Acquisition of a Learned Operant and Critical Flicker-Fusion Rate in the Tuatara (Sphenodon spp.).

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Tuatara (Sphenodon punctatus; Cook Strait).

ABSTRACT

Scientific investigation of the sensory world and behavior of the tuatara is limited. This study incorporates both ecological and psychological perspectives to test learning and visual perception using a novel operant technique and flicker-fusion rates to measure visual discrimination in tuatara. We posed four main questions: (1) can a reliable method examine learning and visual perception in tuatara?, (2) what is the critical flicker-fusion (CFF) rates for tuatara and how does it relate to motion detection ability?, (3) can stimulus control be transferred to a Y-maze from an open field arena?, and (4) what are the implications for behavioral ecology, conservation, and species welfare? Tuatara (Sphenodon punctatus) were trained using an operant conditioning procedure with food reinforcement to respond to discriminative stimuli (S⁺) of various flicker-fusion rates, and ignore a non-discriminative stimulus (S⁻). Tuatara discriminated CFF rates between 2.65-45.61 Hz, but not at 65.09 Hz. The upper threshold between 45.61-65.09 Hz is comparable to other mammalian, avian, and herpetological species. Tuatara demonstrated a learning capacity for acquisition of an operant task as well as cognitive development for learning and memory strategies. Visual discrimination is important to tuatara and may facilitate behavioral responses to many context-dependent ecological processes (i.e., predator/prey/kin recognition, mate selection, environmental discrimination, optimal foraging strategies, and communication). By understanding the importance of visual stimuli, the study provides a better perspective of the tuatara natural sensory world. Additionally, a reliable method was established that can be used for more comprehensive psychophysical experiments to further access visual perception and learning in all reptiles, with the potential to examine other sensory mechanisms such as audition, chemoreception, and tactility.

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Chapter I General Introduction

1.1 Introduction

By examining learning and sensory processes, we can classify elements of reptilian natural history that attempt to bridge the evolutionary paradox of diverging mammalian and avian behavior from reptiles. Tuatara are on the only surviving members of the reptilian order Sphenodontia, and phylogenetically represent a quarter of the reptilian orders. To date, few studies have examined learning and sensory processes in reptiles, and even less have been focused on the tuatara. This study aims to provide basic information governing the tuatara's learning capability and visual capacity. By understanding how tuatara interpret visual stimuli, insight into their discriminative ability elucidates important aspects of evolutionary and adaptive plasticity indicative of species behavioral ecology.

1.2 Learning & Memory

Investigations involving sensory perception can be divided into two categories: those aimed at examining the process of conditioning or other learning phenomena using sensory perception as a tool, and those that investigate discriminatory and sensory abilities such as vision, audition, chemoreception, and tactility using established learning phenomena as a tool (Burghardt, 1977). Although the aims of these research paradigms differ, they are also clearly related. The relationship between these two types of investigation can be best understood by looking at the two most commonly studied learning processes: classical and operant conditioning. Classical conditioning involves animals learning to make a response (i.e., salivation) instinctually elicited by one stimulus (i.e., dry food) in the presence of another stimulus. The adaptive nature of this learning process in natural settings is apparent. For example, if the natural defense against predation of an animal is to freeze when it

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perceives a predator, then it is advantageous that the animal learns to freeze when stimuli signal the likely arrival of that predator.

In a laboratory setting, this process can be studied by taking a stimulus that produces a reflexive response without prior experience (an unconditioned reflex) and systematically pairing that stimulus with a new (neutral) stimulus that initially does not produce a response. Typically after repeated pairings, the previously neutral stimulus comes to elicit the response. Studies concerned with examining this process may vary factors such as the number or timing of pairings or the nature of the neutral stimulus, thereby improving our understanding of this learning process. However, for learning to occur, the animal must be able to perceive the stimuli that are used. Therefore, varying the stimuli in such studies might also provide information about the sensory capacity of the organism. In the operant conditioning paradigm, concern is with how the consequences of an action influence the future probability of the action. General behaviors followed by positive consequences are likely to be repeated, while those followed by negative consequences or no consequences are less likely to be repeated; this control by consequences is often referred to as schedule control. In the natural world, consequences are available for a behavior in some circumstances only (e.g., hunting will only be rewarded if there is prey in the area). When certain stimuli reliably predict these circumstances, then it appears as if those stimuli control the behavior; this control is called stimulus control. In the laboratory setting, operant conditioning is usually studied by choosing an arbitrary response and providing a reinforcer (most often food) when that response is made. Additionally, in order to examine the stimulus control component of operant conditioning, arbitrary stimuli are introduced that correlate with the availability of reinforcement; these stimuli are referred to as discriminative stimuli. When an animal responds in the presence of the stimulus and not in its absence, the animal's behavior is said to be under stimulus control. Studies aiming to understand this learning process may vary factors such as the rate of reinforcement and the reliability of the stimuli in signaling its

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availability. However, as with the classical conditioning paradigm, performance in these operant studies is also dependent on the animal's ability to perceive the discriminative stimuli. Therefore, these procedures provide a means of studying perception. In psychophysical experimentation, learning paradigms frequently provide a methodology by which sensory discrimination is examined. This novel study is the first to use a psychophysical approach to investigate the basic learning and visual perception capacity that govern the behavior of tuatara.

Relatively few reptile species have been used in studies of learning and memory (Burghardt, 1977). Reptilian studies that have examined learning in reptiles apply both classical and/or operant conditioning paradigms. For example, spinal turtles (*Emydoidea blandingi*) were classically conditioned to a reflexive leg response by pairing a stimulus with the administration of a shock (Peretti & Zrout, 1975). This study showed that reptiles can learn under conditioning regimes. Studies have also found that reptiles behave differently compared to mammalian or avian species. Behavioral dissimilarities between reptilian and non-reptilian species maybe attributable to physiology (i.e., physiology is correlated with learning ability; Blau & Powers, 1989), responsiveness, performance ability, or the type of reinforcer (Burghardt, 1977). However, Kaufman, Burghardt, and Phillips (1996) suggest that learning capacities in reptiles are comparable to avian and mammalian behavior, and reptiles can be used as models for studying learning paradigms.

Conditioning experiments with reptiles are challenging because creating a motivational drive or finding the correct reinforcer is difficult (Burghardt, 1977). Food is not always the optimal reinforcer and some ectotherms do not respond to food reward (Northcutt & Heath, 1973). Alternative reinforcers that have been successfully used to maintain operant behavior include shelter (e.g., spotted pythons, *Anteresia maculosus*, Stone, Ford, & Holtzman, 2000), light/dark access (e.g., desert iguana, *Dipsosaurus dorsalis*, Graf 1972; banded geckos, *Colenyx variegatus*, Kirkish, Fobes, & Richardson, 1979), shock (e.g., spinal

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turtles, *E. blandingi*, Peretti & Zrout, 1975), thermal regulation (e.g., common iguana, *Iguana iguana*, Alkov & Crawford, 1965; *D. dorsalis*, Kemp, 1969), and access to air (e.g., water turtles, *Chyrsemys picta*, Morlock, Brothers, & Shaffer, 1968; red-eared turtles, *Pseudemys scripta*, van Sommers, 1963). Additionally, mate selection and competition pressures can motivate reptiles to respond to an aversive stimulus in order to be rewarded with the desired mate. For example, male Iberian wall lizards (*Podarcis hispanicus*) are motivated to fight in order to defend territory and acquire potential mates (López & Martín, 2002). Identifying the correct reinforcer for operant procedures elicits positive responsive behavior from the subject organism and increases their learning potential (Kemp, 1969).

Stimulus control occurs when a neutral stimulus becomes the discriminative stimulus that then governs the response of the organism. This occurs as a result of the stimulus being consistently present when a target behavior is reinforced and absent when that behavior is not reinforced. These cues are learned discriminative stimuli: S⁺ for the cue the organism responds to, and S⁻ for the cue in which the organism does not respond. Stimulus control studies using herpetofauna have shown that subjects can discriminate among various types of stimuli (e.g., *C. picta*, Blau & Powers, 1989; Wise & Gallagher, 1964; *D. dorsalis*, Garzanit & Richardson, 1974;), and appropriately respond as a function of the cue. Herpetological studies incorporating stimulus control methods have used mouse derived (1-month versus 10-month) cues with red spitting cobras (*Naja mossambica pallida*) to measure olfactory discrimination (Stimac, Radcliffe, & Chizar, 1982), a negative reinforcer (shock) to measure sensory thresholds in turtles (Granda, Matsumiya, & Stirling, 1965), color in *Rana* tadpoles (Muntz, 1962), male mounting control in *Anolis carolinensis* (Crews, 1978), and air deprivation control in water turtles (e.g., *C. picta*; Morlock et al., 1968).

Maze studies have provided a basic understanding of learning, memory, and sensory perception processes in reptiles. Dudchenko (2001) suggests that maze performance is based on the use of several cognitive strategies. Navigation through a maze encompasses the use of

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self-motion cues, path integration, extramaze landmarks, environment, and trial and error representations to guide behavior and maintain orientation. However, discrimination is based on a constant relationship between correct task completion and reward system (Scharlock, 1955). Mazes can be used to study memory, spatial concepts for distance and direction, development of cognitive maps, and foraging strategies by predators searching for prey items (Olton, 1979). Maze performance is reflective of behavioral paradigms indicative of the behavioral ecology of the species such as optimal foraging strategies or "win-stay" versus "win-shift" predation (Restle, 1957). Maze studies have examined path selection in water turtles (e.g., *C. picta*, Hart, Cogan, & Williamson, 1969), referential memory in Northern leopard frogs (*Rana pipiens*) using a Morris water maze (Bilbo, Day, & Wilczynski, 2000), and temperature-dependent maze performance in heliothermic lizards (e.g., *D. dorsalis*, Krekorian, Vance, & Richardson, 1968).

Mazes test for ecologically important characteristics such as spatial learning and discrimination. Several reptilian studies have used mazes to examine learning and memory abilities (see Burghardt, 1977). For example, crevice spiny lizards (*Sceloporus poinsettia*) were able to discriminate environmental differences based on previous visits to a particular location (Punzo, 2002). Spatial associative learning contributes to an organism's overall fitness, and allows them to revisit locations where food items were previously and minimize energetic costs. Similarly, sleepy lizards (*Tiliqua rugosa*) were able to remember fixed landmarks to find home ranges, but were also found to discriminate between manipulated environmental cues, shapes, and degrees of brightness (Zuri & Bull, 2000). In a study by Holtzman, Harris, Aranguren, and Bostocks (1999), corn snakes (*Elaphe guttata guttata*) were found to learn and remember spatial landmarks in the wild. These studies provided ecological implications for orientation mechanisms, homing, mate selection, shelter selection, and foraging behavior. In addition, turtles (*P. scripta*) possibly develop spatial learning based on the formation of cognitive maps (high-level of spatial representation), and may

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employ multiple strategies for learning and memory systems (López, Gómez, Rodríguez, Broglio, Vargas, & Salas, 2001; López, Rodríguez, Gómez, Vargas, Broglio, & Salas, 2000). Behavioral adaptations of maze learning reflects an organism's response to ecological niches, and may be a function of several strategies: (1) "guidance strategy" – approaching a single intramaze cue as if it were a beacon, (2) "navigational strategy" – defining a reference goal, (3) "snapshot strategy" – approaching individual or clusters of cues associated with the goal, (4) "conditional response strategy" – repetitive approach to a conditioned goal, and (5) the development of a cognitive mapping system.

Learned behaviors are a function of contextual experience (Shettleworth, 1998b). For example, free-range populations of whiptail lizards (*Cnemidophorus murinus*) were able to discriminate between palatable fruits and artificial replicas (Schall, 2000). Relative to mate competition, Iberian rock-lizards (*Lacerta monticola*) were able to discriminate between individuals, and remember the fighting ability of competitive opponents (i.e., "dear enemy" recognition, Aragón, López, & Martín, 2000; López, & Martín, 2002). Water turtles (*C. picta*) have also shown that reptiles are capable of problem solving activities such as reversal shifts (RS) and concept formation (Graf & Tighe, 1971), reversal learning (Alkov & Crawford, 1966), detour learning (Spigel, 1964a), and escape learning (Spigel, 1964b).

There are three phases to studies investigating learning and memory: acquisition, retention, and retrieval. Most studies focus on the initial stage of acquisition (Domjan, 1998). Learning experiments examine the kind of information acquired and how it is acquired; therefore, learning experiments involve manipulations of the conditions of acquisition that provide insight into cognitive function. Cognition is the process in which animals acquire, process, store, and act on information from the environment (Shettleworth, 1998a). Cognition includes an animal's ability to equate aspects of perception, learning, decision making, and memory. There are two types of memory: working (short-term) and reference (long-term) memory. Forestell and Herman (1988) suggest that animals can develop a

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"memory strategy" for engaging in decision making and problem solving. The ability for an organism to perceive, recognize, learn, and respond appropriately to a stimulus may be a function of cognitive strategies used for survivorship. By applying learning paradigms to cognitive studies, we can use learning as a tool for investigating sensory capacities.

