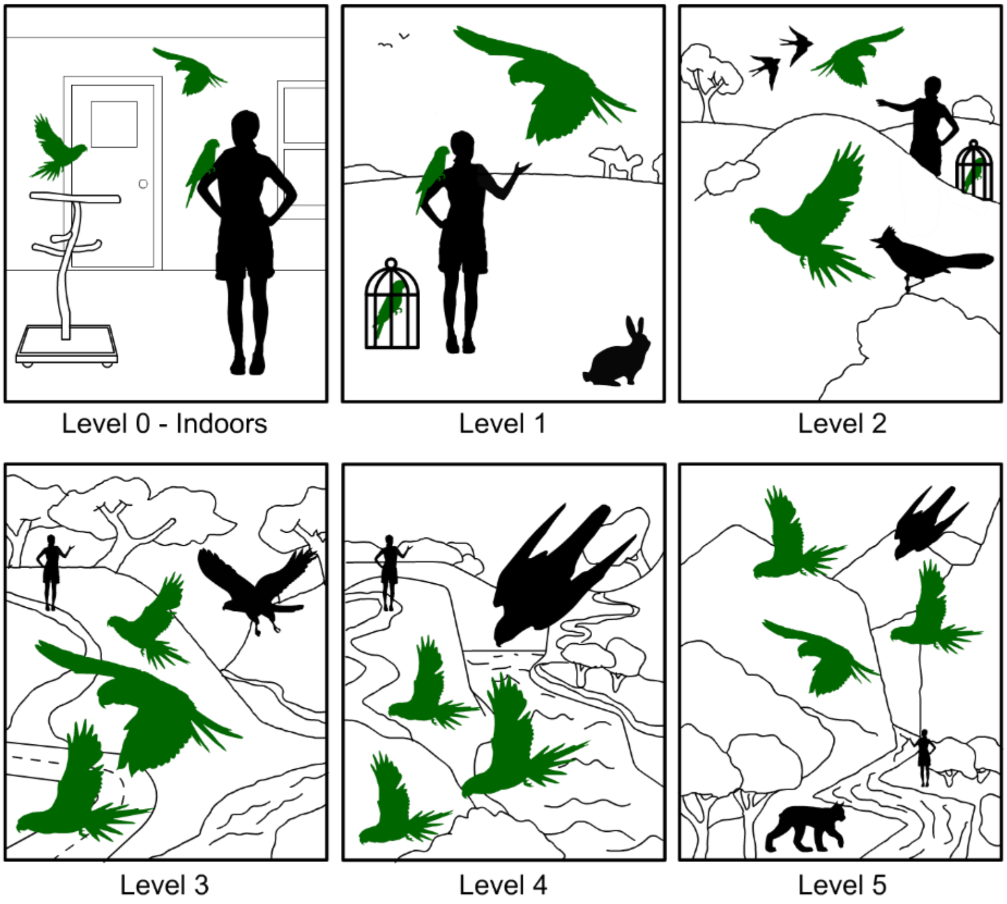

Figure 3.1 Schematic diagram showing the physical and ecological complexity of sites used for training parrots in this study.
Loss of line of sight and landscape feature complexity increases with level. Key level elements include presence of wild animals: harmless at level one; harassing to level two; casual predator investigations in level 3; occasional determined predator at level 4; and immediate predation threat at level 5. Each image in the sequence shows how landscape features influence the ease of retrieving birds by vehicle or by foot, from contained birds indoor at level zero; to retrieval not being possible at level five. Note the caged member of the social group (the "anchor bird") in levels 1 and 2 whose contact calls help keep other released birds near the training site.

3.2.1. Flock descriptions

For this study, we report on the raising and training of 37 individual birds. These birds were flown in three flocks, a large-bodied mixed-species macaw flock, a small-bodied mixed-species parrot flock, and a sun parakeet flock. All birds were reared and trained similarly.

The small-bodied flock included a total of 25 different birds. Sun Parakeets (*Aratinga solstitialis*) n=16, Mitred Parakeets (*Psittacara mitratus*) n=5, Senegal Parrots (*Poicephalus senegalus*) n=3, and a Burrowing Parakeet, (*Cyanoliseus patagonus*) n=1. This group was active for 16 years (1997-2013). Not all birds were intended to be made fully independent, as Biro focused on a sub-group of show flyers and others were less intensively trained.

The large-bodied flock included a total of eight different birds: Hybrid “Calico” macaws (*Ara chloroptera* x *Ara militaris*) n=3, Blue-Throated Macaws (*Ara glaucogularis*) n=2, a Scarlet Macaw (*Ara macao*) n=1, a Blue-and-Yellow Macaw (*Ara ararauna*) n=1, and a hybrid “Shamrock” Macaw (*Ara macao x Ara militaris*) n=1. This group was active over a 13-year period (2000-2013). This flock was trained to be maximally independent. The sun parakeet flock included a total of four birds, all sun parakeets n=4. This group was active for one year (2015-2016). This flock was raised and trained specifically to create documentation of the early rearing process and transition from indoor to outdoor flying. Training for this flock was only for lower level outdoor flying (levels 1-3).

When nesting attempts occurred in mature birds, the birds were not allowed to progress to wild reproduction to avoid creating naturalized populations.

3.2.2. Locations

The large and small-bodied flocks primarily flew in a rural area outside of Moab, Utah, USA. Average temperature during the study period was 14.2 C, with an extreme maximum of 43.9 C and an extreme minimum of -21.11 C. Average annual rainfall was 233 mm (NOAA, 2020).

The birds were also transported by Chris Biro and flown in multiple locations in the Western United States, including locations in Washington State, California, and Oregon. The sun parakeet flock was fledged in College Station and primarily flown outdoors in Dripping Springs, Texas, USA. Average temperature during the study period was 20.1 C, with an extreme maximum of 39.4 C and an extreme minimum of -15.6 C. Average annual rainfall was 1189 mm (NOAA, 2020).

The total number of training sites utilized was large and uncounted. Each group of birds added to the flocks had a different set of location experiences. Property access and site conditions required adaptive practices on the part of the free-flight trainer.

The sites utilized for level one, for example, comprised about 20 sites utilized across all three flocks. Some birds were trained in only one level one area, others were trained in multiple level one locations. For the two longer-term flocks, the small and large-bodied flocks, novel level two, three, and four flights were frequently identified and utilized, increasing the total number of sites for flight training. Site identification included casual recognition of a site while traveling, where birds might only be flown once with permission of a property owner.

The three free-flight flocks varied in their range size based on training. The sun parakeet flock was not trained to travel between locations, while the two long term-flocks were. The large-bodied flock was encouraged to follow a vehicle over multi-kilometer trips, further than was done for the small-bodied flock.

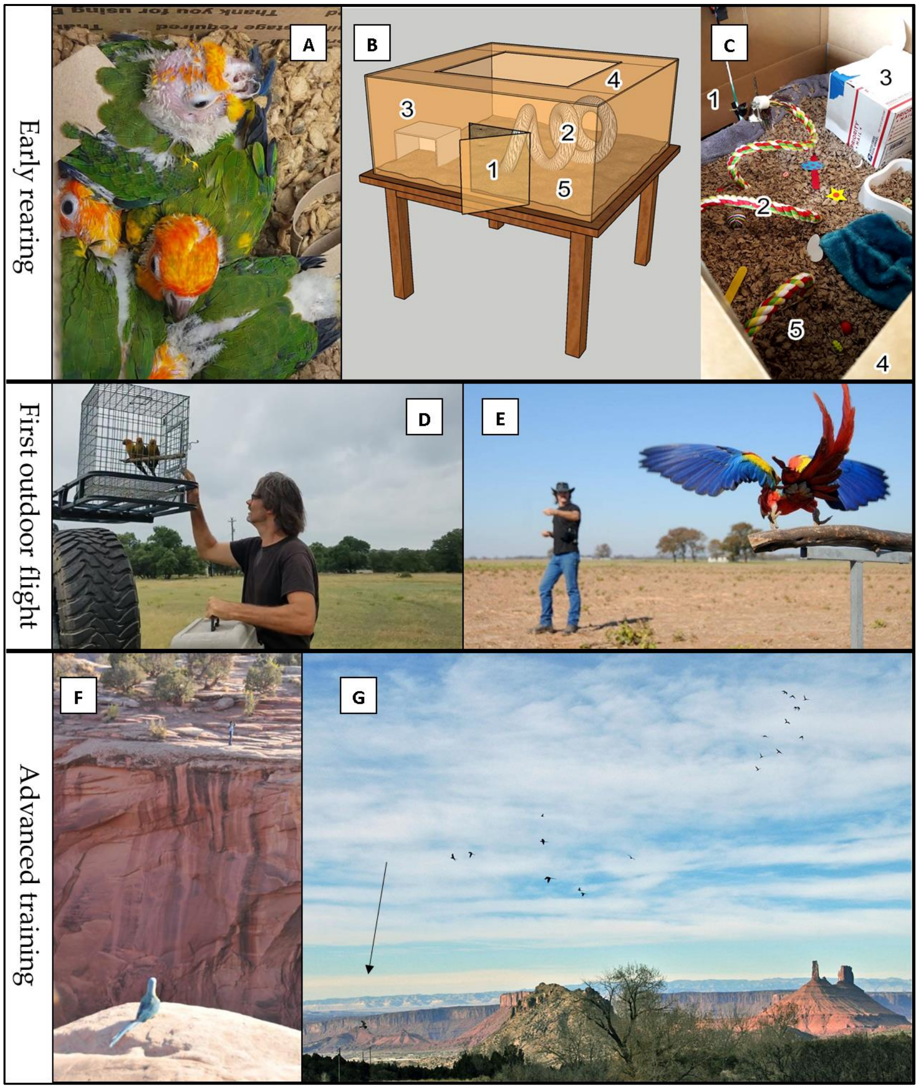

Figure 3.2. Stages during free-flight training.
(a) Sun parakeets flock at the time of acquisition, 33-40 days of age. Chicks show non-human socialization through gaping and swaying as well as cowering; (b, c) Playpen rearing area. 1. Feeding access door. 2. Wire cored rope climbing coil. 3. Brooder box with paper towel entry flap. 4. Overhang to prevent climbing out. 5. Carefresh brand bedding on the floor and in brooder box; (d) Level one area for small birds, an open area of about three hectares. Note the transport carrier and anchor bird’s cage; (e) Level one area large-bodied birds utilizing a much larger open area of about 16 hectares. Note the portable perch for back and forth flying; (f) Complex landscape navigation training (levels 3-5). Trainers on either side of a canyon and cliff complex recall the birds at the safest crossing points to train landscape navigation; (g) The large and small-bodied flock escape from a hawk (arrow) at the home base.

3.2.3. Data types and collection

Data on the large-bodied and small-bodied flocks were drawn from Chris Biro's personal archives and CJW's photography and notes. The archives consisted of dated e-mails, content and meta-data of digital photographs, content and meta-data from videos, and SMS text messages. This information was supplemented by direct interviews with Chris Biro. The data included each birds' name, species, age at first outdoor training, date of each bird's entry into their flock, duration of participation, the reason the bird left the flock, a maximum level reached, and total time spent flying outdoors. To record the sun parakeet flock rearing process, a video camera with a time-lapse recording function was mounted above the playpen to record the chicks and monitor how they utilized the space. Records for the sun parakeet flock consisted of content and meta-data from normal and time-lapse video, content and meta-data data from photographs, and contemporaneous notes taken by CJW.

Total time flying in a natural environment was estimated based on 12 hours of daily flying when not working at seasonal educational shows. Hours flying were calculated per bird, meaning if a group of 10 birds flew for four hours, there would be 40 hours of flying time recorded. To understand how outdoor flight mortality outcomes compare to conservation outcomes of similar outdoor duration, a "flight months" metric was created. The hours of outdoor flying are converted to "flight months," consisting of 30 counts of twelve hours outdoors. Mortality outcomes were analyzed using the Mayfield method (Mayfield, 1961), calculating the risk of death during one year.

3.2.4. Level zero

3.2.4.1. Acquisition

To record the general early rearing process for all flocks, four captive-bred, hand-reared, incubator hatched sun parakeets, from different clutches, were purchased from a commercial bird breeding facility. The sun parakeets were assembled into an aggregated group of young. The hatch dates of the birds were unknown but the developmental stages were roughly estimated as 33 days of age (n=1), and 40 days old (n=3). When acquired, the chicks were able to walk between locations, thermoregulate, and possessed adequate stamina and coordination to climb up and over Carefresh brand bedding (http://www.carefresh.com/) substrate and return to the nest box after play periods. At time of acquisition, the chicks were not yet human-socialized. Gaping, swaying, and cowering in the presence of human beings was observed.

3.2.4.2. An enriched rearing environment

For all three flocks, the rearing setup was intended to maximize opportunities for interaction with the environment. The environment, built as a playpen, was roughly 1m x 1m with 0.5m high walls constructed of cardboard shipping boxes taped together (Figure 3.2). The playpen had an overhang to prevent birds from scrambling to the top and falling out prior to fledging. An access flap for feeding was cut into one side and held closed with plastic coated wire twist ties. Colorful cotton rope, 4cm diameter with a wire core and a bell at the end, was bent to create multiple raised perch areas and taped to the base of the pen. The floor of the playpen was covered in six cm of soft and insulating paper animal bedding, . A small box was turned on its side, and a paper towel flap attached from the top of the box’s opening to create a cavity. The box was routinely refilled with bedding. Various objects were placed on top of the bedding, including small wooden and plastic trinkets, a two-inch diameter red ball, colorful paper cutouts, a plastic dog dish, and a plush Prevue brand Cozy Corner bird cuddle and comfort object. A lamp on a timer was placed near the playpen to provide a 12hr day of direct lighting. The chicks were old enough to thermoregulate so they could be safely reared without a temperature-controlled brooder. This general setup was used because it allowed the chicks to move around the playpen and choose from a variety of activities. Similar configurations were utilized for raising the birds in the large and small-bodied flocks. The focus was on free-choice, where chicks could remain inside a dark box or leave the box and engage with the environment as part of the level goals (Table 3.1).

Table 3.1, Level zero environmental characteristics and mastery criteria for parrot free-flight training.
The birds in this study completed level zero criteria between the time of fledge and weaning, ~70 d for sun parakeets, ~100 d for macaws.

Environmental features	Mastery criteria
• Handfeeding location. • Enclosed spaces such as a living room or outdoor aviary. • No wild species.	• Trainer linked with consistent meeting of care needs through associative learning. • Accepts food and water from the trainer. • Accepts interaction from trainer including snuggles and toy play readily. • Steps up on the trainer. • Approaches trainer on foot or wing when separated. • Returns to trainer with recall cue. • Leaves perch with “get off of that” cue. • Lands on difficult perches. • Flies throughout the entire space. • Orients to other birds in flight (“tagging”, “chasing”). • Aerial maneuvers (i.e., “jinking” sudden turn in the air).

3.2.4.3. Feeding and training

All chicks in all three flocks were hand-fed using commercial parrot hand-feeding formula and offered a variety of solid foods throughout development. The objective of the selected feeding style was to enable normal growth while encouraging beak and tongue use. Daily, solid food including apple slices, breakfast cereals, and Zupreem parrot pellets (https://zupreem.com) were provided to enable a smooth transition to a fully solid diet during weaning and maximize options for chick activities.

Chicks were fed by mouth using a plastic syringe and commercial parrot hand-feeding formula Kaytee brand (https://www.kaytee.com). The total feedings broadly followed manufacturer's recommendations and varied based on individuals' ingested amount per feeding, digestion speed, and age. Observation of chicks' body condition, a common veterinary technique, was utilized to monitor health (Burton, Newnham, Bailey, & Alexander, 2014).

The introduction of a behavioral cue, the recall cue, was paired with feeding times. During feeding times, the chicks would run to the syringe and follow the human hand to different areas of the playpen, and the recall cue was presented. The cue was broken up into a general "here birds" or the bird's specific name to train for individual recall versus full group. Over time the birds would come to the vocal cue whether or not the syringe was present.

The sun parakeet chicks weaned at approximately 60 days of age. To check that the wean was complete, the birds were weighed at the time of cessation of hand feeding and one week later. Weight losses of 1-5% indicated birds were maintaining body condition.

3.2.4.4. Handling

To ensure that the chicks became comfortable interacting with the researchers, chicks were handled several times a day. Handling consisted of petting, holding, carrying, and interacting. When the chicks began to approach human hands spontaneously, about three days after acquisition for the sun parakeets flock, they took 30 minutes trips to indoor or outdoor spaces away from the playpen. Outings occurred roughly every two days, sometimes with one chick and sometimes with all the chicks.

3.2.4.5. Fledging

Once chicks had well-developed wing feathers, approximately 50 days of age for the sun parakeets, they began spontaneously climbing to higher perches and intensely flapping their wings. By the time the chicks fledged they were already responsive to the recall cue, having run to the hand while being called during feedings. Running toward the trainer, chicks were encouraged to flap and climb on the trainer, paired with the recall cue. The running developed into short straight-line flights to the trainer, which was reinforced by feeding, or affectionate handling by the trainer. During outings away from the playpen, the birds were placed on surfaces outside of the playpen, provided with the recall cue, and encouraged to hop, while flapping. As the birds became proficient at hopping, the number of hops a day was increased from two to ten and then became short flights. The goal of this was to create a recall behavior of flying to the human hand when presented..