1.3 Visual Perception and Critical Flicker-Fusion (CFF)

Visual perception encompasses motion detection, colors in the ultraviolet range, color patterns, brightness, and shapes (Zuri & Bull, 2000). Visual perception is closely related to the physiology of an organism's eye; the larger the eye with photoreceptors, the larger an image is cast over a large number of photoreceptors. Motion and pattern detection serve two functions: (1) to draw attention, and (2) to transmit information about a stimulus. This is an example of pattern detection function: the anoline eye has a retina to receive information at 200°, and a lateral monocular field of 180° leaving a 20° overlap, and *Anolis auratus* can detect 0.2° of motion from a visual angle (Fleishman, 1992). The anoline eye has been designed for rod-adapted, high-acuity diurnal vision. Additionally, many cone-adapted lizard species can also distinguish between green, blue, yellow, red, and shades of gray.

Critical flicker-fusion (CFF) is the rate of flicker at which the individual or species ceases to see a flicker. A rapidly flashing light evokes the sensation of a flicker; when the flash frequency increases, the light perceivably ceases to flicker (Sekular & Blake, 2002). Photopic (cone) receptors are scaled by fast sensitivity-regulation and are a function of light adaptation mechanisms (MacLeod & He, 1993). In a feedback mechanism, retinal light adaptation is the adjustment of retinal response properties to variations in ambient illumination (Trachina, Gordon, & Shapely, 1984). CFF perception is processed by a modulation transfer function. First, there is a low pass temporal filter which involves signal processing within the photoreceptors. Secondly, the image is displayed on a high-pass filtering system with an inhibitory feedback network of primarily horizontal and amaerine

cell connections in the inner and outer plexiform layers (Jarvis, Taylor, Prescott, Meeks, & Wathes, 2002). In a study examining CFF and the effects of luminescence, cane toads (*Bufo marinus*) were found to have a duplex flicker response at low intensities for rods, and at higher intensities for cones (Nowak & Green, 1983). CFF incurs a period-doubling feedback (Crevier & Markus, 1998). In salamanders, the retinal ganglion systematically fire on every other flash, and ignore intervening flashes. The retinal responses are synchronized across the retina which poses the "volley theory" or systematic firing of retinal ganglion.

Flicker-fusion rates and thresholds have been studied using psychophysical experimentation in monkeys (Shumake, Smith, & Taylor, 1968), cats (Loop & Berkeley, 1975), rats (Williams, Pollitz, Smith, & Williams, 1985), pigeons (Hendricks, 1966), dogs (Colie, Pollitz, & Smith, 1989), seals (Bernholz & Matthews, 1975), and chickens (Jarvis et al., 2002). Subjects were trained to respond to a clearly recognizable flicker. The flicker rate was then externally varied. Psychophysical experimentation is a reliable method in testing CFF rates. For example, precise and reliable measurements of CFF thresholds were recorded using an avoidance conditioning technique with turtles (*C. picta*, Maxwell, & Granda, 1975).

Examination of two-choice experiment on choice reaction time (CRT) by measuring recognition reaction time and motor reaction time on a two-choice experiment that practice may induce apparent learning effects through habituation to the type of task rather than learned discrimination of stimuli (Parkin, Kerr, & Hindmarch, 1997). Practice reduces CRT in response to discriminating CFF rates. Therefore, it is difficult to determine how many trials are required to delineate a learning effect.

Critical flicker-fusion reflects the ability and proficiency to resolve moving objects. The faster the CFF resolution, the faster the image moves across the retina. Organisms with a higher CFF threshold receive images that are less blurred. Therefore, they are able to negotiate complex environments, identify and catch swift prey, and avoid fast-moving predators. For *Anolis* species, CFF was correlated with ecological adaptiveness (Jenssen &

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Swenson, 1974). CFF was found to be higher threshold for species living in exposed sunlight where organisms may be better able to resolve a variety of images (Table 1).

Species	Mean Upper CFF Threshold (Hz)	Location
A. auratus	42.0	Central America and northern South America in grassy areas
A. sangrei	34.5	Caribbean, southern Florida, and eastern Mexico in open habitats, low bush, low tree trunks
A. grahami	34.6	Jamaica, Cayman Islands, Bermuda, in semi-open habitat and upper tree trunks
A. carolinensis	34.5	Southeastern USA and Bahamas in semi-open habitats from shrubs to arboreal perches
A. valencienni	33.4	Jamaica in semi-open habitat
A. limifrons	28.4	Central America to northern South America in shady, low understory vegetation of closed canopy forest
A. lineatopus neckeri	26.1	Jamaica in heavily shaded area

Table 1. Mean critical flicker-fusion (CFF) rates of *Anolis* lizards (adapted from Jenssen &Swenson, 1974).

Signals must be designed to stimulate the sense organs. Display action patterns (DAPs) transmit visual information about social companions, opponents, mates, specified context, and velocity and acceleration signatures (Peters, Clifford, & Evans, 2002). Display action patterns are ritualized species-specific movements performed in synchronic rhythms, and are a coordination of site, position, posture, movement type, part moved, units of movement, sequence, and cadence (Purdue & Carpenter, 1972). Visual discrimination transmits information about predators (e.g., basking black iguana, *Ctenosaura similis*, Burger, Gothfield, & Murray, 1991), attack strategies (e.g., scincid lizard, *Eumeces laticeps*, Cooper, 1981), prey size and movement (e.g., *A. carolinensis* and *Eumeces fasiatus*, Burger 1964), and allows the use of alternative sensory abilities as compensation for the loss of other sensory mechanisms (e.g., foothill alligators, *Gerrhonotus multicarinatus*, Schultz & Norberg, 1970). Receiving and discriminating between several visual stimuli is important in collecting and interpreting useful information, and appropriately responding in context.

1.4 Behavioral Ecology

The ability to transmit, receive, learn, and respond to cues by other organisms or the environment is critical for the survival of a species. Identifying key features in sensory discrimination has behavioral implications for predator/prey/kinship recognition, mate selection, environmental discrimination, optimal foraging strategies, and communication.

Sensory discrimination is important for predator, prey, and kinship recognition. Predator-prey interactions have revealed that visual cues initiate predator detection, but chemical cues enhance identification. Fright responses can be modified according to the level of perceived threat such as the influence of relative size differences, effect of body condition, running speeds, predator hunger levels, and level of protection provided by morphological features (i.e., spines or defensive armor). The assessed risk for prey should not depend only on distance to a predator, but that it has been detected (Cooper, 1998). Larval newts were able to distinguish among levels of threat, implying a cognitive function of evaluating fitness consequences (Mathis & Vincent, 2000). This anti-predatory response has implications for a cognitive self-defense mechanism. The use of visual cues is important for eliciting an initial anti-predator response, and chemoreceptive cues allow an organism to assess risk (Flowers & Graves, 1997). Equally, the ability to recognize predators is complementary to the recognition of prey items (Burghardt, 1964). For example, A. carolinensis and autarchoglosid skinks E. fasciatus were able to discriminate between prey size and movement of mealworms (Tenebrio molitor). Relative to predator-prey recognition, juvenile Iberian wall lizards (P. hispanica) can recognize familiar conspecifics, and discriminate between familiar and unfamiliar individuals on the basis of chemical stimuli (Font & Desfilis, 2002). In a study of two cross-fostered Australian lizards, T. rugosa and Egernia stokesii, the mothers attended to their own young despite cross-fostering (Main &

Bull, 1996). Animals then have the ability for individual recognition, and this may serve as a component for social organization (Aragón et al., 2000).

Reception and interpretation of various cues are important for mate selection. Both color and odor cues are important for eliciting male courtship in the Iberian wall lizard (*P. hispanica*, López & Martín, 2001). For *P. hispanica*, odoriferous cues are more important than color patterns in sex recognition, but coloration is useful in long range recognition to either deter or elicit a courtship response. Female coloration in the tropidurid lizard (*Microlophus occipitalis*) signals reproductive and behavioral status (Watkins, 1997). Signaling in mate selection may also be a function of the "good-genes" sexual selection hypothesis; the female intends to advertise to a host of males, but reproduce with only the best-fit male. Visual cues play an important role in several aspects of mate selection.

Discrimination of visual stimuli allows an organism to distinguish between characteristics of the physical environment such as other organism, environmental contours, and vegetation. The movement of background vegetation creates visual "noise". Animals need to discriminate, habituate, and desensitize to windblown vegetation which may have an influence on motion patterns of display (Fleishman, 1992). For example, the omnivorous lizard (*L. belliana*) was able to discriminate between prey (e.g., cricket; *Acheta domestica*) and plant (e.g., dandelion; *Taraxicum officinale*) items (Cooper, 2003). The use of distal (visual) and proximal (chemical) cues are both necessary to discriminate between vegetation and non-vegetation objects.

Optimal foraging theory suggests that an organism should be an efficient predator in order to acquire the most profitable prey with the least energy expenditure (Krebs, 1978). Animals have the ability to assess risk and to control decision making reflecting, a trade-off between the risks of predation versus the benefits gained from engaging in a given activity (Lima & Dill, 1990). However, energetic demands are greater for active foragers, than sitand-wait predators. Active foragers must calculate the amount of energy expenditure to the

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benefit of high nutritional value in a prey item. Optimal foraging theory considers two generalizations: (1) the ability and distance of escape of prey item from predator, and (2) the existence of different prey with different energetic values (Goldberg, Hart, & Wilson, 1999).

Animals can develop a memory strategy by using local cues, and adapting to a behavioral response strategy when stimuli or reinforcers change. For example, Australian Ctenotus skinks used tactual, olfaction, celestial and magnetic cues for spatial learning and goal orientation, and suggest that spatial learning reduces the amount of time spent in random foraging patterns, and maximizes foraging activity (Punzo & Madragon, 2002). Patterns in foraging movement in the whiptail lizard (Cnemidophorus tigris), leopard lizard (Gambelia wislizeni), side-blotched lizard (Uta stansburiana), desert horned lizard (Phrynosoma platyrhinos), and zebra-tailed lizard (Callisaurus draconoides) indicate a capacity for shifts in foraging tactics based on learned identification patterns of prey and location (Pietruszka, 1986). Multisensory cues include vision (i.e., motion and pattern) and olfaction for locating prey and identifying location. Prey consumption in *Chalcides ocellatus* was severely impaired when the vomeronasal ducts were sealed suggesting that the vomersonasal system is important for identifying prey and location (Graves & Halpern, 1990). Foraging tactics are influenced by a species' sensory mode and physiological capacities. In a study with two congeneric desert-dwelling lacertids (Acanthodactylus boskianus and A. scutellatus), animals use a spatial adaptation theory suggesting a correlation between underlying neurological substrates and spatially demanding niches become the consequence of selection for navigational demands (Day, Crews, & Wilczynski, 1999).

Sensory discrimination is also important for escape behavior in optimality models. For example, the keeled earless lizards (*Holbrookia propinqua*, Cooper, 1999) and broadheaded skinks (*E. laticeps*, Cooper, 2000) adjust to approach factors of predators and assess the risk of capture (i.e., cost of lost opportunity for escape plus energetic costs of fleeing), predator speed, distance to safe refuge, persistence, direction of movement, and flight-

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initiation distance. For example, *H. propinqua* uses the direction of turning, increased visibility of eye and facial features, and the orientation of predators as cues to gauge risk and optimum flight-initiation distance (Cooper, 1998). Fleeing animals must create an escape strategy where it may prove advantageous to wait as long as possible before fleeing, and an escape trajectory that is either a linear or random route to a refuge, but also adjusts to energetic costs (Martín & López, 2000).

Communication is an important function in the behavior of an organism. Successful transmission of a message is dependent on the ability of the signaler to transmit information that is received and understood by the intended receiver (Carpenter & Ferguson, 1977). Successful communication is dependent on six signal design rules: (1) the range or active space of the signal, (2) locatability of the signal, sender, intended receiver, and/or referent, (3) duty cycle of the signal, (4) sender identification level, (5) modulation level, and (6) degree of form-content-linkage (Bradbury & Vehrencamp, 1998).

The visual design of signals (i.e., DAP, Peters et al., 2002) is dependent on the context in which it is used (Fleishman, 1988a, 1988b). Visual signals evolve with the nature of the environment in which the signal must pass through. The properties of environmental "noise" influence the signal structure. Therefore, various displays serve different functions with more specific informational meaning. Visual modality communication transmits information regarding sexual selection (i.e., vocalization and color in fire-bellied toads, *Bombina bombina* and *B. variegate*, Sanderson, Szymura, & Barton, 1992), territoriality (i.e., vocal display in *Hemidactylus frenatus*, Marcellini, 1977), and to show warning displays (i.e., push-up postural display in sagebush lizard, *Sceloporus graciosus*, Martins, 1993).

1.5 The Tuatara

Tuatara (*Sphenodon*) are on the only surviving members of the reptilian order Sphenodontia, which is represented by two extant species (*S. punctatus* and *S. guntheri*).

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Once widespread throughout the mainland of New Zealand, tuatara now only survive on 32 offshore islands (Gaze, 2001). Tuatara are K-selected species, with slow growth rates, and have slow reproduction rates for females of four to five years (Cree, 1994). Adults may to live 100+ years (Cassey & Ussher, 1999). Tuatara are a ground-dwelling species that are primarily nocturnal feeders (Saint Girons, Bell, & Newman, 1980), but are active during the day and often bask in the open or in entrances to burrows (Cree & Daugherty, 1990). Tuatara metabolism is reflective of ectothermic animals living in cooler temperature climates such as New Zealand (Cartland & Grimmond, 1994). Investigations of tongue morphology in tuatara suggest that taste is important for prey discrimination and possibly courtship as well (Schwenk, 1994; Schwenk, 1986). The tuatara eye is primarily cone dominated, but the fovea is concentrated with long rods and single cones suggesting that tuatara vision is rod adapted (Walls, 1934).

Although there have been many studies on tuatara physiology (Schwenk, 1994; Schwenk, 1986; eye, Walls, 1934), there have been few studies focused on behavior and sensory perception. Behavioral studies have examined feeding ecology (Walls, 1981; Carmichael, Gillingham, & Keall, 1989; in the presence of kiore, *Rattus exulans*, Ussher, 1999), social behavior (Gillingham, Carmichael, & Miller, 1995), predator avoidance (Nelson, 2001), visual predation (in darkness, Meyer-Rochow, 1989; on beetles, *Chaerodes trachyscelides*, Meyer-Rochow & Teh, 1991), circadian rhythm (Goetz & Thomas, 1994), and T-maze performance (Northcutt & Heath, 1973).

1.6 Objectives & Thesis Outline

This study has several objectives focused on increasing our overall understanding of tuatara behavior and natural history by applying psychological, ethological, and biological theory. First, the study aims to provide basic information on tuatara learning capabilities. Secondly, the study seeks to provide basic information on the tuatara's visual capacity,

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acuity, and perception especially from the perspective of motion detection properties. Thirdly, a new established procedure aims to generate a reliable method in which to test learning, memory, and sensory perception in tuatara and other reptiles. Fourthly, by identifying critical aspects of tuatara visual perception, we can ascertain important implications regarding the behavioral ecology of the species. Finally, this study will help increase our knowledge of existing management and husbandry protocols, and provide beneficial implications for conservation and animal welfare efforts. This thesis is written as three independent papers for submission; there may be some repetition of material.

Chapter II focuses on establishing a novel method for experimental behavioral testing. It will examine the extent to which tuatara have the ability to learn under operant conditions. The paper will address the following questions: (1) can tuatara learn to discriminate a visual stimulus using an operant technique? and (2) can a reliable method investigating learning and additional sensory perception such as audition, chemoreception, and tactility in tuatara and reptiles be created?

Chapter III incorporates the same method, but will examine the role in which vision (CFF) and motion detection play a role in behavioral adaptations, and how it applies to interactions such as individual recognition, predator avoidance, environmental discrimination, mate selection, and communication. The paper primarily addresses the following questions: (1) what is the CFF rate in tuatara visual perception? and (2) how does CFF correlate to motion detection properties in tuatara from its natural environment?

Chapter IV also uses the same procedure, but endeavors to transfer the visual discriminative stimulus to a Y-maze apparatus. The paper investigates the successfulness of the transfer, and focuses on the following questions: (1) to what extent do tuatara have the ability to learn? (2) can acquisition of stimulus control be transferred to a Y-maze from an open field arena? and (3) what does maze performance imply regarding species behavioral ecology, and what are the potential applications for this procedure?

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1.7 References

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Chapter II

Establishing a reliable method for testing acquisition learning and visual perception in tuatara (*Sphenodon spp.*).

2.1 Abstract

Learning paradigms can be used for two major purposes: to examine the process of conditioning or learning phenomena, and to use learning as a tool for examining discriminatory and sensory mechanisms. This study established a novel procedure to test learning and visual perception in tuatara. Seventeen juvenile tuatara (*Sphenodon punctatus*) were trained using food reinforcement to discriminate between two visual stimuli under a basic operant task: a discriminative stimulus (S⁺) which flickered at oscillation speeds between 2.65-65.09 Hz and a non-discriminative stimulus (S⁻) represented by a constant light. Tuatara are able to learn a simple operant task and to discriminate between 2.65-45.61 Hz, but not at 65.09 Hz. The study generated a reliable method that can be used to for more comprehensive psychophysical experiments on sensory discrimination, as well as examinations of audition, chemoreception, and tactile responses in tuatara, and other reptiles.

Keywords: tuatara (*Sphenodon punctatus*), reptile learning, oscillation speed, discriminative stimulus (S⁺), operant, methodology

2.2 Introduction

Learning paradigms (i.e., classical and operant conditioning) are used for two major types of study: to examine the process of conditioning or learning phenomena, and to use learning as a tool for examining discriminatory and sensory mechanisms (Burghardt, 1977). Conditioning studies with turtles (*Chyrsemys picta*) have shown that reptiles are capable of problem solving activities such as reversal shifts (RS), concept formation (Graf & Tighe, 1971), reversal learning (Alkov & Crawford, 1966), detour learning (Spigel, 1964a), and escape learning (Spigel, 1964b). Additional sensory studies have also examined the responsiveness of red spitting cobras (*Naja mossambica pallida*) to mouse derived cues (Stimac, Radcliffe, & Chizar, 1982), the use of a negative reinforcer (shock) to measure sensory with thresholds in turtles (Granda, Matsumiya, & Stirling, 1965), color discrimination in *Rana* tadpoles (Muntz, 1962), and classically conditioned air deprivation in *C. picta* (Morlock, Brothers, & Shaffer, 1968). In this study, we investigated the basic learning and visual abilities that govern tuatara behavior, and established a novel operant procedure in which to test the behavioral plasticity of the species.

Identifying the correct reinforcer for operant procedures elicits positive responsive behavior from the subject organism and increases their learning potential (Kemp, 1969). Successful reinforcers used to maintain operant behavior in reptiles include shelter (Stone, Ford, & Holtzman, 2000), light/dark access (Graf 1972; Kirkish, Fobes, & Richardson, 1979), access to heat (Alkov & Crawford; Kemp, 1969), and access to air (Morlock, Brothers, & Shaffer, 1968; van Sommers, 1963). Food is not a recommended reinforcer for ectotherms because of the unreliably to respond to food reward due to temperature-dependent performance, physiology, and behavioral responsiveness (Northcutt & Heath, 1973).

Learning and sensory perception investigations identify important characteristics of behavioral ecology for reptiles such as the ability to negotiate environmental contours (Punzo, 2002), establish orientation (Holtzman, Harris, Aranguren, & Bostocks, 1999), use homing strategies (Stone, Ford, & Holtzman, 2000), find home ranges (Zuri & Bull, 2000), distinguish between food items (Schall, 2000), recognize individuals (Aragón, López, & Martín, 2000; López and Martín, 2002), develop optimal foraging strategies (Krebs, 1978), and learn spatial tasks (López, Gómez, Rodríguez, Broglio, Vargas, & Salas, 2001; López, Rodríguez, Gómez, Vargas, Broglio, & Salas, 2000; Punzo & Madragon, 2002). Tuatara are the only surviving members of the reptilian order Sphenodontia. Once widespread throughout New Zealand, tuatara only inhabit 32 offshore islands (Gaze, 2001), and have also been maintained in captivity for over 100 years (Cartland, Cree, Sutherland, Grimmond, & Skeaff, 1994). However, only a few studies have been conducted on tuatara learning and sensory perception such as feeding ecology (Walls, 1981; Carmichael, Gillingham, & Keall, 1989), social behavior (Gillingham, Carmichael, & Miller, 1995), predator avoidance (Nelson, 2001), visual predation (Meyer-Rochow & Teh, 1991; Meyer-Rochow, 1989), and T-maze performance (Northcutt & Heath, 1973).

Since little is known regarding tuatara learning and sensory perception, we produced a novel approach to examine learning and visual sensory perception. We proposed whether: (1) we can train tuatara to learn an operant task using food reinforcement?, (2) tuatara are able to recognize visually discriminative stimuli?, and (3) we can generate a reliable method for testing reptilian learning and sensory perception, and if so, is this method applicable in testing other sensory mechanisms such as audition, olfaction, and tactility?

2.3 Methods

2.3.1 Experiment 1 - Acquisition

2.3.1.1 Subjects

Subjects were maintained in captive facilities at Victoria University of Wellington. Seventeen (nine males and eight females) individually marked juvenile tuatara (*Sphenodon punctatus*; Cook Strait) four years of age were held in six identical enclosures, ranging from one to four individuals per enclosure based on comparative weight, size, and sex. Enclosures measured 700 x 600 x 350 mm (100-mm of top soil, large pieces of pine bark). Tuatara were held under a 12L:12D light cycle (light between 06:00-18:00 hrs) using TrueliteTM (F40T12/TL, USA) vitamin bulbs. Mass (using an AND HF-300G electronic scale) and morphometric measurements (e.g., tail and snout-vent length) were recorded weekly.

Tuatara were acclimatized to the new facilities for one month before testing, and fed three times a week with either 15-20 mm mealworm larvae (*Tenebrio molitor*) or smallmedium (2-20 mm) locusts (*Locusta migratora*) from a commercial supplier (Biosuppliers – Auckland, New Zealand). Water was provided *ad libitum*. Prior to testing, slaters were introduced into enclosures to supplement feedings. Other food supplements included orthopteran, coleopteran, amphipodan, and hemipteran species. Invertebrate populations were decimated in each enclosure before experimental testing began.

2.3.1.2 Testing Arena

The testing arena was a glass aquarium (900 x 600 x 300 mm). The inside of the glass was covered with neutral brown paper to reduce any use of extramaze cues and reflection from the glass. The arena was divided into four separate quadrants and numerically labeled in conjunction with its respective corner (Figure 1): 1 (NW), 2 (NE), 3 (SW), and 4 (SE). Tuatara were allowed to habituate to the testing environment.

2.3.1.3 Equipment

Tuatara in the testing arena were monitored with an infrared camera powered by a 12V7Ah rechargeable battery (YP7-12 Super SONA[™]). The camera was placed 1.5-m over the testing arena and images were projected onto a JVC[™] Color Video Monitor TM-20PSN. Additional video was recorded with a Nikon[™] Coolpix 4500 and a USB Logitech[™] Quickcam. All video monitoring was viewed out of sight of the testing arena.

2.3.1.4 Feeding Stations

Three identical feeding stations were created. One feeding station was created as the discriminative stimulus (S^+) with oscillation capabilities of 2.68-27.78 Hz, while the second

feeding station presented a non-discriminative constant light (S⁻). The oscillation speed was monitored to a precise frequency using a Hewlett-PackardTM 54601A Oscilliscope. A third feeding station was designed with oscillation capabilities of 17.00-185.02 Hz. Three identical feeding station lights projected 3.5-lum on a white LED bulb, and were powered by a 3.6V LisunTM lithium battery (ER14505). The electronic components were encased in a plastic, neutral gray box (110 x 60 x 40 mm). The lights were positioned in the center of the box. A small wooden platform (120 x 80 x 20 mm) was attached to the bottom of the housing box. The wooden platform (214-mm) had a dugout area to house a feeding dish.



Figure 1. Pictorial of testing arena, feeding station, and procedure. Tuatara were placed in the center of the arena and oriented towards the feeding stations. The testing arena was divided into four quadrants (1-4) with respective directional corner (NW, NE, SW, SE).

The first set of feeding station dishes consisted of two glass dishes: one with a reinforcer without a cover at the S^+ feeding station, and one with a glass cover and reinforcer at the S^- feeding station. The second set was clear plastic with a reinforcer in the S^+ only. The third set of feeding stations consisted of two plastic painted (Mexican Red; Resene Paints Ltd., Lower Hutt, New Zealand) dishes with covers. Covers were attached to thin, plastic-
coated wire and looped through a hole in a piece of wood interlocked at 45° in each corner of the testing arena. The covers were manually lifted via friction-pulley in response to an approach by the subject. A reinforcer was only placed in the S⁺ feeding station. Tuatara were trained to approach and eat from feeding stations, using a shaping procedure.

2.3.1.5 Procedure

Subjects were tested in blocks. Each block consisted of three individual trials. Conditions of block trials were designated over seven phases and one extinction trial (Table 1). One extinction trial was completed between Phases VI and VII. The extinction trial consisted of turning both S^+ and S^- lights off. The extinction trial tests whether tuatara discriminate on the basis of a distinctively discriminative light or respond on the basis of randomized responses, assuming tuatara would choose either the S^+ or S^- at an equal 50% chance ratio. At the start of each trial, the tuatara was placed in the center of the testing arena and oriented towards the feeding stations. The placement of the S⁺ was pseudorandomized while the S⁻ was in either the left or right adjacent quadrant. Each feeding station was angled at approximately 45° towards the center of the testing arena. Each trial was completed by a final S^+ response, irrespective of the number of S^- responses. Tuatara were reinforced with either one mealworm or locust. The hind legs of the locust were excised to prevent them from escaping from the feeding dish. Tuatara were allowed a two-minute intertrial interval between each trial. After each trial, the testing arena and feeding stations were cleaned with 70% ethanol to neutralize chemical compounds. A 20-minute criterion was set to limit the length of each trial. If tuatara did not complete the required task, the trial was terminated at 20 minutes. Tuatara were placed in the center of the testing arena and allowed to approach either the S^+ or S^- . Responses were categorized by the subject being approximately five centimeters from a feeding station. Tuatara were only reinforced for a correct response. For an incorrect response, the S⁻ dish was lifted to reveal no reinforcer, and the subject was

returned to center. Temperature-dependent maze performance was also examined over five different temperature ranges: (1) $<15.9^{\circ}$ C, (2) 16.0-17.9°C, (3) 18.0-19.9°C, (4) 20.0-21.9°C, and (5) $>22.0^{\circ}$ C.