The playpen environment was modified for the fledge once the birds could hop to the hand. To create a landing pad, a second rope perch was added, with a loop extending above the playpen. A perch “tree” made of PVC pipe wrapped in sisal rope, was set up near the playpen for flight practice. By day 60 of age, the Sun Parakeets spontaneously flew to the researcher and areas around the rearing area. The sun parakeets performed an estimated 100 flights a day. As weaning occurs after fledging, parakeets who flew to the researcher for food were fed first, creating a competitive situation that rewarded fast response to the recall cue. After weaning, birds were still eager to take a few drops of food from a syringe and this was used to reward coming to the recall cue. To create a "get off" behavior, birds were spoken to sharply, immediately upon landing in an unsafe location. The harsh volume and tone of voice resulting in them promptly flying off. When birds could fly as a group, engage in aerial acrobatics, be individually recalled, and responded to a "get off" cue, the chicks were ready to transition to a level one environment. The birds were called over for food, touch, or play, then shooed back to the perch, or placed on the perch. Then they were recalled again and given more attention. The flying away and back to the trainer repeatedly developed a habit of back and forth flying to nearby approved objects, called "A to B" flight. Non-approved landing sites were identified through the get off cue. The large and small bodied flocks were raised and fledged in a similar way.

3.2.4.6. Move to outdoor caging

After confirming that weaning was complete, the sun parakeet flock was moved full-time to an approximately 5m by 3.5m by 2.7m tall outdoor aviary in Dripping Springs, TX. The aviary allowed for nearly constant, unmonitored flying, and physiological adaptation to the mild early summer outdoor environment. The two longer-term flocks were split across multiple aviary buildings of roughly similar dimension when not out flying. Back and forth flying was created from the food and comfort-seeking flights to the trainer. The newly fledged chicks would be shooed away or placed on a perch, then when they came back for more attention or food, were rewarded for having left andthen returned.. This informal routine was developed into a trained behavior in the outdoor aviary. Large, portable perches were introduced into the outdoor aviary and utilized for back and forth flying practice, which was performed multiple times a day for each bird.

3.2.5. Level one

3.2.5.1. Landscape setting

The landscape features of these sites were all similar and can be summarized as large, flat areas with few trees or shrubs, similar to prairie or savannah conditions. There were limited opportunities for biotic interactions, and only mild weather (Table 3.2, Figure 3.1). The transport vehicle was parked adjacent to the flying area to train the birds to return to this easily discernable landmark.

3.2.5.2. Goals

The skills the birds gained at level one were foundational skills for flying in an outdoor space and returning to the trainer. Meeting all the criteria in Table 3.2 were needed for the bird to move to a level two environment, Table 3.3.

Table 3.2. Level one environmental characteristics and mastery criteria for parrot free-flight training.
Training occurred as close to fledging as possible, older individuals were observed to be more likely to panic fly or not bond with the group. Birds in this study gained mastery within about three weeks of flying. All criteria must be mastered before moving to the next level environment.

Environmental features	Mastery criteria
• Open field. • Light wind. • No precipitation. • Distant wildlife. • Simple retrieval by foot or vehicle.	• All previous criteria. • Repeated practice flying at low and high altitudes. • Fly with and against the wind. • Demonstrate endurance through multi-minute continuous flapping flight. • Introduced to flocking outdoors with others. • Fly low the majority of the time (high flight is associated with nervous behavior, indicating the bird is unready for more complexity). • Tend to stay near rally point vehicle between flights. • Develop complex movements initiated during aerial play. • Utter alarm and contact calls. • Respond appropriately to flockmate’s contact and alarm calls through increased wariness, reply calling, approaching calling flockmate.

3.2.5.3. A to B flying

Before the training sessions, portable perching stored in the rally vehicle was set up adjacent to the rally vehicle. The bird(s) were taken from the carrier by hand and placed onto a portable perch. The trainer walked a few meters away and began the “A to B” back and forth perch flight routine developed during level 0. This back and forth was utilized as a way to acclimate the birds to the new conditions in level one through a familiar routine. During the first outdoor flights, one bird at a time practiced A to B.

3.2.5.4. Rally vehicle anchor bird

During initial training, not all birds were taken out to fly at once. Birds not being trained were placed in a cage upon the top of the rally vehicle, Figure 2. These caged bird(s) were able to contact call with the bird(s) being trained creating an “anchor.” During training sessions, the birds were reluctant to fly outside of the contact call range of these anchor birds to which they were socially bonded, which helped them remain near the rally vehicle.

3.2.5.5. Recall cue

The recall cue developed at level zero was put into practice at level one. Recall practice began with the back and forth flying routine and continued each time the bird flew off the perch and explored the area. When multiple trainers were available, birds could be recalled between trainers to practice distance flying and build stamina. The constant presence of the vehicle and anchor bird(s), Figure 2, during recall helped reinforce the vehicle as the return point.

3.2.5.6. Get off cue

The get off cue, developed at level zero, was utilized at the level one outdoor location. Birds were cued to “get off” when they entered into dangerous situations such as approaching powerlines or landing on a vehicle that was not the rally vehicle.

3.2.5.7. Human alarm call

Using warning tones while speaking in a louder voice, the trainers could verbally increase the birds’ awareness. For example, if another vehicle approached, but the birds were oblivious, the trainer speaks in a louder, warning tone, and the birds would increase their attention to the environment and notice the oncoming car. Through practice, the birds learned that the warning tone signaled a need for increased vigilance.

3.2.5.8. Flying in a group

Chicks initially flew one at a time. Other socially bonded birds were held back in a cage, set on top of the rally vehicle, or on the tailgate. As the birds explored, they were praised for exploratory flights and increasingly complex aerial maneuvers. Once each bird was competent in outdoor A to B flying, the birds would be placed as a group on the portable perches, flying A to B as a group until they became confident enough to explore the area and expand beyond A to B flights.

Once two or more birds were familiar with the area and recalled reliably, multiple birds could fly at once. These could be from the same cohort of new flyers or a mix of the new flyer(s) and more experienced birds. A socially bonded bird would still be held back in a cage until there was high confidence in level one flying skills and the birds showed no fear-associated behaviors when interacting with normal variations of the level one environment, such as wind, cloud movements and shadows, presence of distant wildlife, etc. Fear-associated behaviors included high flight, increased respiration, raised hackle feathers for moderate fear, completely smooth feathering for strong fear, dilated pupils, panting, tight gripping of the perch or arm, alarm vocalizations, or distress vocalizations (Luescher, 2008).

3.2.5.9. Feeding on plants

Feeding on plants was limited in level one except when birds were flown near lone trees or shrubs present in the landscape. Utilization of sparse trees of shrubs for practicing recall coming down from trees and flying up into them was observed. Upon contacting a tree or shrub the parrot inevitably began chewing on buds, seeds, shoots, and leaves. The “get off” cue was utilized to discourage chewing on a plant that might be toxic.

3.2.5.10. Situations special to level one

The trainer avoided flying apparently overwhelmed birds until they calmed. When appearing fearful, birds were placed back in their carrier or the anchor birdcage to continue to acclimate and watch their socially bonded fellows fly.

When startled, some birds occasionally flew up very high (> 40 m). The anchor bird created back and forth contact calls. The high flying bird would circle the anchor bird and the trainer, eventually tiring and circling and gliding back to the anchor bird and trainer at the rally vehicle. The recall cue was utilized during the high flying to encourage the bird to return.

Uncontrolled flights associated with strong fear states were called panic flights. In a panic flight there was no response to the recall cue. Panic flights tended to be rare. A prolonged panic flight was observed on a single occasion in 2014. A straight-line panic flight away from the rally vehicle was observed by CJW when Biro was flying a macaw. After 13 minutes of flight the bird tired, lost altitude, and landed. The bird was not observed to engage in another panic flight over subsequent weeks. As the bird was being flown in an appropriately wide, agricultural field complex, the bird never left the line of sight or entered a forested area. Nervous flying at unusually high altitudes was only observed at level one.

As the birds habituated to the environment, the parrots would land on the rally vehicle, launch from the perch, and circle back to the perch, or other exploratory activities. Circling flights, increased speed, and increased distance away from the trainer occurred. Eventually, all birds engaged in sudden movements using their tail to maneuver, called “jinking”, recreating the aerial play patterns seen at level zero. This initial pattern of behavior was similar for all flocks.

3.2.6. Level two

3.2.6.1. Landscape setting

Level two landscapes consisted of various shrubby fields, gentle hills, and sparsely treed areas (Table 3). Flying through trees introduced the birds to territorial songbirds, flying in the vicinity of bodies of water provided harassing, curious gulls. Level two landscapes did not contain dangerous predators except as aerial silhouettes on the horizon. Retrieval of birds was possible by off-road vehicle.

The small and large-bodied flock had level two conditions surrounding their home aviary location. Being able to let the large and small-bodied flock out from their home aviaries during early training simplified training, reducing the need to travel. For the sun parakeet flock, the level two areas utilized peripheral areas around the level one field, containing small, spaced apart trees and large shrubs.

3.2.6.2. Goals

The primary goals of level two are to encourage brief, independent navigation when line of sight is broken, build strong flocking skills, introduce interaction with shrubs and trees, and allow interaction with wildlife to begin the development of anti-predation behaviors (Table 3.3).

Table 3.3. Level two environmental characteristics and mastery criteria for parrot free-flight training.
Average time to master level two was three weeks. Mastery time could be extended depending on the exact location and wildlife presence. The frequency of wildlife interactions were a limiting factor.

Environmental features	Mastery criteria
• Hills, shrubs, and small or isolated trees. • Breezy or gusting wind. • Mist or drizzle. • Non-dangerous wild species that follow or harass. • Retrieval by foot or vehicle relatively easy	• All previous criteria. • Recalls to trainer from shrubs and trees. • Chooses perches for easy take-off. • Startle response to strange species. • Joins flock in flight. • Coordinated group escape from curious or harassing wildlife initiated by any flock member. • Recalls after momentary loss of sight of the trainer. • Returns to and follows rally vehicle over short distances.

3.2.6.3. Rally vehicle anchor bird

During level two, typically one bird was an anchor bird while the others were flying. Compared to level one, level two anchor bird use reflected less need to orient birds to the outdoor environment. During level two, birds required less individual monitoring of mood and behavior as panic flights and confusion were less frequent than during level one.

The rally vehicle, similar to level one, was parked close to the trainer, continuing to build an association of returning to the vehicle after periods of activity. The vehicle was often driven a short distance during training, changing the location of both the trainer and the vehicle. These alterations in location began training the flock to follow the vehicle and orient to a changing rally point.

3.2.6.4. A to B flying

Similar to level one, back and forth flying was utilized to adapt the birds to the new environment until they became comfortable with exploring. Birds could be let out individually for training or as a group.

3.2.6.5. Recall cue

The recall was practiced throughout the one to six-hour sessions, with much focus on coming down from trees and shrubs. The birds followed the trainer through areas of trees and learned to follow and recall even when visibility was blocked by trees and hills.

3.2.6.6. Get off cue

Birds were cued to "get off" when they entered into potentially dangerous situations or attempted to consume unsafe items. Observed uses included interrupting perching on a stump near to the ground, landing on dangerous cacti, and landing on powerlines.

The get off cue was utilized to direct the birds in safely utilizing perching in trees and shrubs. Members from all three flocks were not permitted to rest in dense tree cover or other locations where the birds could not see approaching predators. Inexperienced birds would initially perch close to the trunk of a tree and would be discouraged from doing so using the get off cue. Using the get off cue created a habit of perching on outer branches where emergency take-offs were unobstructed by dense branches.

3.2.6.7. Human alarm call

The human alarm vocalizations initially developed in level one were utilized in subsequent levels. By increasing alertness in the flock, the trainer selectively sensitized the birds to dangerous situations. The level of volume and harshness of tone were commiserated with the danger. Birds were alerted to be wary at the approach of harassing wildlife. Bird wariness was increased selectively, such as for a dangerous hawk's silhouette flying far away but intentionally not increased for a harmless vulture silhouette at the same distance, building recognition of predators before close encounters.

3.2.6.8. Flying in a group

Birds from the same cohort were permitted to fly together when each individual showed competence in recallfrom trees or shrubs and recall when there was a break in line of sight to the trainer. Birds were flown as individuals or in subgroups of the full flock to focus on skill development in specific members.

When flying as a group, flocking behaviors appeared to be facilitated by wildlife interactions. Birds from all flocks tended to group together in response to the approach of harassing wild animals. When available, more experienced birds were added to level two birds in training once the newly flying birds showed competency in recalling from trees and broken line of sight. When flying with more experienced birds from outside the study, the sun parakeet flock learned to respond to the alarm calls and escape flights of the macaws and cockatoos. Sometimes, the sun parakeet flock would follow and perch next to the larger birds Biro brought out to go flying, apparently gaining information about how to use the landscape from the more experienced flyers. Even across species of different body sizes, new members would follow the behavior of their more experienced social models.

3.2.6.9. Feeding on plants

The increased diversity of plant life encountered at level two required care to discourage landing on harmful plants, such as those with defensive spines, and to discourage the eating of unknown berries and seeds. Birds would almost always chew spontaneously on the nearest plant parts whenever they landed in foliage.

3.2.6.10. Situations special to level two

Northern mockingbirds, (*Mimus polyglottos)*, blue jays, (*Cyanocitta cristata)*, and various gulls (genus *Larus*), were observed to chase and threaten the parrots. Interactions with aggressive, non-dangerous birds like these allowed the free flight flocks to practice grouping and responding to threats. The flocks spontaneously grouped up and fled or stood their ground in response to harassment. For example, the sun parakeet flock would occasionally group together and chatter or chase harassing wildlife, beginning the development of mobbing behavior.

Through repetition, the flocks learned what stimulus indicated real danger. Initial hypersensitivity to certain kinds of harmless events, such as a vulture high and far away on the horizon, became situationally appropriate after multiple repetitions. Eventually, the birds learned to accept a distant vulture while still reacting to approaching raptors.

3.2.7. Level three

3.2.7.1. Landscape setting

The level three landscapes used were open forest with hills, bluffs, and drainages creating elevation changes (Table 4). Biotic factors included non-dangerous native wildlife, predators that would approach but did not present immediate danger, and an increasing diversity of plant life, such as wetland plants, forest trees and upland shrubs. Non-dangerous predator interaction included juvenile red-tailed hawks (*Buteo jamaicensis*) that made low fly overs but were unable to catch the parrots in the open areas with little forest canopy. The sun parakeet flock's home aviary was in level three conditions.

It was not always possible to have all the desired level three complexity in one location. Utilizing environmental variations over time and traveling to different locations allowed the birds to have all the necessary experiences to develop level three behaviors. Bird retrieval in level three required driving to the edge of the nearest landscape feature then hiking the rest of the way.

3.2.7.2. Goals

The main goals of level three training were escape from low intensity predation attempts, increased independence from the trainer during breaks in line of sight, navigating weather, landforms, winds, and improved flocking (Table 3.4).

Table 3.4. Level three environmental characteristics and mastery criteria for parrot free flight training.
The time for new flocks to meet these mastery criteria ranged from about 2 to 6 months.

Environmental features	Mastery criteria
• Substantial elevation variation. • Open forest. • Small ponds/small streams. • Windy, light precipitation. • Investigative pursuit by aerial predators. • Retrieval by foot and off-road vehicle.	• All previous criteria. • Birds demonstrate exploration and learning of landscape, such circling and exploration patterns. • Birds create consistent routes between features, and preferred perching areas. • Habituation to weather and precipitation, responds by sheltering as appropriate instead of anxiety behaviors. • Ability to fly during wind gusts. • Some mobbing of harassing wildlife. • Complex aerial escape maneuvers. • Recall after 2-3 minutes of loss of sight of the trainer.

3.2.7.3. Rally vehicle anchor bird

The rally vehicle was parked out in the open as much as was possible to keep the return point visible to the birds in the increasingly hilly and forested terrain. Anchor bird use was similar to level two, though mainly utilized during the initial visits to new sites. At some points an anchor bird was carried on the hand by the trainer to encourage other birds to follow while on a hike. Hand carried anchor birds were also used to encourage reluctant birds to fly down from a tree or enter an area with novel features. When a particular bird showed decreased fidelity to the rally point or recall cue, its closest socially bonded flockmate would be kept as an anchor bird to improve fidelity and recall until the behaviors were again reliable.

3.2.7.4. A to B flying

Back and forth flying was seen less often at the start of level three training. The birds were often eager to immediately fly a high circle over a new landscape upon being let out into the area. Rather than being used to introduce the birds to a landscape, back and forth flying was instead utilized as a tool to direct the birds to engage with specific landscape features. One trainer might be at a high elevation, another at a low, and the birds called between them to learn about diving and wind shear. Figure 2 shows how two trainers use A to B flying to train canyon navigation.

3.2.7.5. Recall cue

The recall cue was practiced throughout the flying time. The trainers were careful to keep track of bird locations and distances, using the recall cue to keep birds within range of hearing contact calls. The birds were periodically brought back into the line of sight using recall cues, so birds would not become lost to the trainer among the trees or geologic features. The birds were allowed to have more independent time and longer breaks in the line of sight than in level two.

3.2.7.6. Get off cue

The get off cue was utilized to discourage birds from landing near the ground. When birds perched directly adjacent to tree boles, their behavior was modified using the get off cue. By using the get off cue, the birds developed habits of perching among outer branches to allow for immediate escape flights.