Table 1. Consecutive phase allocations for experimental conditions which included the oscillation speed, number of subjects (&), number of trials, and dish type.

Phase	Hz	Ss	# of Trials	Dish Type
Ι	2.65	17 (9_& 8_)	3	Glass
II	2.65	17 (9_& 8_)	15	Plastic
III	14.08	17 (9_& 8_)	21	Plastic
IV	14.08	17 (9_& 8_)	42	Friction-Pulley
V	25.06	13 (7_& 6_)	33	Friction-Pulley
VI	45.61	13 (7_& 6_)	21	Friction-Pulley
Extinction	$S^+ \& S^-$ lights turned off	6 (3_& 3_)	1	Friction-Pulley
VII	65.09	13 (7_& 6_)	6	Friction-Pulley

2.3.2 Experiment 2 – 65.09 vs. 25.06 Hz

2.3.2.1 Subjects

Thirteen subjects were used in Experiment 2, and were a subset of the original 17 individuals. Four tuatara were excluded for failure to complete the required task.

2.3.2.2 65.09 vs. 25.06 Hz

Tuatara were tested under 12 trials using the same testing arena, equipment, and feeding stations were used as in Experiment 1. In four block trials, the first trial for each subject was tested at 65.09 Hz and the second and third trials were tested at 25.06 Hz. Four trials were completed at 65.09 Hz, and eight trials were completed at 25.06 Hz. Temperature-dependent maze performance was examined over five different temperature ranges: (1) <17.9°C, (2) 18.0-18.9°C, (3) 19.0-19.9°C, (4) 20.0-20.9°C, and (5) >21.0°C.

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2.3.3 Statistical Analysis

Statistics were analyzed using SPSS for Windows (v11.5). Dependent measures included latency, percent accuracy and log d (a point estimate of discriminability independent of bias; White & Alsop, 1993). Statistical analysis incorporated descriptive statistics (n±1SE, %). Accuracy criteria were defined as 75% correct responses. ANOVAs compared performance at each oscillation speed, time variances in completion of each trial, temperature dependent performance (*F*), and S⁺ location. Kruskal-Wallis H chi-squared ($_2^2$) test compared accuracy of performance between trials, S⁺ location, and sex. Analysis using twotailed *t* tests examined differences between sexes. Mann-Whitney *U* nonparametric tests were used to compare extinction trials to each oscillation speed. Post hoc multiple comparison tests were used for latency (Tukey HSD) and accuracy (Bonferroni) nonparametric adjustments. All values, _ = 0.05, are reported to the nearest significance threshold with a minimum value of 0.001. Only mean+1SE was used in graphs for legibility.

2.4 Results

2.4.1 Experiment 1 – Acquisition

Subjects learned the required task (Figures 2A and 2B)and discriminated the S⁺ at 2.65 Hz to 45.61 Hz, but not at 65.09 Hz (Table 2). Tuatara were similarly accurate from 2.65-45.61 Hz, but accuracy significantly decreased at 65.09 Hz (Kruskal-Wallis H, $_2^2(4) = 63.40, p < 0.001$). There was a significant difference in latency ($F_{4,2100} = 88.66, p < 0.001$) with a mean (±SE) difference between 45.61 Hz and 65.09 Hz of 598.24±50.95 s.

Accuracy did not significantly change over successive trials (Kruskal-Wallis H, $_{2}^{2}(3) = 1.28$, p=0.733) nor did latency significantly decrease over successive trials (F_{2,2103} = 1.14, p=0.320) for trial 1 (386.11±15.52 s), trial 2 (359.47±16.02 s), and trial 3 (392.80±17.04 s). Accuracy significantly increased over the successive 14 block trials (Kruskal-Wallis H, $^{2}(20) = 49.53, p < 0.001$). Latency significantly decreased from the first block trial to the last $(F_{20,2085} = 28.63, p < 0.001)$. The S⁺ location did not affect the accuracy (Kruskal-Wallis H, $^{2}(3) = 1.91, p < 0.001$) or latency $(F_{3,2102} = 1.537, p = 0.202)$ of performance.



Figure 2. Acquisition of discrimination task in testing arena: Decrease in latency and increase in accuracy over consecutive block trials for 2.65-45.61 Hz, but high latency and low accuracy for 65.09 Hz and Extinction trials; A) Latency over block of three trials for each oscillation speed and B) accuracy over block of three trials for each oscillation speed.

Hz	Mean (<u>+</u> SE) Latency seconds	Range (s)	% Accuracy
2.65	199.18 <u>+</u> 10.45	10-1000 (N=255)	80.00 <u>+</u> 5.57%
14.08	468.51 <u>+</u> 15.11	8-1200 (N=714)	85.99 <u>+</u> 5.25%
25.06	194.43 <u>+</u> 9.90	8-1200 (N=429)	88.57 <u>+</u> 6.67%
45.61	333.92 <u>+</u> 21.18	11-1200 (N=273)	85.71 <u>+</u> 7.93%
65.09	932.17 <u>+</u> 42.59	59-1200 (N=78)	56.41 <u>+</u> 42.06%
Extinction	996.90 <u>+</u> 81.02	233-1200 (N=21)	52.94 <u>+</u> 4.78%

Table 2. Summary descriptive statistics for CFF phases showing high latency and low accuracy for 65.09 Hz and Extinction phases; N = the number of trials.

2.4.1.1 Feeding Station Trials

Subjects made variable response to the type of dishes (Figure 3). Fewer errors were made on the glass and plastic dishes, and at first, significantly more were made during the friction-pulley trials (Kruskal-Wallis H, $_2^2(2) = 75.20$, p<0.001). Subjects also performed significantly quicker ($F_{2,2103} = 167.470$, p<0.001) during glass dish trials (160.63 ± 20.25 s) and plastic dish (128.13 ± 5.77 s) than with the friction-pulley dish (481.39 ± 12.01 s).



Figure 3. Early phases show low latency and high accuracy, but decreases at the similar Extinction and 65.09 Hz phases; Mean (+SE) latency (s) and accuracy (%) for trial phases: Phase I (2.65 Hz and Glass Dishes), Phase II (2.65 Hz and Plastic Dishes), Phase III (14.08 Hz and Plastic Dishes), Phase IV (14.08 Hz and Friction-Pulley Covers), Phase V (25.06 Hz and Friction-Pulley Covers), Phase VI (45.61 Hz and Friction-Pulley Covers), Extinction Trial, and Phase VII (65.09 Hz and Friction-Pulley Covers).

The sex of the subjects did not influence accuracy (Kruskal-Wallis H, $_{-}^{2}(1) = 0.004$, p=0.949). There was also no significance in latency between sexes (t(2104) = 0.03, p=0.488). Accuracy was influenced by temperature with a decrease in errors with an increase in temperature (Kruskal-Wallis H, $_{-}^{2}(4) = 18.25$, p<0.001). Latency also significantly decreased ($F_{4,2100}=9.04$, p<0.001) when the ambient temperature increased from $<15.9^{\circ}$ C to $>22.0^{\circ}$ C (551.97 ± 91.63 s versus 210.49 ± 15.32 s).

2.4.1.2 Extinction

Similar accuracy and latency responses in the 65.09 Hz and extinction trials, shows that tuatara are unable to discriminate between the S⁺ and S⁻ at 65.09 Hz or Extinction. Tuatara did not meet criterion response accuracy during Extinction trials. Accuracy was overall significantly less (Kruskal-Wallis H, $_2^2(5) = 87.41$, p<0.001) when comparing the Extinction trial to 2.65 Hz (Mann-Whitney, U = 1720.50, p<0.001), 14.08 Hz (Mann-Whitney, U = 6538.50, p<0.001), 25.06 Hz (Mann-Whitney, U = 2415.00, p<0.001), and 45.61 Hz (Mann-Whitney, U = 1591.50, p<0.001). Accuracy during the Extinction trial was not significantly different than 65.09 Hz (Mann-Whitney, U = 751.50, p=0.523). Similarly, latency was significantly different during the Extinction trial ($F_{5,2121} = 81.08$, p<0.001). Latency during Extinction trials was significantly higher when compared to 2.65 Hz (t(274) = -18.55, p<0.001), 14.08 Hz (t(1090) = -4.87, p<0.001), 25.06 Hz (t(448) = -16.69, p<0.001), and 45.61 Hz (t(292) = -8.33, p<0.001). The Extinction trials were not significantly different than latency at 65.09 Hz (t(97) = -0.70, p=0.757).

2.4.2 Experiment 2 - 65.09 vs. 25.06 Hz

Subjects were able to distinctly discriminate between 25.06 Hz and 65.09 Hz (Figure 4A and 4B). Tuatara were more accurate at 25.06 Hz than 65.09 Hz (Kruskal-Wallis H, $_{-}^{2}(1) = 34.41$, *p*<0.001). Individuals correctly identified the S⁺ averaging 90.38% at 25.06 Hz.



Figure 4. Low latency and high accuracy response in 25.06 Hz trials shows distinct S^+ discrimination as opposed to high latency and low accuracy of 65.09 Hz trials; A) Comparison of latency for both 65.09 Hz and 25.06 Hz and B) comparison of accuracy for both 65.09 Hz.

However, subjects correctly responded to the S^+ averaging 48.07% at 65.09 Hz.

Latency was significantly less (t(154) = -18.44, p < 0.001) in 25.06 Hz (243.88±24.23 s) than 65.09 Hz (1071.88±41.06 s). Trial 1 (65.09Hz) showed less accuracy than trials 2 and 3 (25.06 Hz). Trials 1 and 2 show significant differences in accuracy (Kruskal-Wallis H, _2(1) = 18.66, p < 0.001) as trials 2 and 3 did not show a significant difference (Kruskal-Wallis H, $^2(1) = 0.51$, p=0.475). There was also a significant difference over successive trials ($F_{2.153} =$

169.33, p < 0.001) with a mean (±SE) difference between trials 1 and 2 of 840.79±51.97 s and between trials 2 and 3 of 25.58±51.97 s. Log d (5.819) and log bias (0.034) were calculated for 25.06 Hz, showing discriminatory stimulus control and no bias in S⁺ discrimination. Log d (-0.133) and log bias (-0.067) were also calculated for 65.09 Hz, showing no left/right preference.

There was no significant difference in accuracy between sexes (Kruskal-Wallis H, $_{-}^{2}(1) = 0.61, p=0.435$). There was also no significant difference in latency for sex (t(154) = 1.33, p=0.093). There was no temperature-dependent significance in accuracy (Kruskal-Wallis H, $_{-}^{2}(4) = 4.62, p=0.329$) or latency ($F_{4,151} = 0.43, p=0.787$).

2.5 Discussion

We produced a methodology applicable to learning and visual discrimination studies in tuatara. This is the first psychophysical procedure in which to test tuatara learning and visual perception. Having established a reliable procedure for visual perception, the method may be used to test other sensory discriminative mechanisms in tuatara and other reptiles.

2.5.1 Tuatara Can Learn

Acquisition trials demonstrated that tuatara have learning and memory cognitive abilities for engaging in decision making and problem solving activities as reflected by low latencies and reduced errors over consecutive trial periods. Tuatara can adapt to a behavioral response strategy when stimuli change. The ability for tuatara to perceive a stimulus, and appropriately respond may be indicative of cognitive strategies used for survivorship.

Task performance was not temperature-dependent within ambient temperatures from 15.1-23.3°C. However, the natural climatic range for tuatara ranges from approximately 5-28°C (Cartland & Grimmond, 1994), indicative of above or below test temperatures. If the

test temperatures were outside the natural mid-range for tuatara, performance may have shown discrepancies that are temperature-dependent (Northcutt & Heath, 1973).

There was no difference in performance between sexes. Some reptiles do display sexbased dominance hierarchies in social groupings (Stamps, 1977). Dominance hierarchies are prominent in captive situations where an increase in population density alters the response to ecological factors such as food supply, territory, and niche habitat. For example, captivity is a reflection of population densities (i.e., Stephens Island) where there is a large tuatara population in a relatively small area (Goetz & Thomas, 1994). However, males and females were not separately tested, and access to a food was not indicative of natural availability. Sized-based hierarchical difference may influence performance, but was also not tested.

Although tuatara are sit-and-wait predators, juveniles need to locate a proximal food supply, and return to a burrow as quickly as possible to avoid predation by adult tuatara (Walls, 1981), and other species. However, a comparison between adult and juvenile tuatara should examine age-based or ontogeny differences in performance.

2.5.2 Oscillation Speed

Low accuracy in the extinction probe and 65.09 Hz trials suggest tuatara were responding to discriminatively distinct stimuli between 2.65-45.61 Hz and not at 65.09 Hz, and that vision is important in environmental discrimination. Tuatara may use an integration of all sensory mechanisms, but vision is the dominant sensory mechanism in prey discrimination and supports previous observations (Walls, 1981; Meyer-Rochow, 1989; Meyer-Rochow & Teh, 1991).

2.5.3 A New Methodology

Little was known about tuatara learning ability, motivational drive, and sensory mechanisms. Since a new operant procedure was introduced, testing was done on a trial-and-

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error basis until a reliable procedure was continuous administered. Initial open field testing allowed tuatara to learn by maximizing the opportunity to use additional sensory cues such as olfaction and audition to identify reinforcers (Experiment 1). However, subsequent conditions reduced the opportunity to use other cues (Experiment 2) by reducing the number of confounds and having tuatara discriminate solely on the basis of visual stimuli. Responses to glass and plastic dishes accrued faster response times and higher accuracy. By using glass dishes, tuatara could easily see the food reward, and similarly with the plastic dishes, tuatara could see and possibly smell or hear the food reward. Switching to the friction-pulley operation had eliminated the ability to see the food reward, and provided a more well-established procedure. However, tuatara still approached the S⁻ for incorrect responses. If tuatara were also using audition and olfaction as equally discriminative mechanisms, tuatara may have been able to discriminate whether there was a food reward under the pulley-dish. This suggests that vision is an important sensory modality in which to gauge the environment, and may be dominant over other sensory mechanisms in specific situations.

Food proved to be a successful reinforcer despite observations suggesting that ectotherms would not respond to a food reward (Northcutt & Heath, 1973). Although food reward was used as the primary reinforcer, an alternative reinforcer may elicit a more desirable response in other reptiles motivated by another vital stimulus such as access to heat, shelter, air, or water. This method can also be used to test other sensory mechanism, the successfulness of various reinforcers, and ecological preferences. In addition, by understanding how tuatara interpret their sensory world, we have the potential to improve conservation management and animal welfare practices.

By establishing a reliable method, we can examine variations of the S^+ and training regimes. Variations in the S^+ include luminescence, color schemes, black and white patterns, and infrared spectrum. Additionally, this method can be used to examine audition, chemoreception, and tactual responses in tuatara and other reptiles. A more intense

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examination of sensory perception is required, and would examine a forced response to the S⁺ where the two opposing stimuli are suspected within visual range (i.e., 2.65 Hz versus 14.08 Hz). The pineal eye is thought to play an intricate role in behavioral adaptation, but its sensitivity to visual stimuli in tuatara is not understood. Using learning paradigms to examine cognitive theory such as reversal learning and navigational strategies will add further to the natural history of the species.

2.5.4 Summary

Tuatara have demonstrated learning and memory abilities, while also demonstrating visual discrimination at various oscillation speeds. The study generated a reliable method that can be used to for more in depth psychophysical experiments to further access perception and learning in tuatara. The study provides implications for species management and a better understanding of tuatara natural history.

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Chapter III

Critical flicker-fusion (CFF) rate and motion detection properties of the tuatara (*Sphenodon spp.*).

3.1 Abstract

Spatial learning and visual discrimination are both ecologically important characteristics for reptiles that allow for the reception of important sensory information, and thereby allow the individual to correctly respond to appropriate stimuli in their natural habitat. Critical flicker-fusion (CFF) is the rate of flicker at which an individual or species ceases to see the flicker of a flashing light; CFF rate reflects the ability and proficiency by which an organism resolves moving objects. Seventeen juvenile tuatara (*Sphenodon punctatus*) were trained under a simple operant task to respond to various discriminative stimuli (S⁺) flickering between 2.65-65.09 Hz. Tuatara were able to learn the required task and discriminate the S⁺ at CFF rates between 2.65-45.61 Hz, but not at 65.09 Hz. This novel psychophysical methodology has demonstrated that tuatara can learn a basic operant task, discriminate visual stimuli, and show motion detection abilities comparable to other species. Implications of this study facilitate aspects of motion detection and visual stimuli that are important to the behavioral ecology of tuatara. In addition, this method may be used in more comprehensive examinations of visual perception, audition, chemoreception, and tactile responses in tuatara, and other reptiles.

Keywords: tuatara (*Sphenodon punctatus*), critical flicker-fusion (CFF), learning, visual discrimination, operant, methodology

3.2 Introduction

Spatial learning and visual discrimination are both ecologically important characteristics for reptiles. Discriminating important visual cues allows reptiles to negotiate variations in the environment (Punzo, 2002), establish orientation (Holtzman, Harris, Aranguren, & Bostocks, 1999), use homing navigation (Stone, Ford, & Holtzman, 2000), find home ranges (Zuri & Bull, 2000), distinguish between food items (Schall, 2000), recognize individuals (Aragón, López, & Martín, 2000; López and Martín, 2002), adapt and learn spatial tasks (López, Gómez, Rodríguez, Broglio, Vargas, & Salas, 2001; López, Rodríguez, Gómez, Vargas, Broglio, & Salas, 2000), and develop optimal foraging strategies (Krebs, 1978). Behavioral ecology processes facilitated by sensory discrimination and spatial learning are correlated with an organism's ability to perceive motion.

Critical flicker-fusion (CFF) is the rate of flicker at which the individual or species ceases to see a flicker of flashing light. A rapidly flashing light evokes the sensation of a flicker; when the flash frequency increases, the light perceivably ceases to flicker (Sekular & Blake, 2002; Trachina, Gordon, & Shapely, 1984). Flicker-fusion reflects the ability and proficiency to resolve moving objects. If an image moves faster across the retina, it becomes less blurred, enabling animals to negotiate complex environments, and identify and catch swift prey or avoiding fast-moving predators. Understanding CFF rates provide a physiological basis for behavioral implications in species ecology.

Although electrophysical methods are successful in determining CFF rates, psychophysical experimentation is also a reliable method in testing CFF thresholds (Maxwell & Granda, 1975). Psychophysical experimentation has been used to examine CFF thresholds in monkeys (Shumake, Smith, & Taylor, 1968), cats (Loop & Berkeley, 1975), rats (Williams, Pollitz, Smith, & Williams, 1985), pigeons (Hendricks, 1966), dogs (Colie, Pollitz, & Smith, 1989), cane toads (Nowak & Green, 1983), and chickens (Jarvis, Taylor, Prescott, Meeks, & Wathes, 2002). All subjects were trained using a conditioning paradigm to learn and recognize discriminative stimuli, and were reinforced for correct responses to perceivable CFF stimuli. Learning paradigms (i.e., classical and operant conditioning) examine the process of conditioning or other learning phenomena, and can be used as a tool for examining discriminatory and sensory mechanisms (Burghardt, 1977). This is the first novel psychophysical study to use a learning paradigm to investigate the basic visual sensory capacity in the form of CFF rates that govern the behavior of tuatara.

Tuatara are the only living representative of the order Sphenodontia with two surviving species (*Sphenodon punctatus* and *S. guntheri*; Gaze, 2001). Once widespread throughout the mainland of New Zealand, tuatara are now restricted to 32 offshore islands (Gaze, 2001). They have also been maintained in captivity for over 100 years (Cartland, Cree, Sutherland, Grimmond, & Skeaff, 1994). Studies involving tuatara have focused on physiology, but few are focused on sensory perception. However, base sensory investigations have covered feeding ecology (Walls, 1981; Carmichael, Gillingham, & Keall, 1989), social behavior (Gillingham, Carmichael, & Miller, 1995), predator avoidance (Nelson, 2001), and shelter preference using a T-maze (Northcutt & Heath, 1973). Walls (1981) suggested that tuatara attune to the movement of objects as visual conditioning stimuli. Vision has been hypothesized as the primary sensory mechanism tuatara use to hunt and detect prey (Meyer-Rochow & Teh, 1991; Meyer-Rochow, 1989).

We employed a novel, psychophysical approach in examining learning ability and visual sensory perception (CFF) in tuatara. Understanding tuatara learning and discrimination ability may help us recognize relationships between ecological processes and motion detection that have promoted the survival of the species. We examined several questions: (1) can tuatara learn to discriminate visual stimuli under operant conditions and what biological factors influence performance?, (2) what is the CFF rate for tuatara and how does it compare to other species?, (3) how does motion detection ability apply to ecological processes?, and (4) can a reliable method be developed for testing tuatara learning and visual

perception, if so, would this method be applicable for testing other sensory mechanisms such as audition, chemoreception, and tactility?

3.3 Methods

3.3.1 Experiment 1 - Acquisition

3.3.1.1 Subjects

Subjects were maintained in captive facilities at the Victoria University of Wellington School of Biological Sciences. Seventeen (nine males and eight females) individually marked juvenile tuatara (*S. punctatus*; Cook Strait), approximately four years of age, were used for the study. Tuatara were sourced from egg laying and incubation regimes (see Nelson, 2001): natural laying (LNAT) versus induced (IND) egg laying, and artificial (ART) versus natural (NAT) incubation method.

Tuatara were held under a 12L:12D light cycle (light between 06:00-18:00 hrs) using Truelite[™] (F40T12/TL, USA) vitamin bulbs. Individuals were held in six identical enclosures, ranging from one to four individuals per enclosure based on comparative weight, size, and sex. Enclosures measured 700 x 600 x 350 mm (100-mm of top soil, large pieces of pine bark). Mass (using an AND HF-300G electronic scale) and morphometric measurements (e.g., tail and snout-vent length) were recorded weekly.

Tuatara were acclimatized for one month before testing, were fed three times a week, and were given either 15-20 mm mealworm larvae (*Tenebrio molitor*) or small-medium (2-20 mm) locusts (*Locusta migratora*) from a commercial supplier (Biosuppliers – Auckland, New Zealand). Water was provided *ad libitum*. Prior to experimental testing, isopods were introduced into enclosures as food supplements. Isopod populations were decimated in each enclosure before experimental testing began. Other invertebrates introduced as supplemental food included various species of orthoptera, coleoptera, amphipoda, and hemiptera.

3.3.1.2 Testing Arena

The testing arena was a glass aquarium (900 x 600 x 300 mm). The inside of the glass was covered with neutral brown paper reducing extramaze cues and reflection from the glass. The arena was divided into four separate quadrants and numerically labeled in conjunction with its respective corner: 1 (NW), 2 (NE), 3 (SW), and 4 (SE). Tuatara were allowed to habituate to the testing environment for an hour before their first test trial.

3.3.1.3 Equipment

Subjects in the testing arena were monitored with an infrared camera powered by a 12V7Ah rechargeable battery (YP7-12 Super SONATM). The camera was placed 1.5-m over the testing arena and images were projected onto a JVCTM Color Video Monitor TM-20PSN. Additional video recording was done with a NikonTM Coolpix 4500 and a USB LogitechTM Quickcam. All video monitoring was viewed out of sight of the proximal testing arena.

3.3.1.4 Feeding Stations

Three identical feeding stations were created. One feeding station was created as the discriminative stimulus (S⁺) with oscillation capabilities of 2.68-27.78 Hz, while the second feeding station presented a non-discriminative constant light (S⁻). Feeding station lights projected 3.5-lum on a white LED bulb, and powered by a 3.6V LisunTM lithium battery (ER14505). The oscillation speed was monitored and changed to a precise frequency using a Hewlett-PackardTM 54601A Oscilliscope. A third feeding station was designed with oscillation capabilities of 17.00-185.02 Hz. The electronic components were encased in a plastic, neutral gray box (110 x 60 x 40 mm). The lights were positioned in the center of the box. A small wooden platform (120 x 80 x 20 mm) was attached to the bottom of the housing box. The wooden platform (214-mm) had a dugout area to house a feeding dish.

The first set of feeding station dishes consisted of two glass dishes: the S^+ feeding station with a reinforcer without a cover, and the S^- feeding station with a glass cover and reinforcer. The second set was clear plastic with a reinforcer in the S^+ only. The third set of feeding stations consisted of two plastic painted (Mexican Red; Resene Paints Ltd., Lower Hutt, New Zealand) dishes with covers. Covers were attached to thin, plastic-coated wire and looped through a hole in a piece of wood interlocked at 45° in each corner of the testing arena. The covers were manually lifted via friction-pulley in response to an approach by the subject. Reinforcers were placed in the S^+ only. Tuatara were trained to approach and eat from the feeding stations, using a shaping procedure. Refer to Figure 1 in Chapter 2 for a diagrammatic model.

3.3.1.5 Procedure

Subjects were tested in a series of block trials. One block trial consisted of three individual and consecutive trials. Each individual was tested for three trials. Tuatara were tested in five phases designated by different flicker rates and dishes (Table 1). One extinction trial was completed at the end after all phases were tested. The extinction trial consisted of turning both S^+ and S^- lights off. The extinction trial tests whether tuatara discriminate on the basis of a distinctively discriminative light or respond based on randomized guessing, assuming subjects would choose either the S^+ or S^- at an equal 50% chance ratio.

At the start of each trial, the tuatara was placed in the center of the testing arena and oriented towards the feeding stations. The S⁺ placement was pseudorandomized while the S⁻ was in either the left or right adjacent quadrant. Each feeding station was angled at approximately 45° towards the center of the testing arena. Each trial was completed by a S⁺ latency response where the subject makes a final approach to the positively reinforced feeding dish with an invertebrate under the cover, and irrespective of the number of S⁻ responses. Tuatara were reinforced with either one 15-20 mm mealworm larvae or 2-20 mm

locust. The hind legs of the locust were excised to prevent them from escaping from the feeding dish. Tuatara were placed in the center of the testing arena and allowed to approach either the S⁺ or S⁻. Responses were categorized by the tuatara being approximately five centimeters from a feeding station. Tuatara were only reinforced for a correct response. For an incorrect response, the S⁻ dish was lifted to reveal no reinforcer, and the subject was returned to center. Subjects were allowed a two-minute intertrial interval between each trial. A 20-minute criterion was set to limit the length of each trial. If a subject did not complete the task, the trial was terminated at 20 minutes. After each trial, the testing arena and feeding stations were cleaned with 70% ethanol to neutralize chemical compounds. Temperature-dependent maze performance was examined over five ambient temperature ranges: (1) <15.9°C, (2) 16.0-17.9°C, (3) 18.0-19.9°C, (4) 20.0-21.9°C, and (5) >22.0°C.