3.2.7.7. Human alarm call

Through repetition, the birds were trained to associate which circumstances required increased vigilance. Predator response specificity was developed. The human alarm call helped to sensitize birds to dangerous predators. Human alarm calls raised their wariness level, increasingscanning and the chance that the flock would fly. The human alarm call established at level two was utilized to help make the birds aware and wary of predator approaches, helping the birds to begin identifying novel danger sources. The use of binoculars before letting the birds loose and during flying helped the trainers spot danger and direct their gaze and posture toward a threat, while calling out to alert the birds, helping the birds to recognize the features of incoming predator approaches.

3.2.7.8. Flying in a group

Due to the increased predation threat and the fact that the trainer was unable to track individual birds among trees, birds were seldom flown as singletons at level 3. Instead, birds tended to be trained as a large group. Level three flocking extended defensive flocking and mobbing behaviors developed in level two, specifically in response to predators. These skills were practiced in increased landscape complexity, with periods of independent responses out of view of the trainer.

3.2.7.9. Feeding on plants

Feeding on plants continued. Frequent observation and use of the get off cue by the trainer reduced the chances that birds would consume dangerous plant materials. However, as birds had longer periods where they were not in the line of sight, many plant interactions went unobserved.

3.2.7.10. Situations special to level three

Curious predators that were unlikely to harm the birds were permitted to approach the flocks. Care was taken not to fly the birds in areas with local, determined bird predators, such as Accipiter hawks (genus *Accipiter*), peregrine falcons, (*Falco peregrinus*), bobcats, (*Lynx rufus*), and great horned owls (*Bubo virginianus*).

In the large-bodied flock, flying alone at a level three location was utilized to develop specific landscape skills in a skills intensive training setup, where a bird with a poor recall from high elevations would be worked specifically with high elevation change, with an anchor bird present.

To mix experience levels, the novice level three flyers could be intermingled with more experienced birds they had flown with at level two or one locations. Social modeling could speed learning as well as increase safety through the sociobiological benefits of a larger flock.

3.2.8. Level four

3.2.8.1. Landscape setting

Level four conditions were defined by the landscape, predator activity, or weather (Table 5). Weather conditions like rain, fog, or snow caused a loss of sight for the trainer as well as the birds. Precipitation, as a hypothermia risk, made sheltered perching selection imperative. Care was taken to closely observe birds in poor visibility weather and recall them to the shelter of the rally vehicle well before the onset of hypothermia. Sheltering wet or cold birds allowed the birds to learn from mistakes rather than become ill.

The small and large-bodied flocks were let out and recalled near the edges of valleys or steep inclines, to experience intense updrafts. Flying during multiple times of day exposed the birds to daily anabatic and katabatic cycles of wind, wind interaction with landforms, and the conditions associated with strong winds.

3.2.8.2. Goals

The goals of level four (Table 5) include mastery of advanced escape and flocking skills in the face of serious predation risk, including mobbing; strong-flying and safe choices related to weather, landforms, and wind; and independent function during extended loss of line-of-sight with the trainer. As the birds function more independently, they are expected to engage in “intelligent disobedience”, a concept most often encountered in service dog training (Eames, Eames, & Gingold, 1986). The animal should be aware enough of the environment to refuse cues that increase risk until the risk passes.

Table 3.5. Level four environmental characteristics and mastery criteria for parrot free-flight training.
The time for new flocks to meet the mastery criteria ranged from about 2 to 6 months. Most birds in this study mastered level four before one year of age.

Environmental features	Mastery criteria
• Water basins or major streams. • Windy, heavy precipitation. • Chance of pursuit by a determined aerial predator. • Retrieval possible only by foot or specialty vehicle due to limited vehicle access.	• All previous criteria. • Fly up and down cliffs. • Complex diving and escape maneuvers. • Habituation to heavy precipitation. • Strong flight negotiating wind gusts. • Strong flock mobbing, escape, and predator confusion behaviors. • Recall readily after 5-10 minutes out of sight of the trainer. • Intelligent disobedience, refusing to respond to human cues if there are hazards present.

3.2.8.3. Rally vehicle and anchor bird

An anchor bird was occasionally held at the rally vehicle as a location beacon in complex terrain with many visual barriers. The use of an anchor bird was based on the individuals in a flock. Birds less willing to recall had their favorite flock mate kept back as an anchor bird. The favorite flock mate would ride on the trainer through a problem location. The less willing bird would follow its flockmate and become practiced in how to navigate the landscape features.

Birds were recalled while the rally vehicle was in motion. The vehicle drove along access roads between sites, guiding the birds to fly between sites for the small and large-bodied flocks.

3.2.8.4. A to B flying

Back and forth flying practice was utilized to train the birds where and how to interact with complex landscape features (Figure 2). A second trainer was often present to recall the birds to a location where the birds were unlikely to fly alone. Examples include canyon navigation, selection of safest crossing points over or across landscape features, and selection of cliff diving sites to develop diving escape behaviors at appropriate landscape features.

3.2.8.5. Recall cue

The birds were periodically brought back into the line of sight using recall cues, with increasing periods of independence, at a 10-minute maximum. If the birds were wary and did not recall, they were not pressured until the trainers were sure the birds were safe. Whether failure to recall was a behavioral deficient or a beneficial choice not to fly in a dangerous situation was evaluated for each bird. A pattern of refusal that was not related to danger resulted in use of anchor birds or demotion to practice at lower level landscapes to build the behavior until the target bird again met the needed criteria.

Recall occurred while the trainer(s) were hiking through the landscape or when the rally vehicle was driving. Using recall cues, one or more trainers caused birds to engage with a specific route. The birds learned routes within the landscape back to the rally point as well as between rally point locations. Recalling during developing inclement weather conditions appeared to train the birds to associate coming weather events with sheltering.

3.2.8.6. Get off cue

The get off cue was seldom utilized at this level as the birds had learned to perch in safe places that offered easy observation of surroundings and fast escape. The cue was primarily used if the birds behaved inappropriately during a novel situation, such as landing on a stranger’s car.

3.2.8.7. Human alarm call

The human alarm call was utilized loudly and emphatically if the flocks had not yet noticed danger approaching. This occurred rarely, as the birds regularly detected risks before the trainer did by this point in skill development. For small-bodied birds, that bore more predation risk, there was high human vigilance and frequent observation of the birds.

3.2.8.8. Flying in a group

In observations of the small and large-bodied flocks, mixing novice and more experienced birds was done whenever possible to increase the safety of the novice birds. It was observed that behavior acquisition of the first few birds to master level four took months. However, when new birds were added to a group that was already competent at level four, some new birds developed mastery in just a few weeks. Having a flock of similarly sized, socially banded birds, allowed the juvenile birds to quickly learn information about how to react to the environment. Quick mastery also required repeated predator interactions in a short time, which was not always possible.

3.2.8.9. Feeding on plants

Occasionally birds would fly by with berry mash on their beaks, partially full crops, or other evidence of unseen plant consumption. Care was taken that the level four landscape did not provide novel, dangerous plants that had not been trained upon in the level three landscape. Having the ability to follow up on the get off cue by physically reaching a bird improved the frequency of the bird responding to the get off cue and that ability was reduced in level four locations.

3.2.8.10. Situations special to level four

Level four predator interactions were both planned and unplanned, making fundamental level three skills important when flying the birds in a new location that was not thoroughly scouted. For example, during what was supposed to be a low-risk visit to the heavily developed Texas A&M University Campus for a demonstration flight, the sun parakeet flock faced an immediate surprise pursuit before they were oriented to the new landscape. Two accipiter hawks pursued the sun parakeet flock, hunting as a pair. The hawks were unsuccessful and left the area. Practice in level two and three conditions prepared the birds to successfully evade novel predators in a novel location.

The large-bodied and small-bodied flocks were observed successfully avoiding stooping attacks by falcons, extremely close, high speed chasing by hawks, and approach by ground predators. Early mobbing behaviors, of agitated chatter and approach of predators by the flock, observed in earlier levels, in rare circumstances grew to be aggressive, unrelenting mobbing in level four conditions.

3.2.9. Level five

3.2.9.1. Landscape setting

An important difference from level four to level five is the impossibility of emergency retrieval of the birds by a trainer, meaning mistakes made by the birds have a greater chance of being fatal. Landscape features that prevent access include islands surrounded by swift rivers, canyons with no foot trails and steep walls, or dense forest too vast to be searched on foot (Table 6). For example, a level four area may have a body of water with a boat dock and immediate access to a motorboat, while a level five area lake would have no water vehicle access. Non-access was often defined by more extreme landforms, such as deep canyons, or non-traversable mountainous areas. Biotic factors include immediate, serious predator threats, and dense closed canopy forest that obscures active predators.

3.2.9.2. Goals

The goal of level five flying is to train birds to function fully independently of human guidance in situations where retrieval by the trainer is not possible. The criteria and environmental features of the level indicate independent flight (Table 3.6).

Table 3.6. Level five environmental characteristics and mastery criteria for parrot free-flight training.
Some birds were observed to reach five mastery within six total months after fledging but outcomes were highly individual. Level five, if reached, was normally reached before two years of age.

Environmental features	Mastery criteria
• Extreme elevation changes and landforms. • Low visibility due to precipitation. • Large bodies of water or swift-moving water. • Immediate predation threat from determined predators. • Retrieval not possible due to landscape or lack of specialty vehicles.	• All previous criteria. • Function completely independently between recall cues. • Safely negotiate immediate and serious predator threats. • Intelligent disobedience, refusing to respond to human cues if there are hazards present.

3.2.9.3. Rally vehicle and anchor bird

Anchor birds were not typically utilized at level five locations as the landforms and distances involved meant calls between birds were inaudible. To help with fidelity to the rally point, the rally vehicle was typically placed on top of a rim, hill, or in the middle of a valley where the vehicle was readily seen and located again by the birds. A moving vehicle was the most visible way to communicate when vocal recall cues went unheard, and was occasionally used to communicate that the flock was going to head back to home base.

3.2.9.4. A to B flying

A to B flying was occasionally used where the pre-planned placement of humans in different areas allowed birds to fly back and forth in the landscape to desired locations. Otherwise, inaccessibility prevented placing portable perches or other trainers around the area.

3.2.9.5. Recall cue

The recall cue was conditional. If the birds did not feel safe recalling, the trainer was patient and waited until the birds felt safe to return. This might mean being out at the rally point for hours beyond a planned schedule should predator conditions keep the birds defensively perched rather than flying. When birds left contact call distance, trainers waved and visually signaled their locations to the birds to get the birds' attention and encourage the birds to return to audible range.

3.2.9.6. Get off cue

The get off cue was effectively useless due to distance and inability to reinforce the cue. Physically approaching and shooing the birds was no longer feasible should the birds ignore the get off cue.

3.2.9.7. Human alarm call

Level five involved the birds flying out of hearing range, so the trainer was not able to alert them to approaching threats and could not come to help them. Waving to signal a need for increased attention to the environment was utilized as an alternative.

3.2.9.8. Flying in a group

The small and large-bodied flock members that flew at level five conditions flew only in groups due to the imminent danger from predators.

3.2.9.9. Feeding on plants

Feeding on plants was observed to occur fully independent of the trainer’s ability to intervene as the birds might be observed through binoculars, outside of hearing distance.

3.2.9.10. Situations special to level five

Not all parrots in the large and small-bodied flocks reached level five conditions, nor were all possible level five conditions appropriate for the birds. Biro chose not to fly all birds in inaccessible areas with extreme conditions. For example, the smallest birds were not intentionally let out into strong wind shear on the side of a cliff, while macaws were. The greater mass of the macaw made navigating significant wind shear realistic and challenging as opposed to impossible. The macaws were observed to engage in play behaviors in the wind shear, such as hovering above a cliff edge. Matching birds to appropriate level five conditions for training was an individual and species-specific process. Level four conditions are frequently similar to level five, differing only on landscape access for the trainer. The trainer's ability to access an injured or struggling bird was an important factor in choosing a level five location. At level five there was no ability for recovery or rescue, emphasizing the need for fully independently functioning birds.

3.3. Results

A total of 37 parrots across three free-flight flocks logged a total of 501.2 flight months during this study. Total combined mortality during outdoor flying was 6 birds or 16%. The causes of outdoor flying mortality were human environmental hazards (pesticides n=2, powerline n=1, wind turbine n=1) and weather associated with flying birds in cold climates (n=2). Birds that did not suffer mortalities but became house pets were considered retired.

The large-bodied flock was flown over a period of 13 years. For the eight members of the large-bodied flock, total flight months were 147.3, mean ± 3.2 standard deviation. The longest membership was 25.5 flight months over nine years for a scarlet macaw, who was retired, the shortest membership was 15.3 flight months over seven years for a blue-throated macaw, who was also retired.

The small-bodied flock was flown for 16 years. For the 25 members of the small-bodied flock, total flying months were 349.5, mean 15.2 ± 7.6. The longest membership in the small-bodied flock was 38.5 flight months over a 16-year span for a burrowing parrot, who was retired. The shortest membership was 0 flight months for a Senegal parrot and sun parakeet that were not bonded to a human trainer, and who escaped prior to starting outdoor training, and were never recovered. These two bird's zero values of outdoor training duration were omitted for the purposes of calculating means and standard deviations.

The sun parakeet flock was flown for one year, total flight months were 4.4, mean 1.1 ± 0. All birds from the sun parakeet flock were retired after one year, with no early exits from the flock.

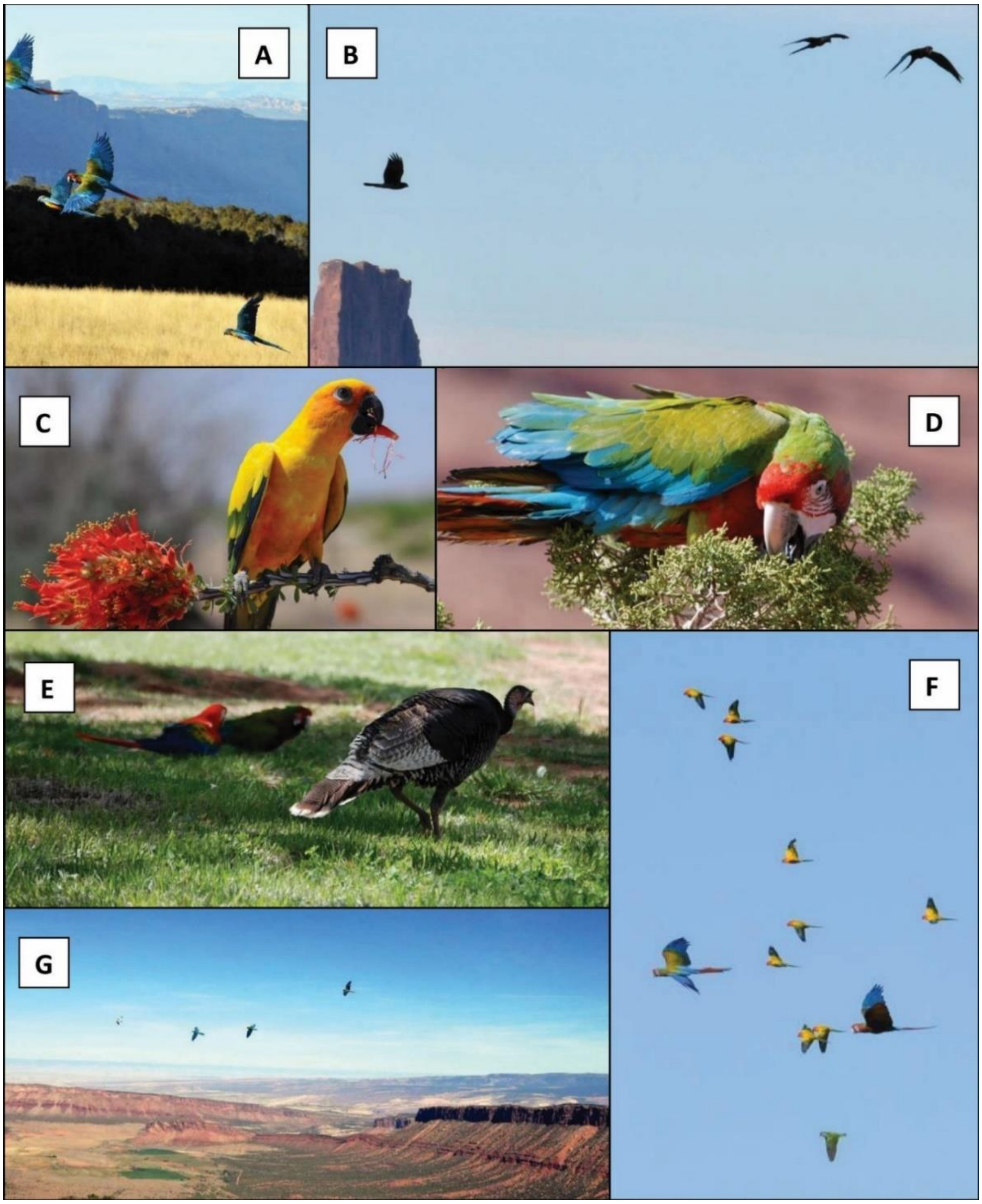

Figure 3.3. Survival behaviors in free-flight trained parrots.
(a) Large-bodied flock coordinated during an escape launch. (b) Blue-throat macaw and hybrid macaw evade a hawk. (c) Sun parakeet foraging on ocotillo (*Fouquieria splendens*) flowers. (d) Hybrid macaw foraging on juniper (Genus *Juniperus*) berries. (e) Scarlet and hybrid macaws forage alongside a wild turkey, *Meleagris gallopavo*. (f) Multispecies flocking in response to a predator. (g) Large-bodied flock engaging in long-distance navigation.