Phase	CFF Hz	Subjects	# of Trials	Dish Type
Ι	2.65	17 (9_, 8_)	a. 3 b. 15	a. Glass b. Plastic
Π	14.08	17 (9_, 8_)	42	Friction-Pulley
III	25.06	13 (7_, 6_)	33	Friction-Pulley
IV	45.61	13 (7_, 6_)	21	Friction-Pulley
V	65.09	13 (7_, 6_)	6	Friction-Pulley
Extinction	$S^+ \& S^-$ lights switched off	6 (3_, 3_)	1	Friction-Pulley

Table 1. Consecutive phase allocations for experimental conditions which included the CFF oscillation speed, number of subjects (&), number of trials, and dish type.

3.3.2 Experiment 2 - 65.09 vs. 25.06 Hz

3.3.2.1 Subjects

Thirteen tuatara were recruited for Experiment 2. The tuatara were a subset of the original 17 individuals. Four tuatara were excluded from the experiment that failed to complete the acquisition task.

3.3.2.2 Procedure

Tuatara were tested under 12 trials using the same testing arena, equipment, and feeding stations, and general procedure were used as in Experiment 1. Under four block trials, four trials were completed at 65.09 Hz (trial 1), and eight trials were completed at 25.06 Hz (trials 2 and 3). Oscillation speed was allocated because tuatara were able to discriminate lights at 25.06 Hz, but not at 65.09 Hz. Temperature-dependent maze performance was examined over five ambient temperature ranges: (1) <17.9°C, (2) 18.0-18.9°C, (3) 19.0-19.9°C, (4) 20.0-20.9°C, and (5) >21.0°C.

3.3.3 Statistical Analysis

Statistics were analyzed using SPSS for Windows (v11.5). Dependent measures included latency, percent accuracy and log d (a point estimate of discriminability independent of bias; White & Alsop, 1993). Statistical analysis incorporated descriptive statistics (n+SE, %). Accuracy criteria were defined as 75% of responses correctly chosen. ANOVAs were used for comparing performance at each oscillation speed, time variances in completion of each trial, temperature dependent performance (F), and S⁺ location. Mixed model ANOVAs assessed performance indicators with type III tests of fixed effects. Small sample sized Akaike's Information Criterion (AIC_c) was used to gauge the importance of sex, birth, incubation, and test temperature as performance indicators for latency with individual tuatara as the random effect (Burnham & Anderson, 1998). Kruskal-Wallis H chi-squared (²) tests compared accuracy of performance between trials, S⁺ location, sex, LNAT versus IND egg laying, and ART versus NAT incubation method. Analysis using two-tailed t tests also included differences between sexes, LNAT versus IND egg laying, and ART versus NAT incubation method. Mann-Whitney U tests were also used to compare extinction trials to each oscillation speed. Post hoc multiple comparison tests were used for latency (Tukey HSD) and accuracy (Bonferroni) nonparametric adjustments. All values, = 0.05, are

reported to the nearest significance threshold with a minimum value of 0.001. Only mean+1SE was used in graphs for legibility.

3.4 Results

3.4.1 Experiment 1 - Acquisition

Subjects were able to learn the required task and discriminate the S⁺ at 2.65-45.61 Hz, but not at 65.09 Hz. Tuatara produced low latencies and were accurate during 2.65-45.61 Hz trials, but had long latency responses and were relatively inaccurate for 65.09 Hz and Extinction phases (Table 2; Figure 1A and 1B). Tuatara were similarly accurate from 2.65-45.61 Hz, but accuracy significantly decreased at 65.09 Hz (Kruskal-Wallis H, $_{2}^{2}(4) = 63.40$, p<0.001). There was a significant difference in latency of performance ($F_{4,2100} = 88.66$, p<0.001) with a mean (\pm SE) difference between 45.61 Hz and 65.09 Hz of 598.24 \pm 50.95 s.

Table 2. Summary descriptive statistics for CFF phases showing high latency and low accuracy for 65.09 Hz and Extinction phase; N = the number of trials.

CFF Hz	Mean (<u>+</u> SE) Latency (s)	Range (s)	% Accuracy
2.65	199.18 <u>+</u> 10.45	10-1000 (N=255)	80.00 <u>+</u> 5.57
14.08	468.51 <u>+</u> 15.11	8-1200 (N=714)	85.99 <u>+</u> 5.25
25.06	194.43 <u>+</u> 9.90	8-1200 (N=429)	88.57 <u>+</u> 6.67
45.61	333.92 <u>+</u> 21.18	11-1200 (N=273)	85.71 <u>+</u> 7.93
65.09	932.17 <u>+</u> 42.59	59-1200 (N=78)	56.41 <u>+</u> 42.06
Extinction	996.90 <u>+</u> 81.02	233-1200 (N=21)	52.94 <u>+</u> 4.78



Figure 1. Acquisition of discrimination task in testing arena: Decrease in latency and increase in accuracy over consecutive block trials for 2.65-45.61 Hz, but high latency and low accuracy for 65.09 Hz and Extinction trials; A) Latency over block of three trials for each oscillation speed and B) accuracy over block of three trials for each oscillation speed.

Accuracy did not significantly change over successive individual trials (Kruskal-Wallis H, $_{2}^{2}(3) = 1.28$, p=0.733). Latency did not significantly decrease over successive trials ($F_{2,2103} = 1.14$, p=0.320) for trial 1 (386.11±15.52 s), trial 2 (359.47±16.02 s), and trial 3 (392.80±17.04 s). Accuracy significantly increased over the successive 14 block trials (Kruskal-Wallis H, $_{2}^{2}(20) = 49.53$, p<0.001). Latency significantly decreased from the first block trial to the last ($F_{20,2085} = 28.63$, p<0.001). The S⁺ location did not affect the accuracy (Kruskal-Wallis H, $_{2}^{2}(3) = 1.91$, p<0.001) or latency ($F_{3,2102} = 1.537$, p=0.202) of performance. The AIC_C predicts that the most important to least important indicator of

performance is test temperature, followed by incubation, birth, and sex, showing that the best

model includes all four main effects (Table 3).

Table 3. Type III tests of fixed effects for examining the main effects of performance indicators during feeding station trials showing the best model using all performance indicators; E = egg laying, S = sex, I = incubation method, T = temperature.

Effect	ΔAIC _C	Source	df	F	р
H _o	170.87	H _o	1,2105	1653.14	< 0.001
S	163.17	S	1,2104	0.01	= 0.974
Е	138.22	Е	1,2104	24.31	< 0.001
Ι	131.36	Ι	1,2104	31.05	< 0.001
Т	84.44	Т	4,2100	9.04	< 0.001
E + I	104.80	E I	1,2103 1,2103	18.15 24.87	< 0.001 < 0.001
E + T	49.82	E T	1,2099 4,2099	26.32 9.54	< 0.001 < 0.001
E + S	125.45	E S	1,2103 1,2103	29.25 4.90	< 0.001 = 0.027
S + I	123.53	S I	1,2103 1,2103	0.14 31.70	= 0.713 < 0.001
S + T	76.75	S T	1,2099 4,2099	0.01 9.04	= 0.978 < 0.001
I + T	42.28	I T	1,2099 4,2099	33.75 9.75	< 0.001 < 0.001
E + I + S	91.55	E I S	1,2102 1,2102 1,2102	23.45 25.36 5.39	< 0.001 < 0.001 = 0.020
E + T + S	36.28	E T S	1,2098 4,2098 1,2098	32.06 9.75 5.68	< 0.001 < 0.001 = 0.017
E + I + T	14.12	E I T	1,2098 1,2098 4,2098	19.72 27.13 10.13	< 0.001 < 0.001 < 0.001
I + T + S	34.39	I T S	1,2098 4,2098 1,2098	33.95 9.76 0.21	< 0.001 < 0.001 = 0.647
E + I + T + S	0	E I T S	1,2097 1,2097 4,2097 1,2097	25.88 27.76 10.38 6.32	< 0.001 < 0.001 < 0.001 = 0.012

3.4.1.1 Feeding Station Trials

Initially, tuatara responded faster and more accurately to plastic and glass dishes Fewer errors were made on the glass and plastic dishes, and significantly more were made during the friction-pulley trials (Kruskal-Wallis H, $_2(2) = 75.20$, p < 0.001). Tuatara also performed significantly more quickly ($F_{2,2103} = 167.470$, p < 0.001) during glass dish trials (160.63 ± 20.25 s) and plastic dish (128.13 ± 5.77 s) than with the friction-pulley dish (481.39 ± 12.01 s).

There were no significant differences in accuracy between sexes (Kruskal-Wallis H, $_^2(1) = 0.004$, p=0.949), egg laying type (Kruskal-Wallis H, $_^2(1) = 0.239$, p=0.625), or incubation method (Kruskal-Wallis H, $_^2(1) = 0.707$, p=0.400). There was also no significance in latency between sexes (t(2104) = 0.03, p=0.488). However, egg laying type (t(2104) = 4.93, p<0.001) and incubation method (t(2104) = -5.57, p<0.001) displayed significant effects. IND juveniles performed faster (261.84 ± 22.20 s) than LNAT juveniles (397.56 ± 10.15 s). ART incubated juveniles also performed faster (362.31 ± 9.65 s) than NAT incubated juveniles (532.65 ± 32.29).

Temperature influenced accuracy, showing a decrease in accuracy with an increase in temperature (Kruskal-Wallis H, $_{2}^{2}(4) = 18.25$, p < 0.001). Latency also significantly decreased ($F_{4,2100} = 9.04$, p < 0.001) when the ambient temperature increased from $<15.9^{\circ}$ C to $>22.0^{\circ}$ C (551.97 ± 91.63 s versus 210.49 ± 15.32 s).

3.4.1.2 Extinction

Subjects did not meet criterion response latency and accuracy during Extinction trials. With similar accuracy and latency responses between 65.09 Hz and the Extinction trials, this suggests that tuatara are unable to discriminate between the S⁺ and S⁻ at 65.09 Hz or Extinction, and the high CFF threshold for tuatara is between 45.61-65.09 Hz. During the Extinction trial, accuracy was significantly less (Kruskal-Wallis H, $^{2}(5) = 87.41$, p < 0.001) than the trials at 2.65 Hz (Mann-Whitney, U = 1720.50, p<0.001), 14.08 Hz (Mann-Whitney, U = 6538.50, p<0.001), 25.06 Hz (Mann-Whitney, U = 2415.00, p<0.001), and 45.61 Hz (Mann-Whitney, U = 1591.50, p<0.001), but was not significantly different from 65.09 Hz (Mann-Whitney, U = 751.50, p=0.523). Similarly, latency was overall significantly higher during the Extinction trial ($F_{5,2121} = 81.08$, p<0.001). Latency during Extinction trials was significantly higher when compared to 2.65 Hz (t(274) = -18.55, p<0.001), 14.08 Hz (t(1090) = -4.87, p<0.001), 25.06 Hz (t(448) = -16.69, p<0.001), and 45.61 Hz (t(292) = -8.33, p<0.001). The Extinction trials were not significantly different than latency at 65.09 Hz (t(97) = -0.70, p=0.757).

3.4.2 Experiment 2 - 65.09 vs. 25.06 Hz

Subjects displayed a distinct discrimination between 25.06 Hz and 65.09 Hz (Figure 2A and 2B). Tuatara were more accurate at 25.06 Hz than 65.09 Hz (Kruskal-Wallis H, $_2^2(1) = 34.41, p < 0.001$). Individuals correctly identified the S⁺ averaging 90.38% at 25.06 Hz. However, subjects correctly responded to the S⁺ averaging 48.07% at 65.09 Hz. Latency was significantly less (t(154) = -18.44, p < 0.001) at 25.06 Hz (243.88±24.23 s) than 65.09 Hz (1071.88±41.06 s). Trial 1 (65.09Hz) showed less accuracy than trials 2 and 3 (25.06 Hz). Comparably, trials 1 and 2 showed significant differences in accuracy (Kruskal-Wallis H, $_2^2(1) = 18.66, p < 0.001$) as trials 2 and 3 did not show any significant differences (Kruskal-Wallis H, $_2^2(1) = 0.51, p = 0.475$). There was also a significant difference in successive trials ($F_{2,153} = 169.33, p < 0.001$) with a mean (±SE) difference between trials 1 and 2 of 840.79±51.97 s and between trials 2 and 3 of 25.58±51.97 s. Log d (5.819) and log bias (0.034) were calculated for 25.06 Hz, showing discriminatory stimulus control and no bias in S⁺ discrimination. Log d (-0.133) and log bias (-0.067) were also calculated for 65.09 Hz and show lack of discriminability between the S⁺ and S⁻ with no bias for left/right preference.

Subjects showed no significant distinction in accuracy between sexes (Kruskal-Wallis H, $_{-2}^{2}(1) = 0.61$, p=0.435) and incubation type (Kruskal-Wallis H, $_{-2}^{2}(1) = 1.78$, p=0.182). There was also no significant difference in latency for sex (t(154) = 1.33, p=0.093) or incubation type (t(154) = 1.58, p=0.058). There was no temperature-dependent significance in accuracy (Kruskal-Wallis H, $_{-2}^{2}(4) = 4.62$, p=0.329) or latency ($F_{4,151} = 0.43$, p=0.787).