3.3.1. Predation and mortality

No birds were killed by predation even though they flew in predator-rich environments. Resident predators at the Moab, Utah location included observed accipiter hawks, buteo hawks, peregrine falcons, golden eagles, coyotes, foxes, and bobcats. The two long-term flocks, small-bodied and large-bodied, were primarily flown in a hawk migration area. The largest observed migration was a kettle of 197 hawks, counted by CJW from photos. The Dripping Springs, Texas location included various observed *Buteo* hawks, accipiter hawks, feral domesticated cats, foxes, and coyotes. Mortality (Table 7) was primarily due to husbandry issues. Of the 37 birds studied between 1997 and 2017, 11 died during this study period during captive management. These deaths occurred unrelated to outdoor training, such as dying naturally during sleep, or accidental escape of a young bird before any training began. The death during husbandry and training combined translates into a mortality rate of about 45%.

To understand this mortality in terms of risk over time in outdoor environments, the Mayfield method (Mayfield, 1961) was utilized. For accuracy, the calculation did not include the two fledged chicks that escaped before the start of outdoor training. During birds' first year of flight months, there was 100% annualized daily survival probability during outdoor training. During the first year, 6 birds were considered husbandry-related moralities, creating a 59% annualized daily survival probability related to handling and care. Post-first year, survival probability in training decreased to 77%. Post-first year captivity and husbandry survival probability was 60%.

3.3.2. Flocking and responses to predator threats

Flocking skills were built incrementally during training stages. During level zero training, hand-fed chicks flew as a group to be fed when formula was presented, practicing the fundamentals of group flight. The birds also tended to follow one another around the human home while expanding their activity area from the playpen. Social play during flight consisted of chasing, following, and pouncing, such as landing by grabbing the tail of a flockmate. During level one, the groups became more cohesive, with birds increasingly seeking to remain with the group. During level two defensive flocking was developed through repeated interactions with harassing birds. Sometimes, coordination would be developed from a single, prolonged set of interactions with a particularly tenacious wild bird, such as a black vulture that hopped from tree to tree following the sun parakeet flock over the course of an hour. In other cases, interactions with multiple wild birds formed the basis of a predator response. After each iteration or harassment, flocking behavior became more cohesive, forming coordinated vigilance, escape, and mobbing behaviors as seen in wild birds. A gull or a jay that might initially scatter the birds during early interactions, would face a coordinated, alarm calling group during subsequent interactions. Once birds gained level two mastery, flocking behavior was highly developed and constant in all three flocks, with birds seldom leaving the line of sight of the group. Coordinated alarm calling and escape occurred at that time. Level three training created discrimination between non-dangerous wildlife and animals that posed a predation threat.

Interactions with predators were primarily avian predators. It is estimated that over 100 aerial predation attempts were observed across the three flocks, primarily hunting attempts by bird-hunting *Buteo* species and *Accipiter* hawks. When a predator was observed, typically one bird would alarm call and launch into flight, immediately its fellows launched as well. All three flocks responded to predator observation with a pattern of identification, alarm call, launching and forming tight flying groups, predator avoidance, effective perching for escape, and exhibiting wariness. All three flocks utilized loud, continuous vocalizations in the presence of predators. If the birds were already airborne when a predator was observed, an initial bird would alarm call and the birds would form into a group while already in the air.

Table 3.7. Outcomes for three free-flight parrot flocks from 1997-2016 flown in the continental United States. Of 37 birds, six died due to abiotic hazards in the environment, eleven died due to husbandry-related issues. LB is large-bodied flock, SB is small-bodied flock, S is sun parakeet flock. Flight months are defined as 30 twelve-hour days flying in wildland spaces. Age level 1 is the age, in months, when a bird began flying outside. The level attained is the highest level on the free-flight Biro system of 0-5 environmental complexity.

Species	Flock	Age level 1	Start level 1	End training	Membership months	Flight months	Level attained	Fate
Blue & Yellow Macaw	LB	3	Apr-00	Apr-07	84	21	4	Wind turbine mortality
Scarlet Macaw	LB	3	Oct-04	Mar-13	102	25.5	5	Retired
macaw hybrid	LB	3	Jul-06	Mar-13	78	19.5	5	Retired
macaw hybrid	LB	3	Jul-06	Jan-12	66	16.5	5	Aviary fight mortality
Blue-Throated Macaw	LB	3	Jul-06	Mar-13	66	16.5	5	Retired
Blue-Throated Macaw	LB	3	Dec-06	Mar-13	61	15.25	5	Retired
macaw hybrid	LB	12	Oct-07	Mar-13	66	16.5	5	Retired
macaw hybrid	LB	3	Oct-07	Mar-13	66	16.5	5	Retired
Mean ± SD		4.1 ± 3.0				18. 4± 3.2	4.9	
Patagonian Parrot	SB	3	Jun-97	Mar-13	154	38.5	5	Retired
Mitred Parakeet	SB	3	Jun-97	Aug-06	99	24.75	4	Electrical line mortality
Mitred Parakeet	SB	3	Jun-97	Aug-04	75	18.75	5	Pesticide mortality
Mitred Parakeet	SB	3	Jun-98	Jul-07	87	21.75	4	Aviary fight mortality
Mitred Parakeet	SB	3	Jun-98	Aug-04	63	15.75	4	Pesticide mortality
Sun Parakeet	SB	3	Apr-99	Mar-07	94	23.5	4	Aviary fight mortality
Sun Parakeet	SB	3	Nov-04	Mar-13	101	25.25	5	Retired
Sun Parakeet	SB	3	Nov-04	Nov-06	24	6	4	Husbandry issue

Table 3.7. Continued

Species	Flock	Age level 1	Start level 1	End training	Membership months	Flight months	Level attained	Fate
Sun Parakeet	SB	3	Nov-04	Nov-06	24	6	4	Husbandry issue
Mitred Parakeet	SB	4	Feb-08	Mar-13	61	15.25	5	Retired
Sun Parakeet	SB	3	Mar-08	Mar-13	60	15	4	Retired
Sun Parakeet	SB	3	Mar-08	Mar-13	60	15	4	Retired
Sun Parakeet	SB	3	Mar-08	Mar-13	60	15	4	Retired
Sun Parakeet	SB	3	Mar-08	Mar-13	60	15	4	Retired
Sun Parakeet	SB	3	Mar-08	Aug-10	30	7.5	4	Natural death
Sun Parakeet	SB	3	Mar-08	Feb-13	60	15	4	Weather mortality
Sun Parakeet	SB	3	Mar-08	Feb-13	60	15	4	Weather mortality
Sun Parakeet	SB	3	Mar-08	Mar-13	60	15	4	Husbandry issue
Sun Parakeet	SB	3	Nov-08	Nov-08	0.1	0	0	Husbandry issue
Senegal Parrot	SB	3	Mar-08	Mar-11	36	9	3	Husbandry issue
Sun Parakeet	SB	3	Mar-10	Mar-13	36	9	4	Retired
Sun Parakeet	SB	3	Mar-10	Mar-13	36	9	4	Retired
Senegal Parrot	SB	5	Mar-10	Mar-13	34	8.5	5	Retired
Senegal Parrot	SB	5	Mar-10	Mar-10	0.1	0	0	Husbandry issue
Mean ± SD		3.8 ± 0.57				15.2 ± 7.6	4.2	
Sun Parakeet	S	3	Jul-15	Jul-16	12	1.1	3	Retired
Sun Parakeet	S	3	Jul-15	Jul-16	12	1.1	3	Retired
Sun Parakeet	S	3	Jul-15	Jul-16	12	1.1	3	Retired
Sun Parakeet	S	3	Jul-15	Jul-16	12	1.1	3	Retired
Mean ± SD		3.0 ± 0				1.1 ± 0	3.0	

The large and small-bodied flocks were observed in some cases mobbing predators and strange animals that approached the flock. Mobbing was a spectrum of behavior, ranging from tentatively approaching the target while the group alarm called to the extreme of chasing and biting. Typically, the flock's alarm called and stood their ground, facing the target as a group. CJW only observed one instance in the large-bodied flock and one instance in the small-bodied flock where flock members aggressively chased down a target.

In one instance of extended mobbing, the large-bodied flock drove a golden eagle that approached the flock out of a valley and up over the cliff rim about 2km away for approximately 10 minutes before breaking off pursuit. The small-bodied flock showed high aggression when they chased a pet parrot that flew into their midst. The flock surrounded the bird in the air, physically pushed the offending bird to the ground and forced it to land, where a trainer broke up the skirmish.

3.3.3. Behavioral outcomes

The two long-term flocks were outdoors regularly for long durations. The large and small-bodied flocks would be regularly free-flown in the area around the home base, ranging up to 2km normally. The two flocks occasionally flew further away when at the home base but excursions were difficult to verify due to a lack of telemetry. The conditions at the Utah home base ranged from level 2-4, based on predator presence and weather. The normal flying day was approximately 12 hours a day of flight time, varying on seasonal day length. Outdoor flight time for the small and large-bodied flocks involved periods of no supervision, estimated to be up to two hours, while trainers were in a nearby building. There were almost always more experienced birds present at the home base when new juvenile birds were let out to free-fly at the Moab, UT, home base. Occasionally, birds would not recall at the end of the day and would overnight outdoors but the frequency of these overnights was not recorded. The sun parakeet flock home base in Dripping Springs, TX, was adjacent to a forest area ranging from level three to four, requiring the development of level three skills prior to flying at the home base. Their free-flight sessions were up to six hours a day, with no overnights outdoors. Experienced birds were less often present at the home base when the sun parakeet flock was free-flown due to the difficulty of casual tracking of birds in among the dense trees.

3.3.3.1. Landscape navigation

All three flocks showed strong site fidelity to the home base. The behavioral control of the recall cue allowed the birds' behavior to be shaped. No birds permanently left the home base site during training. Non-fidelity was seen in two fledged birds that escaped from the small-bodied flock prior to the start of formal outdoor training, a sun parakeet and a Senegal parrot, in Table 3, showing zero hours of outdoor training.

Physical fitness was the pre-requisite for distance flying and was developed early in training, starting at level one. The birds in all three flocks made extended flights as a form of social or individual play. Play flying was indicated by non-aggressive aerial dog-fighting and jinking. Aerial circling in response to novel situations or wildlife presence was common, with investigative flights greater than 10 minutes of length regularly observed.

The free-flight training occurred in multiple spatially disparate landscapes. Through experience, the birds learned to navigate in novel landscapes. Through repetition, birds learned to travel between adjacent training locations. Travel involved repeating the route of the rally vehicle, the foot route of the trainer, or through aerial observation (developing novel direct aerial routes between landmarks). The large and small-bodied flock could return home or fly to the next location spontaneously. The large-bodied flock executed the longest spontaneous navigation, an 11km independent flight, to return to the home base after training rather than following the rally vehicle along the winding road.

Practice within the landscape focuses on navigating cliffs, canyons, hills, trees, and other landscape features at each level, emphasizing staying up high and enabling maximum line of sight for the three flocks. Birds flew over and not through heavily treed areas when navigating between locations, stayed above narrow canyons, and perched at the highest point of landforms whenever possible. The only flock that did not go between identified training locations spontaneously was the sun parakeet flock, as they were in a semi-rural residential area, where it was not possible to fly between areas without disturbing property owners. These birds were trained to return to a bird carrier when training was completed outside their home base, as opposed to following a rally vehicle to learn a route home. The birds mastered these features at each level of increasing complexity before moving forward. For example, macaws would dive off a localized 4m bluff with a hiking trail at a level three location until the usage of that landscape feature was mastered, while a trainer above and below uses A to B flying to encourage diving. For level four, those same macaws dove and rode the air currents down a landscape-size, steep cirque, where the trainer had less access and ability to interact. At level five macaws fully and independently navigated major canyons that took up all the visible geography and was not accessible to the trainer.

3.3.3.2. Foraging on wild foods

All three flocks were observed feeding on local plants (Figure 2). In all three flocks, all the birds routinely consumed the berries of junipers (Genus *Juniperus*), and specific individuals occasionally ate maple (genus *Acer*) seeds. The birds of all flocks daily chewed on leaf buds, seeds, and catkins of local trees but it was difficult to tell if birds were consuming items or only destroying them. The birds from the small and large-bodied flocks were seen feeding on flower buds and fruits from a variety of trees, shrubs, cactus, and ornamental fruit trees. The two longer-term flocks were also regularly seen with red or purple staining on the beak suggesting they consumed a variety of berries of unknown species.

The three parrot flocks were daily joined by other wild birds that were nearby foraging on the ground, in adjacent trees, or the same tree as the parrots. These associations withwild birds appeared to create temporary foraging assemblages with other species, where the wild species could transmit information or model behaviors to the parrots. At least one time the large-bodied flock was observed dropping to the ground to search for food in the grass with a wild turkey, (*Meleagris gallopavo*), despite the flock's training to stay off the ground (Figure 2). The turkey and macaws foraged safely within this novel multi-species complex, the macaws' non-wary behavior suggested this event had previously occurred. When foraging wild birds, most often doves and songbirds, flushed or alarm called, the flocks being trained increased wariness or might, themselves launch into the air demonstrating learning of hetero-specific signals and behavioral cues.

3.4. Discussion

The free-flight training methods appear to have successfully developed necessary skills in flocking and predator evasion, navigation of complex landscapes, and wild food use in these hand-raised parrots. These successes align with the goals of parrot pre-release training (Brightsmith et al., 2005; White et al., 2012) and show a methodology that can avoid skill deficiency and aberrant behavior associated with hand-raised parrots (Snyder, Koenig, Koschmann, Snyder, & Johnson, 1994). This method of human-guided learning could have conservation applications.

3.4.1. Flocking, predation and mortality

Captive-bred pet trade parrots may lack vital survival skills, such as being able to form a cohesive flock (Snyder et al., 1994) and are considered unsuitable for release due to inadequate behaviors that defend against predation. Predation is a major cause of failure in parrot releases. A review of 100 releases for 10 species showed that high predator presence was the main predictor of release program failure (White et al., 2012). Related to predator avoidance, the birds in the three flocks demonstrated appropriate behaviors including identification of predators; flocking behaviors that led to escape, increased vigilance, mobbing; and evasive landscape use by the group. There were zero predation events in the studied flocks despite multiple observed interactions with predators, a contrast to projects with drastic declines of released birds and failure to establish a second-generation due to predation (Snyder et al., 1994; Ziembicki, Raust, & Blanvillain, 2003) Flocking behavior is a key part of predator evasion.

3.4.2. Landscape use

In the Yellow-shouldered amazon releases (Sanz & Grajal, 1998), birds that ranged farther had higher survival, indicating that there may be beneficial applications of training free-flight flocks to navigate between human-selected areas. Home base level appeared to effect range size in the free-flight flocks. The level three environment around the sun parakeet flock home base was a limitation. The sun parakeets could not be easily tracked in the dense trees so they were given more limited flying time at the home base and recalled before going very far. By contrast, the small-bodied and large-bodied flocks, had open level two surroundings at the home base and could be easily tracked. The long-term flocks spent more time expanding their home base area through exploration, in addition to taking specific training trips away from the home base.

Ranging in the landscape is different than leaving the intended release area. The free-flight trained flocks did not have issues leaving the intended flight areas. During thick-billed parrot and scarlet macaw releases (Brightsmith et al., 2005; Snyder et al., 1994) birds permanently left the intended release area, reducing the success of the projects.

There are animal behavior theories related to why released animals leave high-quality release sites, never to be seen again. Natal habitat imprinting (Davis & Stamps, 2004) has been studied in multiple bird species. The theory is that young animals develop a preference for habitat features from their early experience and will seek to find conditions that match their early experiences. Translocated or aviary held birds might not be able to find their preferred conditions after release. The free-flight flocks experienced landscapes during their early development. They may have been imprinted on the environments they encountered during their early development and been attracted to the features, assisting in site fidelity. The behavioral control through the socially bonded trainer combined with recognition of the landscape as an acceptable environment, were likely pressures that kept the birds local to the rally points and home base.

Human-directed landscape use in the free-flight flocks trained the two long-term flocks to find high-quality patches in a semi-arid and marginal landscape. The birds flown near springs and streams with fruiting trees and shrubs knew how to travel between these patches. White et al (White et al., 2012) identified habitat quality as an indicator of release success. Free-flight training to utilize distant, high-quality patches suggests a new way to improve habitat quality by training the birds to go between specific locations of high quality.

Mixed species foraging assemblages have been theorized to have multiple benefits (Diamond, 1981) but these community-level interactions are not discussed in parrot release literature. Experimentally, multispecies bird flocks are more likely to successfully utilize a novel food source (Freeberg, Eppert, Sieving, & Lucas, 2017). Multi-species species flocking might help naïve released birds utilizes food sources and is an area in need of study. During foraging in the landscape, all three flocks free-flight flocks foraged alongside other prey species engaged in similar foraging behavior. When not physically near other groups of birds, the three flocks had increased wariness and scanning in response to alarm calls or flushing of other birds. Eavesdropping on the signals of other animals, when not participating in a multi-species flock, can also confer survival benefits. Inter-species information transfer between bird species (Fallow & Magrath, 2010) as well as information transfer between different taxonomic classes (Schmidt, Lee, Ostfeld, & Sieving, 2008) is believed to improve predator avoidance.

3.4.3. Similarity of free-flight training to previous methods

The level of human effort for free-flight training is comparable to other intensive bird management schemes utilizing hand-rearing, wild nest management, cross-fostering, or utilization of an intensive soft release program. (Ewen, Armstrong, Parker, & Seddon, 2012; Fund et al., 1983; Lloyd & Powlesland, 1994; White Jr et al., 2005). The echo parakeet methodology included a recall cue to bring birds to a home aviary, where supplemental food was provided (Woolaver et al., 2000). The echo parakeets had increasing exposure to the home base environment, through longer and longer outdoor periods between recall, until they were free-living. In the yellow-shouldered Amazon project, the conspecifics in an outdoor aviary appeared to function as anchor birds, as immediately after release birds perched on caging that still contained conspecifics. Released birds returned to the home aviary months after release. The fidelity to the home aviary site was greatest with younger birds (Sanz & Grajal, 1998). Non-lethal exposure to predators to learn they are dangerous is the state of the art in parrot release, as demonstrated by the Puerto Rican parrot project (White Jr et al., 2005). First the trainers passed a silhouette of a hawk over the cage while playing a hawk call. Then, a captive hawk attacked the aviary. The next training event was passing a hawk silhouette over the bird cage then having the captive hawk attack an armored Hispaniola parrot (*Amazona ventralis*). Whereas White et al utilized a captive raptor, free-flight training utilizes non-dangerous harassing birds present in the environment to build early group skills, then utilizes increasingly dangerous predator interactions in the field to train aversion to specific species. Predator pre-exposure training has been identified as a predictor of increased first-year survival after release (White et al., 2012).

3.4.4. Human-guided learning

The free-flight training method of skill gain involves shaping a flock's landscape use through human knowledge and intent. Effectively, the human is creating the landscape usage patterns the birds normally learn from their parents and conspecifics. Translocation of wild birds may be cited as a more useful tool than breed and release because adult wild birds possess a full complement of survival skills and knowledge (Silvy, 2012).

The flexible, "plastic" development of young parrots is not spontaneous, developing from extended environmental and social interaction (Mason et al., 2013). While translocation appears to be a more straightforward solution, historically, bird translocation success rates are low (Wolf, Griffith, Reed, & Temple, 1996) and perversely, the stressful act of translocation negatively impacts the survival behaviors that make it a desirable option, such as reduction of the fight-or-flight response (Wolf et al., 1996). In some species, conservation under the small population paradigm (Dickens & Romero, 2009) means there are not enough wild individuals to draw upon for translocation, leaving captive-release as the only remaining option.

The intensive developmental process that parrots undergo allows them to adapt to widely varying circumstances. Naturalized populations of parrots can adapt to strikingly dissimilar environments to their ancestral range, such as Amazon parrots in Germany (Martens & Woog, 2017) and other locations throughout the world (Jones, 2021). In the wild, behavioral flexibility allows wild parrots to quickly adapt to human-altered environments (Renton et al., 2015; Salinas-Melgoza et al., 2013) and transmit behavior socially between individuals.

Parrots are adaptable and develop survival skills special to their locations. The free-flight technique presented here appears to successfully utilize parrots' adaptive abilities to customize the birds' behavior to the locations and resources that the trainers want them to exploit.

Animal learning and culture can be considered a fourth level of biodiversity in addition to the ecosystem, species, and genetic diversity (Laiolo & Tella, 2007). Free-flight training may be a way to create learned behaviors necessary for a self-sustaining population.

3.4.5. Potential use of free-flight training in conservation

The levels system of 0-5 is a useful comparison of animal survival skills to the complexity of the environment. Looking back at the thick-billed parrot releases (Snyder et al., 1994), in light of the level system, the release cohorts lacked coordinated flocking responses to threats seen at level two. Based on the level system, where environmental predator presence was level 4 to 5, the thick-billed parrots would not be considered ready for release.

A parrot flock, as seen through free-flight training, can be behaviorally adapted to specific release conditions, using human knowledge to shape how the animals use the landscape. Free-flight training provides an opportunity to adapt de novo populations to human-altered landscapes. The method itself could be adapted to release. Free-flight trained birds could serve as a kernel of a wild flock, either producing their own young at the release site or being supplemented with human-wary birds. Depending on project needs, eventually, the human-socialized kernel could be recalled and removed from the site so all birds present would be wary.

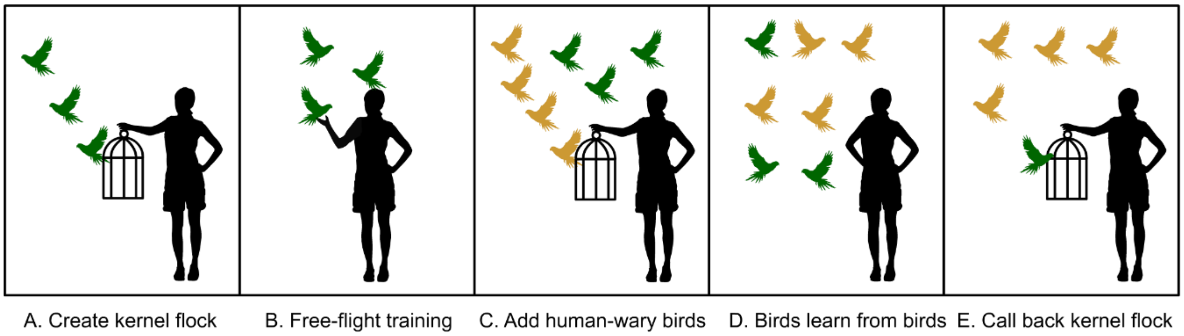

Figure 3.4. Potential integration of free-flight training for conservation. (a) A kernel flock is created from captive-produced birds. (b) Having trained a free-flying flock as a kernel, (c, d) human socialized birds are used to enculturate human wary birds with limited human interaction to produce a truly wild behaving, locally adapted, population. (e) Human socialized birds are recovered and held in reserve for future work or breeding efforts. The process could create a fully wild pioneer flock.

Generally, free-flight training opens new avenues for use of captive-bred parrots, and an alternate method of intensive management for translocation. Further work can illuminate if this privately developed method of sport flying captive-bred parrots can help increase success in releases of imperiled parrots.

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4. CONCLUSION: EXPLORING THE SOURCES OF DISCORD THAT AFFECT CONSERVATION PROJECTS

4.1. Introduction

We face massive environmental change and species extinction (Dirzo et al 2014) described as an emerging crisis for humanity (Dominey-Howes 2018). A large number of new conservation problems are expected (McCarty 2001) and will correspondingly need solutions. Teams of people with different expertise and access to different jurisdictions of knowledge and different types of natural resources will need to work together. In the past, the success of scientific problem-solving depended on the scientific community developing and sharing the same view, or paradigm, of the what's, how's, and why's that unite them. Shared paradigms are the basis for Normal Science (Kuhn 1970).

Today, conservation research exists in a Post-Normal Science (Funtowicz & Ravetz 1990; Ravetz 1999), with wicked time-critical problems that do not interact well with the pacing of the scientific method of Normal Science (Laurance et al 2012). These types of problems demand flexible, novel approaches. Ecological catastrophe cannot wait for scientific community-level paradigm shifts and the formation of new disciplines. While we deal with non-traditional conservation problems, we remain scientists. "A strong commitment to the application of the scientific method, including hypothesis testing and careful consideration and development of basic concepts, is essential." (Drew 1994).

This situation creates a dilemma for problem-solving researchers. There are questions about how conservation research teams should operate when problem-solving does not resemble Normal Science. How can team members interact if they do not share the value sets, language, and research approaches that make up each other’s paradigms? By understanding where and why conflicts occur and what research frameworks can sidestep those conflicts, we can design successful projects that minimize conflicts among team members.

Early-career scientists can be confused and surprised by the conflicts they encounter and may not have the concepts or background in scientific philosophy to understand what is happening when disparate disciplines interact. Rather than taking conflict as a sign of personal failure, I suggest reflecting upon scientific identities, roles, team building, and scientific philosophy to put conflicts in context. By understanding the basis of conflicts, strategies to reduce or eliminate the conflicts can be deployed. When conflict stops conservation progress, the crises created by wicked problems that loom over us continue to escalate.

4.2. Conservation research brings together different viewpoints

Conservation occurs across communities that necessarily encompass differing paradigms and or identities. Understanding the distinction between single disciplinary, interdisciplinary, and cross-boundary work sets up the actors who engage in conflict.

Conservation research is frequently team-based and interdisciplinary (Green et al 2015) or cross-boundary. Interdisciplinary work uses the resources of two or more disciplines of study (National Academy of Sciences 2005) such as hydrologists and biologists working together to find a method to best define a protected area for salamanders. Cross-boundary is different from interdisciplinary work. Cross-boundary is working across jurisdictions (Harris, Huntely, Mangle & Rana 2001). An example would be an ecosystem scientist from a university system working with a governing board of a protected land area to study what management strategies could apply to salamanders.

Cross-boundary problem-solving is well-described in conservation. An example is the coproduction of actionable science by scientist and resource manager teams (ACCCNRS 2015; Beier, Hansen, Helbrecht & Behar 2017). Conservation boundary organizations exist to bridge gaps between science and other areas, such as linking science to politics, policy, law, and resource management (Guston 2009). Conservation research is interdisciplinary and cross-boundary to such a degree as to be unusual to other disciplines.

4.3. Examples of conflict fueled by different viewpoints and beliefs about identities

Each academic discipline has its value systems, theory base, and research approaches, called domain specificity (MacLeod 2018), that form paradigms (Kuhn 1970) of normal operation. When people utilize different paradigms, their working together is complicated. The complications caused by disconnected disciplines, researchers, and practitioners of conservation are silo effects (Halpern, Hodgson & Essington 2019). A given conservation project may be fraught with contrasting, discipline-based perspectives related to the salience, credibility, and legitimacy of information used by one discipline or the other (Cook et al 2013). For example, the intersection of the disciplines of conservation and behavioral ecology is limited even though they appear related. Both deal with organisms functioning in their environments.

4.3.1. The trouble with disciplinary silos

When research foci have dissimilar primary goals, such as preserving biodiversity or evaluating evolutionary theory, respectively, the gap has been described as a bridge too far (Caro 2007) for successful overlap.

Silo effects can cause privileging of one discipline's paradigm over another's. In some cases, one discipline will overpower and subsume other forms of knowledge creation. The subsuming discipline will push to impose its discipline-specific practices, in place of existing strategies, as though colonizing the other discipline (Scott, Brown, Lunt & Thorne 2002).

The author's experience provides a concrete example. Imagine that young, captive-bred animals are being prepared for release into the wild as part of a study. The team's ecologist believes that animal learning systems are each a unique, species-specific product of evolution. The team's behavioral psychologist uses behavior modification techniques, a part of Behaviorism, to prepare the animals for release. The behaviorist believes that animal learning systems follow a general theory across species and operate by similar sets of rules. Both approaches work successfully in the respective areas of study. The ecologist finds the general rules for learning defined by Behaviorism to be flawed and considers that Ecology's methods offer the "true alternative" to "dethrone a dominant paradigm" of general learning theory (Johnston 1981). Trouble is brewing. The methods developed through psychology's Behaviorism are widely and successfully used in animal management programs (Pryor & Ramirez 2014). Due to the ecologist's perception that the basis for psychology's Behaviorism is flawed, the ecologist believes that Behaviorism does not have a place in a scientifically informed endeavor. The behaviorist quotes from a memorable publication, Behaviorism isn't Satanism (Barrett 2012). The ecologist insists their paradigm, the better form of animal behavior study, should be driving both the animal colony management and behavior comparison activities. The conflict becomes personal. They can no longer work together. This is a functional example of siloed perspectives leading to disciplines trying to subsume one another. The ecologist's opinion and the behaviorist's defense stem from disciplinary privileging and silo effects.

4.3.2. How a practitioner versus scientist dichotomy can cause conflict

Implementation is different from discovery. We call implementation, the practical use of the knowledge, applied science. Discovery is called basic science. They are two of the three kinds of research and development (NCSES 2018). Discovery and implementation interact to support each other. Research into a specific product or process is called experimental development, the third kind of research and development.

Where practical applied work belongs relative to theory development in science is an old debate. Sir Robert Boyle, a founding influence in modern science, wrote about the value of integrating both theory and hands-on work in response to others who felt they should be separate. Boyle noted that a true scholar of nature "[. . .] does not only Know many things, which other men ignore, but can Performe many things that other men cannot Doe;" Boyle stated how far he was from "effeminate squeamishness" of the "nice" people who do not do hands-on work (Boyle, 1663). Today, trends in academic science show the continuing tension between application and theory, such as fieldwork publications becoming less prestigious and popular in academia (Ríos-Saldaña, Delibes-Mateos & Ferreira 2018).

When people try to separate applied and basic sciences, it is the presentation of a "false choice" (Powell 2017). Though they are different approaches, they are symbiotic to one another (Leibowitz 1996) and both are key to scientific progress. A pure application of scientific findings, without research, may be called practice. However, the spectrum of theory, research, and practice can be understood in many ways.

Theory development, hands-on basic research, hands-on applied research, and technical practice differ, and academic programs integrate them differently into degree programs.

Doctoral degree programs exist where research and practice are strictly separate. Practitioner doctorates exist for areas such as education, business, and medicine (Lester 2004). For example, the M.D. degree has high engagement with theory and practice but low engagement in basic research. Alternatively, there are increasing university communities of practice, formed around a shared topic and interest, where members view themselves as practitioners within the topic area (Wenger 1999; Etzkowitz, Webster, Gebhardt & Terra 2000; Buckley & Du Toit 2010) even though they may be researchers or theorists. For some disciplines, there are historic models where research and practical work co-exist. In psychology, there is a well-established scientist-practitioner role, following the 1949 Boulder Model that blends research and practice (APA 2015).

Different assumptions about the integration of theory, research, and practice cause difficulties for conservation scientists. Problems arise when conservation scientists work in partnership with siloed traditional academic environments. Conservation scientists' work does not resemble their peers' when there is an intensive practical component or a primary identification as a practitioner. In a study that involved characterization of conservationists' identities as a scientist or a practitioner, participation in any practical conservation activity bestowed a practitioner identity (Meredith, Collen & Griffiths 2018). Such simplistic rules may be useful for study statistics but troublesome for identity politics. When viewed from the perspective of a too simplistic practitioner versus scientist dichotomy, there may be an uncomfortable implication that by directly engaging in conservation outcomes a practitioner identity wholly replaces a scientist identity. When the term practitioner is meant to imply non-scientist, of less scientific value, or separate from scientists despite engaging in research, important work may lose credibility. Such arbitrary hierarchies are detrimental to the overall goals of conservation.

Failure to integrate practice into science interrupts the discovery and application cycle. One artifact of that disconnect is the science-practice gap. Two kinds of science-practice gaps render field activities uninformed by research. The first type occurs when those in the field do not use existing scientific knowledge often due to a lack of access or awareness (Knight et al 2008; Arlettaz et al 2010; Esler et al 2010). Factors leading to the first kind of gap in conservation activities, including research, include lack of access to academic resources, such as articles and relevant experts. The second type of science-practice gap occurs when requisite scientific knowledge does not yet exist. There may be a lack of research relevant to the problem or management decision at hand (Fazey & Lindenmayer, 2005; Braunisch et al 2012; Laurance et al 2012). In the first case, those carrying out practical activities are un-informed through lacking the typical resources available to members of academic faculty. In the second case, existing research does not speak to the problem, and stronger interaction between the practitioner and the larger research community could lead to better solutions.

4.3.3. How we value parts of the scientific method causes conflict

How people value theory or practice can cause conflict as discussed above. Similarly, the enormous scope of modern science means one person may not be able to participate in all aspects of the scientific method. Scientists may engage with either the whole scientific method, primarily theory development, primarily testing, or primarily observation. Figure 1 outlines a relevant model of the scientific method.

Working in a single area of the scientific method may be required to move science forward. There is a paucity of information related to many conservation areas (Hawksworth & Rossman 1997; Morais et al 2013; Tedesco et al 2014; Bland, Collen, Orme & Bielby 2015) requiring the gathering of empirical data. Empirical conservation data may take many years to acquire through work in remote locations. It is possible to spend a fruitful and necessary career in the observation portion of the cycle, becoming specialized in collecting observations that are the foundation of the scientific method. This work can be highly rewarding and affect immediate conservation actions, such as when observing a population of thought to be extinct "Lazarus species" (Ryan & Baker 2016).