Figure 2. Low latency and high accuracy response in 25.06 Hz trials shows distinct S^+ discrimination as opposed to high latency and low accuracy of 65.09 Hz trials; A) Comparison of latency for both 65.09 Hz and 25.06 Hz and B) comparison of accuracy for both 65.09 Hz.

3.5 Discussion

Tuatara have demonstrated basic acquisition of a simple operant task. In addition, tuatara have learned the task by perceivably discriminating visual stimuli of CFF rates

between 2.65-45.61 Hz, but not at 65.09 Hz. Visual discrimination and motion detection abilities are comparable to other species. The methodology provided a basis in which to test visual discrimination, but may also be applicable in testing other sensory mechanisms.

3.5.1 Tuatara Learning Capacity

Tuatara are capable of learning a simple operant task in addition to discriminating CFF rates between 2.65-45.61 Hz. In the acquisition and CFF trials from 2.65-45.61 Hz, tuatara produced low latencies and errors over consecutive trials. Tuatara can develop cognitive abilities and adapt to a memory strategy for engaging in decision making and problem solving activities. The ability for tuatara to perceive a stimulus, and respond appropriately may be a function of cognitive strategies used for survivorship.

Task performance in this study was not temperature-dependent across tested range (15.1-23.3°C). Comparably to other ectotherms, the natural temperature range for tuatara has a higher and lower range in New Zealand, which was outside the experimental range (Cartland & Grimmond, 1994). If the ambient temperature during experiments was modified to higher or lower temperatures, there may have been a discrepancy in ectotherm performance. For example, cold ambient temperature may produce lethargic behavior in ectotherm performance (Northcutt & Heath, 1973; Kemp, 1969; Krekorian, Vance, & Richardson, 1968). However, performance in temperatures outside experimental conditions should be tested to observe any temperature-dependent task performance.

There was no difference in performance between sexes. Some reptiles display sexbased dominance hierarchies in social groupings. Sex-based dominance hierarchies are more prominent in captive situations where an increase in population density alters the response to ecological factors such as food supply, territory, and niche habitats (Stamps, 1977). Captivity reflects population densities similar to Stephens Island where there is a large tuatara population in a relatively small area (Goetz & Thomas, 1994). However, sex-based competition for food resources was not reported. Males and females were not separately tested, and access to food was not a reflection of availability in their natural habitat. Additionally, size-based differences may be more important for examining hierarchy.

Induced egg laying and artificially incubated individuals performed feeding station trials faster than ones laid naturally or naturally incubated individuals. For tuatara held in captivity, the survival rate is substantially higher than tuatara in the wild (Goetz & Thomas, 1994). In captivity, subjects held in semi-natural areas were monitored for health and changes in environmental variability. IND and ART individuals may respond and adapt to the task faster than LNAT and NAT individuals because the level of fitness is higher for the former group than the latter.

3.5.2 Critical Flicker-Fusion Rates and Motion Detection

Tuatara were able to discriminate CFF rates between 2.65-45.61 Hz. The upper CFF threshold is between 45.61-65.09 Hz, but the lower threshold was not tested. Low accuracy in the extinction probe and 65.09 Hz trials in Experiment 1 and distinct discrimination of oscillation speeds in Experiment 2 suggested that tuatara can discriminate on the basis of visual stimuli, and may rely on visual discrimination in receiving context-dependent cues from the environment. Tuatara may use an integration of all sensory mechanisms, but vision is the dominant sensory mechanism in prey discrimination and social behavior, consequently supporting previous observations (Walls, 1981; Meyer-Rochow, 1989; Meyer-Rochow & Teh, 1991; Gillingham et al., 1995).

Tuatara visual perception is comparable to other species such as chickens, cats, humans, and *Anolis spp.* (Table 4). Motion detection in tuatara is comparable to avian, mammalian, and herpetological species that evolved separately from the tuatara. Primarily a nocturnal species (Cree & Daugherty, 1990), tuatara displayed CFF rates comparable to the motion detection parameters similar to species that are both rod and cone adapted. Tuatara

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are known as one of the oldest living reptiles, and this study has shown that visual perception in the species may have evolved to accommodate discrimination for diurnal and crepuscular cycles, and evolutionary changes in the natural environment. Visual ability also suggests a possible bridge between the evolutionary paradox of diverging mammalian and avian species from reptiles. Comparative research with reptilian species not only exemplifies learning and sensory capacities in living species, but provides a basis of understanding the evolution and adaptive importance of these behaviors.

Species	Upper CFF Threshold (Hz)	Retinal Adaptation	Reference
Startling (Sturnus vulgaris)	>100	Cone	Maddocks et al., 2001
Rhesus Monkey (Macaca mulatta)	95	Cone	Shumake et al., 1968
Dog (Canis familiaris)	70-80	Cone	Colie et al., 1989
Pigeon (Columba livia)	77	Cone	Hendricks, 1966
Chicken (Gallus g. domesticus)	71.5	Cone	Jarvis et al., 2002
Human (Homo sapien)	50-60	Cone	Sekular & Blake, 2002
Tuatara (Sphenodon puncatus)	45.61-65.09	Rod (Walls, 1934)	This study
Cat (Felis domesticus)	40-55	Rod	Loop & Berkeley, 1975
Anolis spp.	26.1-42	Rod	Jenssen & Swenson, 1974
Japanese Rice Fish (Oryzias latipes)	37.2	Rod	Carvalho et al., 2002
Tiger Salamander (Ambystoma tigrinum)	30	Rod	Crevier & Meister, 1998
Sprague Dawley Rats (Rattus norvegicus)	20-30	Rod	Williams et al., 1985
Common Eel (Anguilla anguilla)	~14	Rod	Adrian & Matthews, 1928
Cane Toad (Bufo marinus)	6.7	Rod	Nowak & Green, 1983

Table 4. The upper CFF thresholds (Hz) for a list of various taxonomic species from highest to lowest rates in comparison to the tuatara.

3.5.3 Visual Perception and Behavioral Ecology

Juvenile tuatara need to locate a proximal food supply or item, be able to discriminate between food item and surrounding environment (i.e., substrate, vegetation, leaf litter), and quickly return to a burrow to avoid predation by adult tuatara (Walls, 1981), and other

species. Although tuatara are sit-and-wait predators (Walls, 1981), it is beneficial for individuals to discriminate between environmental differences (i.e., leaves) and prey items to minimize energetic expenditure or the risk of capture. Early frequent exposure to a stimulus may allow an individual to habituate to environmental cues during ontogeny (Cooper & Lemos-Espinal, 2001). However, a comparison between adult and juvenile performance should be made to establish whether juvenile learning and memory ability is a function of ontogeny, or related to age-based differences in behavioral ability.

Sensory discrimination in tuatara has implications for behavioral ecology, including such processes as optimal foraging strategies (Lima & Dill, 1990; Kaufman, Burghardt, & Phillips, 1996; Punzo & Madragon, 2002, Cooper, 2000; Day, Crews, & Wilczynski, 1999), predator-prey interactions (Cooper, 1998; Burghardt, 1964), kin/non-kin/individual recognition (Main & Bull, 1996; Aragón et al., 2000), mate selection (López & Martín, 2001; Watkins, 1997), environmental discrimination (Fleishman, 1992, 1988a, 1988b) and communication (Bradbury & Vehrencamp, 1998; Peters, Clifford, & Evans, 2002; Sanderson, Szymura, & Barton, 1992; Marcellini, 1977; Martins, 1993). Learning and discrimination ability in tuatara bridges a relationship between ecological processes and the evolution of behavior that has promoted the survival of the species.

3.5.4 A Novel Method

The methodology should be applicable to additional learning, memory, and visual perception studies in tuatara. Tuatara responsive behavior to visual stimuli suggests that this method may also be used to examine other sensory mechanisms such as audition, chemoreception, and tactility. However, since this study employed a novel methodology, testing transpired on a trial and error basis until a reliable procedure was identified. Little was known about tuatara performance in operant tasks. A more intense examination of visual perception would be to force a response to the S^+ where the two opposing stimuli are known

within the CFF range (i.e., 2.65 Hz versus 14.08 Hz). In this case, two-choice reaction time (CRT) tasks may induce learning effects (Parkin, Kerr, & Hindmarsh, 1997). Practice may influence CRT in response to CFF thresholds; therefore, it is difficult to determine how many trials are required to delineate a learning effect.

Further studies and improvements would include examining variations of the S⁺ and training regimes. Variations in visual stimulus such as luminescence, color, black and white, and infrared discrimination may further provide insight into the visual ability of tuatara. Additionally, the pineal eye plays an intricate role in behavioral adaptation, but its function and sensitivity to visual stimuli in relation to tuatara is not understood. Testing learning theory such as place versus cue discrimination, reversal learning, and navigational strategies will add further to what is known about cognition in tuatara. Previously, little was known about tuatara performance in operant tasks and perceptual ability. Comparable to other species, tuatara can develop a memory strategy by using local visual cues, and possibly adapt to behavioral response strategies when stimuli or reinforcers change.

3.5.5 Summary

Tuatara have demonstrated learning and memory abilities, while also demonstrating visual discrimination at various CFF rates. Tuatara motion detection is comparable to an array of diverse species, suggesting a significant evolutionary adaptation of importance and behavior. An established procedure allows for comprehensive examinations of other visual processes, audition, chemoreception, and tactual perception not only in tuatara, but in other reptiles. Implications of this study provide a better understanding of tuatara natural history.

3.6 References

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Chapter IV

Transfer of visual discriminative stimulus to a Y-maze in tuatara (*Sphenodon spp.*).

4.1 Abstract

Maze studies can be used to examine discrimination, learning, and sensory perception that reflect ecologically important characteristics of an organism. Identifying the mechanisms underlying visual discrimination facilitates an understanding of both the sensory capacity and cognitive processes of a species. Seventeen juvenile tuatara (Sphenodon punctatus) were trained using a simple operant conditioning technique to respond to various visual discriminative stimuli (S^+) in an open field. The stimuli were flickering lights with rates between 2.65-65.09 Hz. A distinguishable S^+ (25.06 Hz) was then transferred to a basic Ymaze where a subset of six subjects were required to correctly choose the arm signaled by the S^+ from an arm signaled with a non-flickering light (S⁻). Open field results showed that subjects were able to discriminate between a constant light and flicker rates between 2.65-45.61 Hz, but not at 65.09 Hz indicating that the upper threshold is between 45.61-65.09 Hz. Y-maze trials showed a successful transfer of the S^+ at 25.06 Hz, and not at 65.09 Hz. Tuatara have demonstrated learning a simple operant task, discrimination of flicker rates, and a transfer of a learned visual stimulus in an open field arena to a Y-maze apparatus. The study elucidates aspects of visual stimuli and cognitive strategies that are important to the behavioral ecology of tuatara, while producing a novel procedure in which to test sensory discrimination with the potential for improving animal welfare practices. This is the first psychophysical methodology to examine learning and visual discrimination in tuatara.

Keywords: tuatara (*Sphenodon punctatus*), critical flicker-fusion (CFF), operant conditioning, Y-maze, visual discrimination, reptile learning
4.2 Introduction

Maze studies have provided a basic understanding of learning, memory, and sensory perception processes in reptiles. Maze performance and navigation is based on the use of several cognitive strategies such as the use of self-motion cues, path integration, extramaze landmarks, environmental cues, and trial and error representations to guide behavior and maintain orientation (Dudchenko, 2001). Mazes are used to examine memory, spatial concepts for distance and direction, the development of cognitive maps, ecological relationships (Restle, 1957), and optimal foraging strategies by predators searching for prey items (Olton, 1979).

Maze performance studies using herpetological species have suggested that sensory discrimination is functionally important to the behavioral ecology of the species. For example, visual cues allow organisms to negotiate variations in their environment (e.g., crevice spiny lizards, Sceloporus poinsettia, Punzo, 2002), establish orientation (e.g., corn snakes, Elaphe guttata guttata, Holtzman, Harris, Aranguren, & Bostocks, 1999), use homing navigation (e.g., juvenile spotted pythons, Anteresia maculosus, Stone, Ford, & Holtzman, 2000), find home ranges (e.g., sleepy lizards, *Tiliqua rugosa*, Zuri & Bull, 2000), distinguish between food items (e.g., whiptail lizards, Cnemidophrous murinus, Schall, 2000), recognize individuals (e.g., Iberian rock-lizards, Lacerta monticola, Aragón, López, & Martín, 2000; López and Martín, 2002), adapt and learn spatial tasks (e.g., turtles, Pseudemys scripta, López, Gómez, Rodríguez, Broglio, Vargas, & Salas, 2001; López, Rodríguez, Gómez, Vargas, Broglio, & Salas, 2000), and develop optimal foraging strategies (Krebs, 1978). Turtle (*Chrysemys picta*) performance has shown that reptiles are capable of problem solving activities such as reversal shifts (RS) and concept formation (Graf & Tighe, 1971), reversal learning (Alkov & Crawford, 1966), detour learning (Spigel, 1964a), and escape learning (Spigel, 1964b). Additional maze studies with herpetological species have investigated path

selection and preference in water turtles (e.g., *C. picta*, Hart, Cogan, & Williamson, 1969), referential memory in Northern leopard frogs (e.g., *Rana pipiens*, Bilbo, Day, & Wilczynski, 2000), and temperature-dependent maze performance in heliothermic lizards (e.g., desert iguanas, *Dipsosaurus dorsalis*, Krekorian, Vance, & Richardson, 1968).