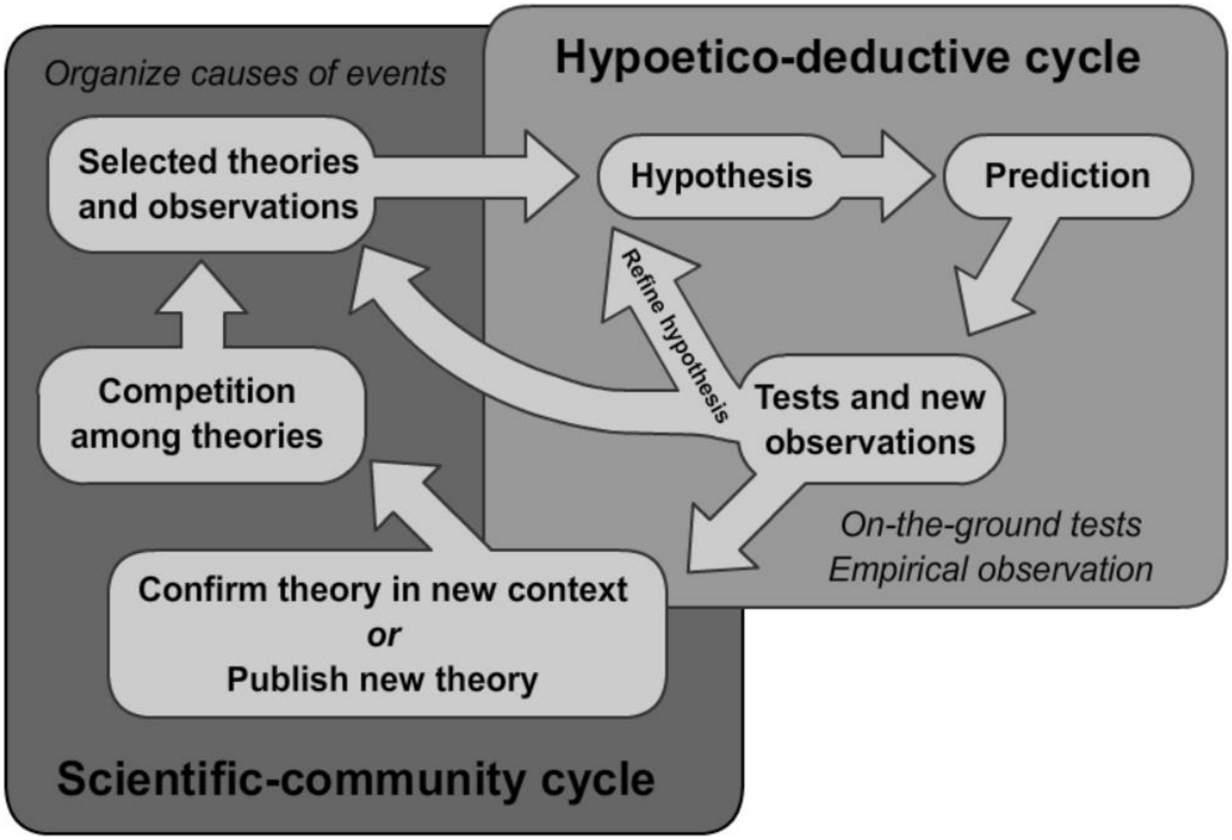

Figure 5.1 The scientific method, after Dodig-Crnkovic (2003).
The first step of this process is an observation to create empirical evidence. Observation can take the form of immersive roles, ranging from intensive data-collection field transects to participation in non-scientific activities to understand applicable methods or find knowledge gaps. Once there are observations, those observations can form hypotheses, and may act upon theory. If there is a paucity of observation, the theory will lack a rational basis and its utility is compromised. Especially in new, highly specific, non-generalizable conservation problems, theory development may be a secondary concern to creating the foundational body of observations.

Devaluing working in a single area of the scientific method can be traced back to long ago scientists. The philosopher Henri Poincare stated, “Science is built up of facts, as a house is built of stones; but an accumulation of facts is no more a science than a heap of stones is a house” (Poincaré 1905). This is an attitude that warns against observation without theory. Activities that lack the use of theory could be pure observation, sample collection, and cataloging. Those who perceive theory as intrinsic to science may devalue the individual who engages in observational activities, considering the individual as a non-scientist even if the larger body of science requires the observations to move forward. Philosopher Immanuel Kant advocated for balancing the extremes of only thought-driven knowledge creation versus only observation-driven knowledge creation, called pure rationalism and pure empiricism, by noting that each requires the other. His synthesis is popularly summarized that perception without conception is blind while conception without perception is empty (Kant, 1855).

The fieldwork scientists who conduct on-the-ground testing of an intervention, or collect observations, may refer to the theoreticians who do little experimenting or fieldwork as armchair scientists. The term armchair scientist is sometimes derisive, meaning an amateur non-scientist consumer of others’ work (Zerbe 2007, pp 125). The related and non-offensive concept, working from the armchair, describes a non-experimental approach to developing scientific information that has been used across time and disciplines (Nadel 1956; Mandelbaum 2006).

Recognizing that theory and practice activities are complementary parts of the scientific method, and that observation is foundational for theory development, can help ground non-traditional activities and scientist roles as valid parts of the scientific method.

4.4. Contexts that make conservation necessarily interdisciplinary, interjurisdictional, and different

Unlike traditional basic science, which fits Kuhn’s Normal Science model, conservation science may fit the definition of Post-Normal Science. In Post-Normal Science, the stakes are high, facts are uncertain, values are in dispute, and decision-making needs are urgent (Funtowicz and Ravetz 1990; Ravetz 1999). Scientifically-driven problem-solving in conservation has been called “use-based science”, “translational science”, and “actionable science” (Cook et al 2013). Time-critical and wicked problems that are hard to address with the scientific method may be those conservation problems most in need of scientific research (Laurance et al 2012).

4.5. The context of super wicked problems

The concept of wicked problems originated in the 1970s (Rittel & Webber 1973). A wicked problem has no stopping rule where the problem’s solution means that the work is over. There is no opportunity to learn through trial and error, no ability to immediately test the efficacy of proposed solutions, and being wrong is not an option.. The wicked problem concept has been extended for environmental problems that are super wicked (Levin, Cashore, Bernstein & Auld 2012). In super wicked problems, those who cause the problem are creating the solution, needed central authority is weak or nonexistent, while time is running out. Just before addressing the problem, its severity is irrationally discounted, so direct action is pushed into the future. Climate change and biodiversity loss are examples of these wicked and super wicked problems.

4.6. The conservation activities that don't resemble traditional science

Researching the efficacy of a conservation intervention may require the scientist to carry out the intervention. In conservation research, experimentally testing different conservation interventions is a requisite activity to preserve biodiversity (Drew 1994) and may cause the scientist to test a method in the field. Workers and supplies are often limited in the field, which could comprise situations ranging from a tundra field station to an open-sided two-person tarp shelter in the Amazon. Small teams, working in remote locations, necessitate that the scientist participates in implementing the intervention. This participation creates experts. Scientists who engage in interventions becomes competent to evaluate the efficacy of the intervention. Through participation, the feasibility and effects of an intervention are more apparent to conservation scientists.

The innovation and discovery that occurs while implementing a conservation intervention do not typically resemble traditional laboratory research, nor should they, as implementation differs from reductive hypothesis testing. Field-based activities lack the physical trappings of scientific disciplinary identity (Trowler 2001, pp 45-47) such as working in a building with other discipline-adherent individuals, working in an environment decorated with discipline supportive artifacts on desks or representations of shared idols such as a Darwin poster.. Fieldwork occurs without the physical identifiers of disciplinary science.

The activities to carry out an experimental conservation intervention may be what some consider technician practice. Universities are hierarchical and create conditions where support staff often face devaluing and bullying by those in superior positions (Björkqvist, Österman & Hjelt-Bäck 1994; Thomas 2005). Conservation researchers who do their own practical work may take the role of greenhouse manager, forester, zookeeper, animal breeder, behavior modification specialist, veterinary technician, or wildlife rehabilitator and may be placed lower on the academic hierarchy by those who devalue the practical and applied work as non-scientific. The theme of the invisible technician in science (Shapin 1989; Wilson 2012; Morus 2016) shows how extreme the disciplinary separation of carrying out work and conceptualizing work can become, where those who do the work may garner limited credibility as scientists even if they are contributing original innovations and methods.

When questions regarding the scientific merit of practical work arise, objective measures can help communicate the value of the work. The United States Environmental Protection Agency outlines when technician activities are elevated to scientific activities, requiring recognition of authorship. If an individual is “adapting or developing new techniques or equipment”, working on the “development of new methods or significant modification to existing methods essential to the research", or "analysis and interpretation of data" (Grifo, Russo & Otto 2016) then they should be authors on a paper. These guidelines illuminate the difference between creative, cerebral scientific activities that generate new knowledge, either basic or applied, versus carrying out repetitive technical tasks. Conservation scientists in this position may need to eloquently describe their practical activities as having scientific merit to establish or retain external validation of scientific identity.

The strong integration of practice and research is a successful approach to designing research interventions. An example is raptor conservation. The largest collection of successful techniques for working with raptors resides in the practice of falconry. Conservation has crossed jurisdictional boundaries to work with falconers. Falconry methods applied to conservation performed better than techniques developed from outside of falconry (Boyd & Schwartz 1991; Kenward 2009). Tom Cade, the founder of the Peregrine Fund (Anderson 2006), was a practicing falconer and had a Ph.D. (Cade & Blount 2018). The Mauritius kestrel project (Jones et al 1995), one of many falconry-utilizing projects was borne of accepting knowledge from across jurisdictions and building that knowledge into applied conservation research.

4.7. Research approaches to reduce interdisciplinary conflict

Funding a community of practice can deal with gaps such as the science-practice gap, and decreases the distances separating disciplinary silos or between jurisdictions. A funded community of practice covers the costs of bringing diverse individuals related to the topic. The individuals come together for regular communications that allow knowledge to be harnessed and shared. Watkins, Zavaleta, Wilson, and Francisco (2017) suggest forming funded communities of practice to manage information strategically, share experience, and share skills, among those who work within the shared topic.

4.7.1. A funded community of practice

They suggest that commitment and competence of individuals unified under the shared topic create a shared identity, which is probably a useful alternative to siloed identities. Benefits of a conservation community of practice introduce scientific findings into policy, unify vocabulary, and allow researchers who focus on knowledge gaps and practitioners who focus on implementation issues to share their needs with policymakers.

4.7.2. Transdisciplinary frameworks

Another method to work across disciplines is the transdisciplinary framework. The goal is to dissolve boundaries between traditional disciplines to focus on solving real-world problems, often in a research context. The setup acknowledges that the work done within the framework will rely on knowledge from many disciplines but will not follow the boundaries and rules of any one discipline. Transdisciplinary frameworks are widely discussed as valuable to conservation work (Reyers et al 2010; Torkar, G. & McGregor 2012). A review of transdisciplinary frameworks in conservation noted that relating to conservation research frameworks, there is a lack of a commonly shared framework, glossary, or communication platforms (Brandt et al 2013) so there is not a single best approach known for transdisciplinary conservation frameworks.

It may be possible to use elements from developed transdisciplinary frameworks that are not conservation-focused when setting up research projects for conservation. Developed biomedical transdisciplinary frameworks include One Health, Conservation Medicine, EcoHealth, and Global Health (Kaufman, Epstein, Paul-Murphy & Modrall 2008). One Health seeks to unify human, animal, and ecosystem health under the concept that health unites all species, creating shared ecosystem-level health (Zinsstag, Schelling, Waltner-Toews & Tanner 2011; Kahn, Monath, Bokma & Gibbs & Aguirre 2012). A detailed, step-by-step One Health Framework, from identifying collaborators, to choosing methods of analysis, has been outlined by Lebov et al (2017). Lebov et al note that those who have on the ground knowledge needed for research development should be included.. Such individuals might include a SCUBA diver, wildfire fighter, or a plant worker.

4.7.3. Translational science

Biomedical sciences offer a sophisticated problem-solving science model. The United States' National Center for Advancing Translational Sciences (NCATS 2020), in the National Institutes of Health, focuses on interdisciplinary problem-solving science. Austin (2018) discusses that in the NCATS approach, the role of basic research is to understand and the role of translational science is to fix, in a complementary, mutually informing relationship. NCATS defines translational sciences as “the multi-step process of turning observations in the lab, clinic, and community into interventions.” Team members each have a domain of expertise and work together to apply their expertise to scientific problem-solving.

The translational scientist's skills are identified by NCATS as being a boundary crosser, team player, process innovator, domain expert, skilled communicator, rigorous researcher, and systems thinker (NCATS 2020). Major ethics inherent to the NCATS model of translational science include process transparency and data sharing (NCATS 2020). The interdisciplinarity, systems thinking, and domain expertise overlap with foci for conservation communities of practice, as well as transdisciplinary frameworks.

4.7.4. Growing past conflict to transform science

As conservation scientists, we have varied approaches, identities, and wicked problems to work through. Post-Normal science requires us to do novel, unexpected things to ameliorate the biodiversity crisis while validating our actions through the scientific method. How we manage the conflicts that arise is based on our ability to recognize where the problems come from and utilize frameworks that will reduce conflicts and maximize the success of our work.

As we solve individual conservation research problems, we find new methods, develop technologies, and can more effectively preserve biodiversity. Through new approaches and innovation, science transforms itself. Transformational science results in revolutionizing existing fields, creating new subfields, causing paradigm shifts, supporting discovery, and leading to radically new technologies (National Science Board 2007). With such new attitudes and integrated approaches, conservationists will be better equipped to solve the wicked problems of massive environmental change and species extinction, creating humanity's most profound emerging crisis.

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APPENDIX A

WATER QUALITY FOR GUEST HEALTH AT REMOTE AMAZON ECOTOURISM LODGES[*]

A.1. Introduction

Ecotourism can be a major income generator and support local economies (C. A. Kirkby et al., 2010) in one case offering nearly double the income of existing opportunities (Hunt, Durham, Driscoll, & Honey, 2015). Conservation scientists note the positive effects of ecotourism beyond building local economic capacity. Ecotourism businesses can reduce threats to the environment such as logging and the hunting of local wildlife. In some instances, the continued survival of individual animals or species is contingent upon the presence of ecotourism (Buckley, Castley, de Vasconcellos Pegas, Mossaz, & Steven, 2012). In addition, eco-tourism can provide research stations and funding for scientific research that furthers conservation as a science (Brightsmith, 2004). However, remote tourism lodges may have relatively small profit margins and relatively high fixed expenses (C. A. Kirkby et al., 2011). This can make remote ecotourism lodges susceptible to income interruptions.

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Preventing remote rainforest ecotourism failure is increasingly important to local economies and ecosystems as the number of lodges increase. Remote rainforest tourism is a growing form of ecotourism. (Torres-Sovero, González, Martín-López, & Kirkby, 2012). A simple search on TripAdvisor.com for “rainforest camp” returned the website’s maximum display of 1,020 locations. These accommodations typically involve a camp or lodge setting in intact forest located outside of traditional utility grids. Being “off-grid” requires highly technical management to produce potable water for guests and managing use of local water bodies. Without careful management, diseases acquired by tourists from drinking, bathing, and swimming, can have direct effects on economic development (World Health Organization, 2013) and severely impact these remote lodges. A fundamental global practice to reduce disease in water is chlorination. When water is underchlorinated, disease microbes can exist and reproduce in the water (WHO, 2011). Though many developed countries have water quality standards in place, many of the countries in the Global South are still lacking necessary support to chlorinate water (Bhalotra, Diaz-Cayeros, Miller, Miranda, & Venkataramani, 2017).

The World Health Organization (WHO) considers small community-managed water sources, such as those utilized in remote ecotourism, especially prone to contamination (WHO/UNICEF Joint Water Supply & Sanitation Monitoring Programme, 2014). The most common illness acquired by tourists to the developing world is traveler's diarrhea (TD) (Connor & Riddle, 2013). Bacteria are thought to account for 80%–90% of TD cases (Connor & Riddle, 2013), which are often thought to be *E. coli*. When caused by

E. coli bacteria, TD will usually clear spontaneously but in some cases it can cause some serious or fatal diseases, including hemorrhagic colitis, hemolytic-uremic syndrome, and long term disease such as irritable bowel syndrome (Connor 2017; Nataro & Kaper, 1998; Noris & Remuzzi, 2005). Some destinations can have illness rates up to 70% for tourists (Connor & Riddle, 2013). Tourists from the developed world may expect a greater than 50% chance of becoming ill. (May, 1989).

In the context of ecotourism, guest health should be central to destination management to ensure the sustainability of tourism (WHO, 2011; Musa, Hall, & Higham, 2004). Ill guests can cause many difficulties for individual companies. When guests become sick they may change their itineraries, (May, 1989), and even brief bouts illness may lead to costly litigation such as in the case of a lodge's settlement of $10,000 per guest (Pineiro-Zucker, 2016). A severe disease outbreak can affect the industry through bad publicity and loss of revenue (Marti, 1995). In Mexico, an H1N1 flu outbreak in 2009 caused an estimated loss of 2.8 billion USD (Rassy & Smith, 2013). Tourists can return home carrying water-acquired diseases with them, causing international disease outbreaks, such as the case of hotels in the Canary Islands which shared an unsafe well. Guest of these hotels returned to four countries, bringing with them 15 cases of Vero cytotoxin-producing *E. coli* O157. (Pebody et al., 1999).

Overall, the tourism literature has limited discussions on tourist health. A "lack of research" connecting health and tourism was noted during the 1992 conference on Food

Protection and Tourism (Spivack, 1994). Over the last 25 years, arguably little advancement on the subject has occurred. Our search of the leading tourism journals (*Journal of Travel Research*, *Tourism Management*, *Journal of Sustainable Tourism*, *Annals of Tourism Research*, and *Journal of Ecotourism*) using terms, “E. coli” and “traveler’s diarrhea” returned only 11 results. While papers mentioned *E. coli* in various contexts, there was no focus on tourist health despite this being an extremely common tourist ailment.

The general water sanitation literature shows that relatively simple treatments remove the risk of waterborne disease (WHO, 2011). Typically, large particles are removed through settling if the water is not clear. Once visually clear, water is put through a nanopore filter to remove larger organisms, such as parasite eggs. Then, the water is chlorinated to inactivate bacteria and viruses. Enough chlorine is added so that after inactivating microorganisms there is still extra chlorine (“residual chlorine”) to prevent re-contamination of stored water by hands or utensils (Centers for Disease Control, 2014). When combined with hygiene of hands, drinking cups, and used water bottles (Rufener, Mäusezahl, Mosler, & Weingartner, 2010) most sources of illness can be eliminated.

When caused by water, as opposed to foods (TD is not always caused by the drinking supply), natural water bodies can be a source of disease. The water ingested while swimming (Dufour, Evans, Behymer, & Cantu, 2006) or boating (S. Dorevitch et al.,

2011) can cause illness. A study showed 1.5% of those who engaged in limited contact freshwater activities in natural water, such as kayaking or fishing, became ill afterwards (S. Dorevitch et al., 2012), whether or not the water body was directly impacted by sewage.

The lodges in the Madre de Dios region of Peru are heavily studied, with over 700 papers mentioning ecotourism in the region. The frequent publications make the region a model for remote rainforest ecotourism. We visited four lowland tourism lodges within Madre de Dios, to collect information concerning the utilization of chlorination, guest education, health tracking, and hygiene. (*combine this and the next paragraph) We tested drinking water, tap water, and rivers for fecal bacteria. We interviewed lodge staff about water issues and investigated health issues through anecdotal tracking of our own group participants and online guest reviews.

A.2. Methods

The study was conducted in the watershed of the Madre de Dios River in the lowlands of southeastern Peru in the areas surrounding the city of Puerto Maldonado (12° 35.226' S, 69° 11.820' W). Areas of forest are grouped into reserves and a park (SERNANP, 2018), most notably Manu National Park, (1 716 295 ha), Tambopata National Reserve (274,690 ha) and Bahuaja-Sonene National Park (1,091,416 ha). The region contains lowland tropical rainforest, with average annual rainfall up to 3,000 mm (Brightsmith, 2004; Vuohelainen, Coad, Marthews, Malhi, & Killeen, 2012). Certain charismatic megafauna can be reliably observed by visitors, including three large macaw species (genus *Ara*), giant river otters (*Pteronura brasiliensis*), five monkey species (suprafamily Ceboidea), and caiman species (subfamily Caimaninae). The region is also home to a form of strip mining along rivers to attain gold rich silt which encroaches upon protected lands (Gardner, 2012). The study area was visited during a 22-day period in May 2015. Three authors were present for the travel, CJW, AMV, DJB. CJW took and incubated samples, AMV interviewed Spanish-speaking lodge and protected area staff. DJB oversaw research activities and made introductions for interviews.

A.2.1. Study area

Four independently-owned lodges were included in this study. Each lodge required access via river boat and was off the traditional utility grids. As a result, all lodges provided their own electric, water, and sewage. Lodges could accommodate an approximate range of 10-50 guests per night. Per night stay cost between $40 and $160. To help ensure anonymity of the lodges who allowed us to test their water, the names and exact locations of lodges are withheld. Lodge A was a newly constructed primitive forest camp with an open, roofed dining and cooking area. Commercially bottled “water cooler” style bottles were hauled in by boat and not re-used. Wastewater treatment was a commercial biodigester. It was located at the intersection of a river and a tributary creek. The creek water was the source for the lodge’s washing water system. The creek was also used for swimming and occasional bathing. This was the only lodge with an open kitchen, all others had screening.

Lodge B was a high-end, resort-style lodge blending some features of a primitive camp with high-end vacationing, such as open-air structures with limited satellite internet, and an on-call masseuse. Local stream water was used for washing and showers. The stream water was filtered and ozonized and placed in reusable water cooler style dispensers for drinking water. Biodigesters processed wastewater. The lodge was adjacent to a major river, river swimming by guests was apparently possible, but discouraged by the company, and was not seen during the visit. This lodge was accredited through the Rainforest Alliance Certification Services for Tourism Businesses.

Lodge C was a bed-and-breakfast style accommodation built on a lake inland from the river system with a totally enclosed kitchen. Lake water was used for washing and showering. The lake water was treated with chemical tablets, placed in reusable dispensers, and offered as drinking water. Wastewater treatment was through a series of connected cesspits. The lake was utilized for boating, wading, and swimming.

Lodge D was a high-end lodge. Local creek water was used for washing and showers. The creek water was filtered and ozonized, placed in reusable water-cooler style dispensers, and offered as drinking water. Two biodigesters processed wastewater. The kitchen area was screened. The lodge was adjacent to a major river. River swimming occurred during the visit. This lodge was accredited through the Rainforest Alliance Certification Services for Tourism Businesses.

A.2.2. Sample collection

With permission from the lodge management, samples were taken from lodge water sources and the river access near each lodge. Samples were also collected from the river near Puerto Maldonado. Due to portable incubator size limitations, a single measurement was taken for each category. For tap water collection, researchers ran the faucet for 30 seconds then collected 50 ml water samples in sterile test tubes. Drinking water samples were collected in similar tubes directly from water coolers or water pitchers depending upon how they were offered at each lodge. River samples were obtained by boating to the center of the river, and dipping a sterile collection tube into the water, swirling the tube under water, and then capping the tube immediately.

A.2.3. Indicator bacteria enumeration

Water samples were chilled in a cooler on ice or refrigerated within four hours of collection to reduce bacterial die-off (Flint, 1987) and incubated within 30 hours of acquisition following US EPA guidelines for holding water test samples. (EPA 2013). pH of the samples was checked because the process depends on acidity of the sample. Utilizing 3M Petrifilm product instructions for sampling and incubating, 1 ml amounts were taken from each sample using a sterile-tipped micro-pipette and spread on 3M Petrifilm *E. coli*/Coliform Count Plates. All pipetting was done adjacent to a burning candle or large grilling lighter to draw in falling dust and prevent particles settling on the plate during pipetting. For incubation, a Jameson brand portable field incubator was used for 24 hours at 44.5 C, using 12-volt car batteries when electricity was not available.

Incubated plates were photographed, and counts carried out. The types of bacteria identified were fecal bacteria, *E. coli*, or non-fecal bacteria, based on their color and gas production.

A.2.4. Anecdotal human health

To understand if people were becoming sick with TD or other ailments associated with poor water quality, authors spoke with staff members at each lodge. Authors spoke with lodge owners, lodge managers, two trail guides, a research director, and a staff member that supervised special multi-week stays. The authors were traveling with a group of 18 people and noted the health status of their own group throughout the trip.

A.2.5. TripAdvisor methodology

To determine the incidence of disease self-reported by tourists, the researchers read all relevant reviews on TripAdvisor.com from for each of the four study lodges. At the time of data collection, January 2015, this included 391 reviews from 2007 onward. For each review, any mention of illness, gastrointestinal issues, or drinking water quality was recorded. Lodge A was new and did not have a TripAdvisor profile at the time of data collection, so no reviews were available.

A.2.6. Practice and beliefs

To determine local knowledge and practice regarding water quality, AMV and CJW, spoke with six lodge tour guides, three field scientists, two lodge managers, two lodge owners, one park system guard, and one park system administrator. Discussions were open-ended about water, guest health, and relevant laws and rules. Discussions lasted approximately fifteen minutes and were transcribed

A.3. Results

A.3.1 Drinking water

Of the 11 lodge water samples tested, seven (64%) showed fecal coliform bacterial levels estimated to be in excess of 2000 per liter (Table 1). The treated drinking water offered by three lodges contained measurable levels of fecal coliform (mean 15000, standard deviation ±15811 fecal coliforms per liter, $N = 3$ samples). At lodges B and C, total coliform counts were zero in the untreated water from the tap but high in the treated drinking water (9000 and 44,000 respectively). In Lodge D fecal coliforms were high in the tap water (33,000) and lower in the treated water (2000 and 11000, Table 1). The only lodge where coliform bacteria were not discovered in the drinking water was Lodge A which provided commercially bottled water for drinking.

A.3.2. Lodge practice

No lodges provided treated showering or hand-washing water ($N = 4$), no lodges tested their water for microbes ($N = 4$), each lodge manager and owner was confident of high drinking water quality ($N = 4$), and one lodge (C) used water treatment tablets in their water. The high-end lodges (B and D) utilized nanopore and ozone treatment for drinking water. None of the lodges utilized residual chlorination, the global standard for water treatment (WHO, 2011).

No lodge used sanitizing soaks on their water storage containers. Lodge B was observed swishing a water storage jug with soapy water and rice, using the rice to “scrub” away contamination.

Alimentary (eating and drinking) education was provided to new guests at all four lodges. Guests were instructed to drink only from water coolers and served drinking vessels, as the tap water was not treated and the lodge administrators considered it unsafe to drink. Guests were not provided with a way to wash their personal water bottles nor encouraged to do so (N = 4). No lodge offered a telemedicine solution or on-site health worker to address guest illness. The two higher-end lodges (B and D) had small plaques in the showers reminding guests that the washing water was not treated. (N = 2) At lodge D an “edible sanitizing dip” was used after washing dishes. However, this dip made water and food distasteful which led to some staff rinsing off their dishware with untreated tap water to remove the flavor.

A.3.3. Septic systems

All lodges used bacteria to break down sewage, either using commercially-purchased biodigesters ($N = 3$), or a series of primitive cesspits connected by a buried pipe ($N = 1$). Managers at lodges with biodigesters were concerned that adding chlorine to the water could inactivate the bacteria in their septic systems.

A.3.4. Practice and beliefs

Lodge staff, park and reserve staff, and scientists working under research permitsall indicated that the use of chlorine bleach was “banned” at the lodges (*N* = six individuals across the four lodges). This included the director of Bahuaja Sonene National Park, as well as a guard from the Tambopata National Reserve. Some suggested it was because the lodge owners forbid it due to the damage it could cause to the septic systems, some said it was banned by the ecotourism accreditation agency because of its potential damage to the environment, and others stated it was banned by Peruvian law because it was a chemical used in the making of illicit drugs. However, no one could offer precise clarity about the legal basis of the ban or what group enforced it.

Further research by the authors after completion of field work identified some of the drivers behind these perceived prohibitions. The law behind the “bleach ban” is Peruvian Law Number 29037 for regulated chemicals and controlled substances. The law strictly regulates chemicals associated with the production of cocaine and heroin. As translated by the authors, the list included the active ingredient in household bleach, sodium hypochlorite, in “any amount, shape, or presentation” (Chapter 1, article 4.). The law requires registration, rigorous daily record keeping, and immediate reporting of quantity changes such as accidental spills. As multiple individuals in the lodge system avoided bleach to a widely known but vague “ban,” the law has appeared to have negative effects on the use of liquid chlorine bleach for sanitation in the remote lodges.

There may have been additional influence for not using chlorination based on a Global Sustainable Tourism Council (GSTC) accreditation criteria (“Criteria for Hotels”, 2012). These guidelines require that “the use of harmful substances,” including “swimming pool disinfectants, and cleaning materials, is minimized, and substituted when available, by innocuous products or processes.” A suggested companion performance indicator to the GSTC is that “There has been a review of each chemical used to identify available alternatives which are more environmentally innocuous.” The regional accrediting body uses a GSTC aligned criteria according to their website, and following such guidelines could logically lead to a reduction of chlorination.

A.3.5. River fecal bacteria

The river water samples (N = 4) all contained *E. coli* counts (Average 633 standard deviation ±560 fecal coliforms per liter, N = 5 samples, Table 2) above the 100 per ml recreational water body limit set by the United States Environmental Protection Agency (EPA 2012).

A.3.6. Health anecdotes

During the 18-day trip conducted by the researchers, six of the ten travelling companions self-reported having acquired a diarrheal illness. Some illness was debilitating, requiring bed rest. One of the authors required medical attention as the illness did not clear after 10 days. The long-term guest supervisor reported that all the research assistants from the USA who stayed at lodge D for more than two weeks acquired some sort of diarrheal illness during their stay. Staff reported that relatives visiting lodge employees also became ill. A trail guide at lodge B did not drink from the lodge water explaining it was not healthful. The guide instead drank from a small water source by a trail behind the lodge.

A.3.7. TripAdvisor results

Each lodge manager identified TripAdvisor.com as a major source of reviews and feedback regarding their facility ($N = 4$). The newly built primitive camp lodge did not yet have a TripAdvisor listing and were eager to create one. Of the 391 TripAdvisor reviews that were examined for the three lodges, only four posts mentioned illness associated with lodge stays (~1% of reviews). Only one was obviously related to a gastrointestinal issue, referring to a “stomach bug” picked up “somewhere in Amazonia” (~0.3%). By comparison, five posts mentioned water being clean and safe to drink.

Table A.1 Total fecal coliforms in lodge water samples from southeastern Peru.

Measurement	Total fecal coliform bacteria per 1ml	*E. coli* =per 1ml	Source	Total fecal coliform bacteria per 1ml	*E. coli* per 1ml
EPA Maximum Contaminant Level Goal per liter (EPA 2013)	0	0	Lodge A commercially bottled drinking water	0	0
Lodge A untreated tap water	7	1	Lodge B ozonated and filtered drinking water cooler	9	0
Lodge B untreated tap water	0	0	Lodge C tablet treated and filtered drinking water cooler	44	6
Lodge C untreated tap water	0	0	Lodge D ozonized and filtered room pitcher	2	0
Lodge D untreated tap	20	13	Lodge D ozonized and filtered drinking cup	11	0
Port city municipal tap	0	0	Port city municipal source in a restaurant drinking cup with ice	5	0

Total fecal coliforms per liter were calculated as a count of the number of bacterial colonies that grew from 1 ml of water. The EPA goal of zero colony forming units per liter is provided for comparison.

Table A.2 Natural water body fecal bacteria counts, with scaled EPA water quality criteria.

Measurement	E. coli	Total Coliforms
Scaled 2012 US EPA Recreational Water Quality Criteria for E. coli, and 1986 criteria for total coliforms.	1	2
Port city river center.	3	6
Upriver of port city.	3	2
River tributary in reserve.	3	1
Upstream of river tributary in reserve.	13	9
Most upstream tributary within reserve, farthest from port city.	16	13

The quality criteria is the geometric mean of *E. coli* colony forming units per 100ml. This level cause 23 illnesses per 1000 people (EPA 2012). Scaled from “colony forming units” of 100 cfu/100ml to 1 cfu/1ml for comparison to the 1ml mobile water test size. Similarly, the 1986 total coliform criteria has been scaled from 200 cfu/ml to 2 cfu/ml. The 2012 criteria do not include total coliforms.

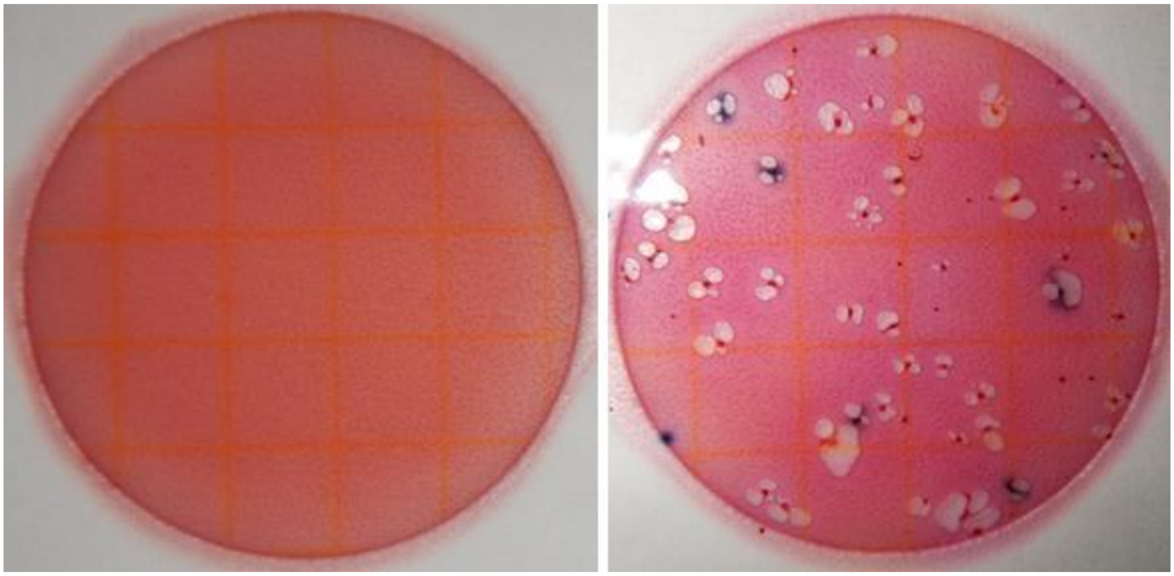

Figure A.1 Incubated samples from lodge C.
Incubated samples from lodge C. 3M Petrifim dishes inoculated with 1ml of untreated tapwater (left) and chemically treated tap water from the water cooler (right) after 24 hours of incubation at 44.5 C. In the image on the right, blue colonies with gas indicate *E. coli*, red colonies with gas indicate coliform bacteria. The count was 44 total colony forming units, six being *E. coli*.

A.4. Discussion

A.4.1.How guests are exposed to the bacteria that cause illness

Lodge water and river exposed guests to fecal bacteria, including *E. coli*. These levels were above the safety levels suggested by the EPA in the United States. Exposure to unsafe levels began during the boat ride to the lodge, where tourists were lightly and occasionally heavily sprayed with water from the bow splashes for up to seven hours. Upon reaching a lodge, the tourists could refill their personal bottle or cup and self-contaminate due to the vessel being unclean (Rufener et al., 2010). When lodges processed water for guests, in a cooler or a jug in the room, there was more exposure to bacteria. Hand washing before a meal or after use of the bathroom would put hands in contact with bacteria in the tap water. At meals, lodge processed drinking water provided further exposure, as would any items that were made with water but not cooked enough to kill bacteria, such as juice mix. During showering, untreated tap water would further expose guests. During any river baths, wading, or swimming, guests would again be exposed. The only drinking water free of fecal contamination was purchased bottled water in a non-reusable jug.

A.4.2. Health anecdotes

Through conversations with staff, and observing travelling companions, it became apparent that guest illness was common. However, the majority of guests only stayed at each lodge for a short duration (2-4 days). As illnesses like traveler's diarrhea can occur as much as 2 weeks after infection, guests might not develop an illness until days after leaving the lodge, making it difficult for tourism companies to know the full extent of the illness acquired at their lodges (Connor, 2017).

Managers thought their purification systems worked. They responded with surprise when we shared the bacterial counts. Staff, researchers, and long-term guests seemed to expect illness during their extended stays but there was not an obvious understanding that this posed a danger, and that a more dangerous illness could be spread the same way TD was being spread.

A.4.3.TripAdvisor

The TripAdvisor reviews, which are heavily utilized by lodge managers, appear to provide an unreliable story with respect to guest health. The lack of discussion of illness in the reviews seem to provide an inaccurate recounting of guests' experience. Months after our trip, our companions did not write about their experiences despite the majority becoming ill, not even the companion who was bedridden with violent vomiting for two days while at a lodge. We expect that other guests, like our companions, may choose not to report their illnesses. In this way, TripAdvisor may mask health problems as the platform is not designed to report guest health.

Guest satisfaction may remain high despite illness. In a mountain adventure tourism study, where illness rates were known, 92.9% of the 448 people surveyed were satisfied with their experience. This was despite 89.4% of surveyed guests reporting illness (Musa, Hall, & Higham, 2004). High guest satisfaction and positive reviews do not indicate the guests are healthy.

A.4.4.Natural water

All river samples tested during this study were above EPA guidelines for recreational water. Counts of 100 *E. coli* per 100 milliliters of water are associated with 32 illnesses per 1,000 people (EPA 2012). These *E. coli* guidelines are part of a number of factors used by the EPA to help U.S. state governments determine when and where to close beaches.

In our study, the highest *E. coli* count was from the sample taken the furthest upstream close to the mountains, flowing from a protected area which is basically uninhabited and off-limits to tourism. As samples from further up-river contained more, not less bacteria, there is a contrast with the purity narrative that water from a ‘virgin ecosystem’ far from polluting civilization is more healthful and desirable (Wilk, 2006). This purity narrative was echoed in the statements to the authors from experienced visitors who suggested that the authors engage in natural water baths while at the lodges. River bathing was conveyed as entirely positive, being “cleansing,” and “life changing,” as well as hygienic, by other guests. Authors did not encounter cautions against the realities of diarrhea or gastritis. Guests may be arriving at lodges with a cultural belief system that encourages contact with untreated water, without knowledge of the consequences. Frequent televised and internet-based marketing for bottled water has focused on the commodification of nature as a way to deliver purity. This is referred to as a “modern medicine show” (Gleick, 2010) due to the false claims associated with the purity narrative.

A.4.5. Lodge practice and barriers to best practice

No lodge staff utilized residual chlorination techniques for water treatment. This seems to have been the product of a complex mix of beliefs, guidelines, regulations, and other cultural elements that are worthy of consideration here.

Lodge staff stated and believed that their current drinking water quality and handling was adequate and for that reason likely saw no reason to improve water treatment. This was reinforced by TripAdvisor which did not suggest gastrointestinal illness was common. The aforementioned purity narrative also supports the assumption that the rainforest environment is pure and healthful.

Many of the lodge staff did not come from highly developed urban areas. For some employees, running water might not be present at home and many may have reduced access to best hygiene practices such as on-demand access to laundering with detergent, heat or chemical sanitizing of dishes, and unlimited soap, hot water, or alcohol sanitizer for handwashing. As a result, fecal contamination is common in rural Peruvian homes (Lanata, Ochoa, Lozada, Pineda, & Verastegui, 2014) and for many lodge employees, technical maintenance of sanitation and water treatment may not be well understood. It is likely that an interaction of beliefs and culture downplayed recognition of TD and other ailments and removed the perceived need for improving water treatments.

Even if the lodge staff wanted to improve water treatment there were many factors that would work against them. Most lodge staff had the mistaken belief that drinking water chlorination would cause undue harm to the biodigesters (personal comm. with three U.S. based water treatment companies.) In addition, the use of chemicals was discouraged through the language of accreditation, which suggests finding “alternatives” to sanitary chemicals. This likely reinforced managers’ fears that chemicals would inactivate the biodigestor systems or cause other unwanted harm. In addition, Peruvian law functionally banned all use of bleach at these remote sites, eliminating the easiest and most common water treatment method. Without training in water quality chemistry, the average person would not know how to substitute related chemicals associated with swimming pool maintenance nor could they do so safely. The combination of beliefs, guidelines and regulations probably had a cumulative effect, leading to a state of “chemonoia” (Ropeik, 2015). Chemonoia is a psychological and cultural phenomena where people reject the use of chemicals despite evidence that the chemicals are safe and improve quality of life. These factors, combined with the warm and humid rainforest setting, likely led to the high levels of contamination we found in this study and create a “perfect storm” that could lead to a major health crisis.

A.4.6. Limitations

The situation surrounding water in remote rainforest lodges is complex and poorlyl characterized. Our study provides only single sample snapshots of a small area. Additional studies across a wider area and longer time frame are needed to better assess the extent, causes and potential consequences of the water quality problems we discovered here.

A.4.7. Recommendations

We suggest that regional lodge associations, and the leading lodges, engage in water chlorination with residual chlorination. Those with the most resources can pave the way for smaller lodges. For lowland Peru, the use of a legally acceptable alternative chemical, such as Calcium Hypochlorite, could avoid the “bleach ban.” Using such chemicals, the entire water system could be treated at the storage tower.

Using plastic on the front of the boat to block spray from contacting guests and discouraging river baths or swimming are potential tools to avoid natural water body-related health issues. Lodge managers might consider “closing” access to natural waterbodies if the waters consistently have high levels of indicator bacteria. Guest education about natural water related illness risks as part of the initial welcome education talk may be useful. The tourism community should rethink “green” accreditation or certification for remote sites. As part of an accreditation process, agencies should instruct staff on theory and practice of water quality, chlorination, hygiene, and water quality testing. Sustainability depends on healthy guests and staff. The agency could help each lodge create a plan to carry out water quality activities. During the inspection period, the accrediting agency could count bacteria in tap, drinking, and natural water bodies for lodges who may not be able to do so themselves.

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APPENDIX B

SOFTWARE TRACKING ACCURACY IN PRACTICAL CONDITIONS, A REPORT TO THE NATIONAL SCIENCE FOUNDATION

B.1 Report summary

The software developed during the AIR-TT process utilizes computer vision to track animals. In April, 2019 two tests were utilized to better understand tracker performance when compared to a trained human observer. The purpose of these tests was to validate how best to use the motion trackers for experimental data collection and when a human scorer would be appropriate to check tablet data outcomes.

To validate the number of birds detected, two multi-hour recording sessions (approximately 5 hours total recording) were scored by a human observer for a total of 106 motion events. This human detection was compared to tablet detection. Single bird tracking was adequately able to match a human observer's perception of movement 80% of the time, while multi-tracking was less effective, agreeing with the observer for 72% of samples.

To understanding how distance from the tablet to the subject changed movement recordings, two tablets at different distances were set up to record bird movement during a two hour recording session. A distance of 100cm or more away from the bird created the fewest errors. At close distance, the center of mass created by the pixel detection method could jump enough to create 2000% more movement than at 100cm. These enormous % movement increases appeared to be driven by shifts in the pixel contrast shape center of mass due to high detail edges in up-close videos that shifted rapidly during contrast detection, regardless of movement. The Center of mass change due to pixel detection shift was multiplied by true movement close to the tablet lens, which made a few centimeters of movement up close be recorded similar to a meter of movement at 100cm. By reducing the complexity of shape geometry through detection from a distance, the tracker was less likely to have a shifting center of mass each time the computer vision software updated the pixel outline. A method to cut off close-up detection through the determination of pixel areas sizes is established.

B.2. Validation test 1: Accuracy of movement detection in single and multi-tracking of long-tailed birds.

B.2.1 Method for validation test 1

A tablet and cellular phone equipped to record video were set up adjacent to a cage holding two client-owned juvenile parrots, Blue-throated Conures (*Pyrrhura cruentata.*) The species is a long-tailed parrot (Figure B1).

Figure B.1. Photo of client-owned conures

The tablet was set to detect the birds’ motion. The tracking software can track up to four different moving bodies simultaneously. We set the software to track two moving bodies and recorded over two hours per day for two days, producing a total of approximately five hours of both video and tracking data. To determine how successful the software was at tracking the birds, we selected 106 six-second samples of video, which represented periods of alternating movement and stillness. We scored if zero, one, or two birds were moving in the cage during these six seconds. For these same 106 six-second periods, we used the tracking software data to determine if zero, one, or two trackers recorded motion. The software was set to show the camera’s perception on-screen during bird motion recording, allowing for a detailed review of how false positives or negatives occurred (Figure B2).

During movement recording, a human observer watched the data recording for one hour.

To better understand how the computer vision “saw” movement, a setting was enabled to see the live video analysis on the tablet. This was done to understand how the tablet might misinterpret motion.

The laboratory utilized the Asus Zenpad 8.0 P001 tablet for experiments. As many animal enclosures do not have wireless network access, the tablet cannot check online to update its clock. The accuracy of the tablet’s internal clock is important to data validity as the data is timestamped. To check that this model of tablet can maintain its clock while running the software, the data timestamps were also evaluated. An accurate digital clock was placed so it was viewable on video. The tablet ran offline for the two day recording period, allowing adequate time to detect if the clock ran fast or slow.

Figure B.2. Tablet view and video view of the cage set up, before the addition of birds and clock.

B.2.2. Data analysis for validation test 1

For each of the 106 six-second periods, the human movement score was compared to the tablet software movement score to determine the percentage of time both human and tracking reported motion of zero, one, and two birds. The timestamped data from the tablet was checked against video of the clock to see if the tablet remained accurate over the recording period.

The largest distance movement data were checked against the video to see if the recorded time for peak movement matched the time on the digital clock in the video, to see if peak movement was accurately recorded by the tablets when compared to human observation.

B.3. Results for validation test 1

The single tracker was in agreement with the human scorer 80% of the time out of 106 human detections of movement. The double tracker agreement with human 72%, for 9 human detections of movement. Review of disagreements between the single tracker and human scorer mainly appeared to be smaller movement amounts, such as head and body swaying, which were marginal cases. Inaccurate detection by the tablet was primarily false negatives occurred, whereupon review the bird moved its center of mass several centimeters across the cage but the tracker did not report movement.

The internal clock appeared accurate throughout the recording period, allowing us to confidently use the tablets' internal clocks for data recording when wireless internet is not available.

B.4. Discussion for validation test 1

These long-tailed birds can incorrectly cause the tracker to record two birds are moving. The number of birds recorded by the multi-tracker was imperfect. Approximately 5% of all recordings were false-positive detections of two birds moving when only one bird was moving. The human observer noted that two trackers occasionally appeared, one in the center of the bird, and one in the center or end of the bird's tail, which is equal to the length of the bird.

While pairs of long-tailed birds should be fine for consumer pet interaction, for early scientific studies, single, short-tailed birds are prescribed to increase the accuracy of the system. The single bird tracking scored a "B" grade and is acceptable for future use. The movement tracker is accurate for the recording of “moving” or “not moving” to usefully shows animals’ activity change on a fine scale.

B.5 Validation test 2: Distance and accuracy of movement detection for a single short-tailed bird.

B.5.1. Method for validation test 2

On the first day, tablet positions and recording data was taken from different distances to define the experimental setup, utilizing a client-owned Galah Cockatoo, *Eolophus roseicapillus* shown in figure B3. On the second day, a different client-owned Galah Cockatoo was recorded in a cage, for approximately two hours. As shown in figure 4, recording utilized a tablet in the cage tracking motion, a tablet outside of the cage tracking motion, and a video recording capturing the entire cage.

Figure B.3. Photo of a short-tailed, client-owned cockatoo.

The near tablet was 75 cm distant from the birds' perch, mounted to the cage, and far tablet was 105 cm away from the bird, mounted a distance away from the cage. This allowed for two comparisons. The first comparison studied viewing angle and amount of movements captured. It was hypothesized that the limited viewing angle of the tablet internal tablet would miss movement when compared to the wide view of the far tablet. The second comparison was of distance affecting the amount of movement recorded The hypothesis for the second comparison was that distance would be magnified by the closer proximity tablet.

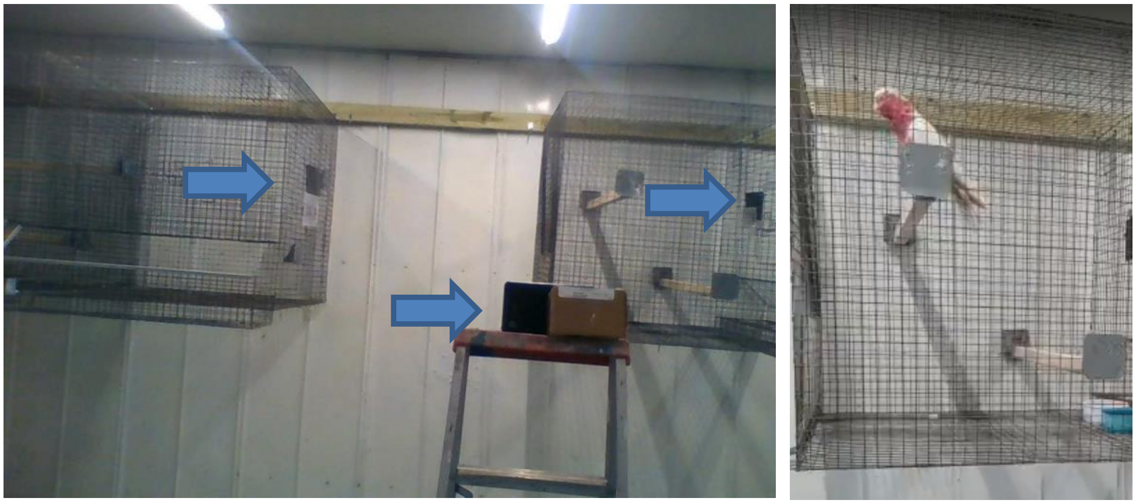

Figure B.4. Arrangement of two tablets and video recorder
The leftmost arrow is the far tablet at 104cm from the perch. Center is the video recording tablet for validation. Rightmost arrow is the 75cm distance tablet. In the right image, there is a cockatoo on the perch.

B.5.2. Data analysis for validation test 2

The data from the two tablets for the same time period were compared. The percent different between the data was computed. The largest disagreements of data between the two tablets, video was reviewed.

B.6 Results for validation test 2

Distance away from the tablet had a major effect on the amount of motion detected. The tablet nearer to the birds recorded movement totals approximately 10-400% greater than the farther away table, with a single extreme of 2000% more movement for one data point. These differences were due to center of mass detection as well as the faster crossing of the field of view when the subject was close to the tablet.

At far distances the tablet recorded distance change in a more accurate and useful way. At close distance, center of mass change may be erroneously recorded moving from edge to edge of the field of view during changes in pixel geometry between frames. The distance from the tablet at which small comfort movements (i.e., scratching, yawning) of a medium-sized pet bird no longer register as center of mass movements was >100 cm.

Surprisingly, >90% movement detected by the far tablet was also detected by the near tablet, despite the near tablet having a limited field of view of the cage interior. This appeared to be because the highest perch is preferred by birds. By aiming the limited viewing angle toward the high perch, all movement along the length of the perch, upper cage walls, and ceiling were recorded.

B.7. Discussion for validation test 2

The software currently has a setting to eliminate close objects from the tracking. This is done by ignoring moving objects that are over a certain pixel area size. This can be used at short distances to eliminate a problem we found in previous versions of the exercise game, where birds got very close to the camera and bobbed back and forth to win the game, without effectively exercising. For a medium bird in a typical house cage, settings can be used to only reward movement when the bird is small compared to the field of view. The 100 cm distance seems to be a useful minimum for a medium-sized parrot.

Video review showed that some activities, that did not result in a location change, such as wing stretching, registered as movement on the motion tracker for the close tablet, but not for the far tablet. As wing stretches and body feather puffing result in increased apparent body size, we believe we can remove those comfort motions from exercise detection by closely matching the size limit to the size of the bird at the minimum desired distance. We propose that for future experimental setups, recording movement will start by taking a video still from the tablet's camera when the bird is at the desired distance, then counting the area of pixels using Adobe Photoshop's selected pixel reporting, and setting the pixels detection area to be plus or minus 60% of the desired body size relative to the field of view.

Small cage set ups (~95 cm^3) with medium or large birds (>200gm) will require careful calibration of the "too close" cut off to prevent feather scratching, fluffing, wing stretches and other non-exercise movements from being interpreted as center of mass change. This will likely be a non-issue for small birds, such as those parrots weighing near 30 gm, as their caging is typically many times larger than their beak-tail length, so the bird will often be farther away from the camera. For small birds, the cut off size could be two or three times their body area when on the preferred perching, without causing issues.

At this time we are confident in the software and are beginning to run experimental trials to show exercise can be encouraged through the technology. We predict exercise games will not be as time-intensive as testing the habituation app. Training “neophobic” animals to accept something new can take many repetitions which required daily attention for 21 days. The exercise game, in anecdotal single bird tests, has shown nearly immediate interaction with birds. It is hoped this will allow for faster testing per bird, similar to what was seen in our initial pilot data submitted for the grant, where a bird went from near zero accuracy to approximately 25% accuracy for a game within eight gameplay sessions (unpublished Data Woodman, Strange, & Brightsmith, 2016).