Learning paradigms (i.e., classical and operant conditioning) are used for two major types of study: to examine the process of conditioning or learning phenomena, and to use learning models as a tool for examining discriminatory and sensory mechanisms (Burghardt, 1977). This was the first novel study that used a psychophysical approach to examine basic visual discrimination and learning in tuatara, using the species as a model organism.

Stimulus control occurs when a neutral stimulus becomes the discriminative stimulus that governs the response of the organism. This occurs as a result of the stimulus being consistently present when a target behavior is reinforced and absent when that behavior is not reinforced. Various stimulus control methods in herpetological studies have used a negative reinforcer (shock) in turtles (Granda, Matsumiya, & Stirling, 1965), mouse derived cues (1month and 10-month) for red spitting cobras (e.g., Naja mossambica pallida, Stimac, Radcliffe, & Chiszar, 1982), color in Rana tadpoles (Muntz, 1962), and the male mounting behavior of Anolis carolinensis (Crews, 1978). The current study used a flickering light as the S^+ for stimulus control over a series of conditions and experiments, the flicker rate was varied. A rapidly flashing light evokes the sensation of a flicker; when the flash frequency increases, a pinnacle point occurs where the light perceivably ceases to flicker (Sekular & Blake, 2002). Critical flicker-fusion rate is the rate of flicker at which the individual or species ceases to see a flicker. A strategy taken here was to first establish whether flicker rate could be used successfully in an open field as a means to assess whether foraging behavior could come under stimulus control in this manner with tuatara. On the basis that stimulus control could be demonstrated in the open field, we proceeded to transfer these stimuli to a

Y-maze task in order to further demonstrate that tuataras had actually learned the relevant contextual relationship (Scharlock, 1955).

Tuatara are the only surviving members of the reptilian order Sphenodontia. Once widespread throughout the mainland of New Zealand, tuatara only survive on 32 offshore islands (Gaze, 2001). Tuatara have also been maintained in captivity for over 100 years (Cartland, Cree, Sutherland, Grimmond, & Skeaff, 1994). However, studies on behavior and sensory perception in tuatara have been limited, with many studies focused on physiology. Behavioral investigations have examined feeding ecology (Walls, 1981; Carmichael, Gillingham, & Keall, 1989; Ussher, 1999), social behavior (Gillingham, Carmichael, & Miller, 1995), predator avoidance (Nelson, 2001), visual predation (Meyer-Rochow & Teh, 1991; Meyer-Rochow, 1989), circadian rhythm (Goetz & Thomas, 1994), and T-maze performance (Northcutt & Heath, 1973).

The current study was the first to employ psychophysical procedure to examine tuatara discrimination learning, sensory perception, and maze performance. In this study, we addressed several questions: (1) could tuatara be trained using operant conditioning to learn to recognize visually discriminative stimuli, and what are the biological factors that influence performance (i.e., egg laying type, incubation method, temperature, or sex)?, (2) what is the flicker-fusion rate for tuatara?, (3) can stimulus control be transferred to a Y-maze from an open field arena?, (4) what were the implications for the behavioral ecology of tuatara?, and (5) can a reliable method for testing learning and sensory perception in tuatara and reptiles be generated? Procedures selected in initial experiment were designed to maximize the opportunity for tuatara to learn. However, multiple cues were available for tuatara to make a choice. First, subsequent conditions reduced the opportunity to use other cues. Secondly, the change in the stimulus guided the change in behavior of the tuatara (Experiment 1). After establishing stimulus control, confounds were reduced across conditions and experiments (Experiment 2). The final discrimination task (i.e., Y-maze) was to see if tuatara can learn a task in one context, and then be able to adapt the responsive behavior to another contextual experience (Experiment 3).

4.3 Method

4.3.1 Experiment 1 – Acquisition

4.3.1.1 Subjects

Subjects were maintained in captive facilities at the Victoria University of Wellington School of Biological Sciences. Seventeen (nine males and eight females) individually marked juvenile tuatara (*Sphenodon punctatus*; Cook Strait), approximately four years of age, were used in the study. Medical records were known for each individual. Subjects were known from birth and incubation regimes (e.g., head-started by Nelson, 2001): natural laying (LNAT) versus induced (IND) egg laying, and artificial (ART) versus natural (NAT) incubation method. Individuals were held in six identical enclosures, with one to four individuals per enclosure based on comparative weight, size, and sex. Enclosures measured 700 x 600 x 350 mm with 100-mm of top soil and large pieces of pine bark on the floor. Subjects were held under a 12L:12D light cycle (light between 06:00-18:00 hrs) using twofoot and four-foot TrueliteTM (F40T12/TL, USA) vitamin bulbs. Mass (using an AND HF-300G electronic scale) and morphometric measurements (e.g., tail and snout-vent length) were recorded weekly.

Subjects were acclimatized to the new facilities for one month before testing. The subjects were fed three times a week, and were given either 15-20 mm mealworm larvae (*Tenebrio molitor*) or small-medium (2-20 mm) locusts (*Locusta migratora*) from a commercial supplier (Biosuppliers – Auckland, New Zealand). Water was provided *ad libitum*. Prior to experimental testing, isopods were introduced to the compounds to

supplement feedings. Other invertebrates introduced as supplemental food included various orthopteran, coleopteran, amphipodan, and hemipteran species. Isopod populations were allowed to be decimated in each enclosure before experimental testing began.

4.3.1.2 Open Field Testing Arena

The open field testing arena was a glass aquarium (900 x 600 x 300 mm). The inside of the glass was covered with neutral brown paper to reduce any use of extramaze cues and reflection from the glass. The arena was divided into four separate quadrants and numerically labeled in conjunction with its respective corner: 1 (NW), 2 (NE), 3 (SW), and 4 (SE). Subjects were allowed to habituate to the testing environment.

4.3.1.3 Equipment

Subjects in the testing arena (Experiment 1 and 2) and Y-maze (Experiment 3) were monitored with an infrared camera powered by a 12V7Ah rechargeable battery (YP7-12 Super SONATM). The camera was placed approximately 1.5-m over the testing arena and images were projected onto a JVCTM Color Video Monitor TM-20PSN. Additional video was recorded with a NikonTM Coolpix 4500 and a USB LogitechTM Quickcam. All video monitoring was viewed out of sight of the proximal testing arena.

4.3.1.4 Feeding Stations

Three identically designed feeding stations projected 3.5-lum on a white LED bulb, and powered by a 3.6V LisunTM lithium battery (ER14505). Luminescence was constant. One feeding station was created as the positively reinforced S⁺ with oscillation capabilities of 2.68-27.78 Hz, and the second feeding station presented a non-reinforced constant light (S⁻). The oscillation speed was monitored and changed to a precise frequency using a Hewlett-PackardTM 54601A Oscilliscope. A second S⁺ feeding station was designed with oscillation capabilities of 17.00-185.02 Hz. The electronic components were encased in a plastic, neutral gray box (110 x 60 x 40 mm). The lights were positioned in the center of the box. A small wooden platform (120 x 80 x 20 mm) was attached to the bottom of the housing box. The housing box was detachable and transferable to the Y-maze. The wooden platform (\sim 214.18-mm) had a hollowed out area in which a feeding dish was placed.

The first set of feeding station dishes were two glass dishes: one with a reinforcer without a cover at the S⁺ feeding station, and one with a glass cover that prevented access to a reinforcer at the S⁻ feeding station. The second set of dishes were clear plastic with a reinforcer in the S⁺ only. The third set of dishes were two plastic painted (Mexican Red; Resene Paints Ltd., Lower Hutt, New Zealand) dishes with two covers. The covers were attached to thin, plastic-coated wire and looped through a hole in a piece of wood interlocked at 45° in each corner of the testing arena. The covers were manually lifted via friction-pulley in response to an approach by the subject. A reinforcer was only placed in the S⁺ feeding station. Subjects were trained to approach and eat from the feeding stations using a shaping procedure.

4.3.1.5 Procedure

Subjects were tested over blocks of trials with each block trial having a total of three individual trials. Table 1 shows the consecutive phase allocations for the number of trials within each oscillation speed, number of subjects, and dish type. One extinction trial was completed between Phases IV and V. The extinction trial consisted of turning both S^+ and $S^$ lights off. The extinction trial tested whether subjects discriminated the S^+ on the basis of a distinctively discriminative light or responding on the basis of randomized guessing (i.e., subjects would choose either the S^+ or S^- at an equal 50% chance ratio).

At the start of each trial, the subjects were placed in the center of the open field testing arena and oriented towards the feeding stations. The placement of the S^+ was pseudorandomized while the S^- was in either the left or right adjacent quadrant. Each feeding

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station was angled at approximately 45° towards the center of the testing arena. Each trial was completed by a final S⁺ response, irrespective of the number of S⁻ responses. Subjects were reinforced with either one mealworm or small-medium locust. The hind legs of the locust were excised to prevent them from escaping from the feeding dish. Subjects were allowed a two-minute intertrial interval (ITI) between each trial. After each trial, the testing arena and feeding stations were cleaned with 70% ethanol to neutralize chemical compounds. Subjects were placed in the center of the testing arena and allowed to approach either the S⁺ or S⁻. A 20-minute criterion was set to limit the length of each trial. If subjects did not complete the required task, the trial was terminated at 20 minutes. A response was considered to have occurred when a subject had approached within approximately five centimeters of a feeding station. Subjects were only reinforced for a correct response. For an incorrect response, the S⁻ dish was lifted to reveal no reinforcer, and the subject was returned to the center. See Figure 1 in Chapter 2 for a diagrammatic model. Temperature-dependent maze performance was examined over five different temperature ranges: (1) <15.9°C, (2) 16.0-17.9°C, (3) 18.0-19.9°C, (4) 20.0-21.9°C, and (5) >22.0°C.

Phase	Hz	Ss	# of Trials	Dish Type
Ι	2.65	17 (9_& 8_)	a. 3 b. 15	a. Glass b. Plastic
Π	14.08	17 (9_& 8_)	42	Friction-Pulley
III	25.06	13 (7_& 6_)	33	Friction-Pulley
IV	45.61	13 (7_& 6_)	21	Friction-Pulley
Extinction	$S^+ \& S^-$ lights switched off	6 (3_& 3_)	1	Friction-Pulley
V	65.09	13 (7_& 6_)	6	Friction-Pulley

Table 1. Consecutive phase allocations for experimental conditions which included the CFF oscillation speed, number of subjects (&), number of trials, and dish type.

4.3.2 Experiment 2 - 65.09 vs. 25.06 Hz

4.3.2.1 Subjects

Thirteen subjects were tested used in Experiment 2. Subjects were a subset of the original 17 individuals. Four subjects were excluded from the experiment for failure to complete the required task.

4.3.2.2 65.09 vs. 25.06 Hz

The same open field testing arena, equipment, and feeding stations were used in this experiment as in Experiment 1. However, Experiment 2 identified possible controlled possible confounds such as olfactory and auditory cues able to be acquired from the reinforcer. The new friction-pulley dishes had limited the ability for subjects to discriminate reinforcers on the basis of other discriminatory cues by removing the glass and plastic dishes. Subjects were able to discriminate between the constant light (S⁻) and the flickering light (S⁺) when the S⁺ was 25.06 Hz, but not at 65.09 Hz. The first trial for each subject was tested with the S⁺ at 65.09 Hz. The second and third trials were tested with the S⁺ at 25.06 Hz.

At the start of each trial, the subject was placed in the center of the testing arena and oriented towards the feeding stations. The placement of the S^+ was pseudorandomized while the S^- was in either the left or right adjacent quadrant. Each feeding station was angled at approximately 45° towards the center of the testing arena. Each trial was completed by a final S^+ response, irrespective of the number of S^- responses. Subjects were reinforced with either one mealworm or small-medium locust. The hind legs of the locust were excised to prevent them from escaping from the feeding dish. Subjects were allowed a two-minute ITI between each trial. After each trial, the testing arena and feeding stations were cleaned with 70% ethanol to neutralize chemical compounds. Subjects were placed in the center of the

testing arena and allowed to approach either the S⁺ or S⁻. A 20-minute criterion was set to limit the length of each trial. If a subject did not complete the required task, the trial was terminated at 20 minutes. Responses were categorized as correct or incorrect as soon as the subject traveled to five centimeters from a feeding station. Subjects were only reinforced for a correct response. For an incorrect response, the S⁻ dish was lifted to reveal no reinforcer, and the subject was returned to the center. Temperature-dependent maze performance was examined over five different temperature ranges: (1) <17.9°C, (2) 18.0-18.9°C, (3) 19.0-19.9°C, (4) 20.0-20.9°C, and (5) >21.0°C.

4.3.3 Experiment 3 – Y-maze

4.3.3.1 Subjects

Six subjects (three males and three females) were tested on the transfer procedure. Subjects were selected based on their proficiency to acquire the initial performance discrimination task. Subjects were part of the initial 17 groups of animals.

4.3.3.2 Y-maze

The Y-maze was of standard construction, with each of the three arms being 540 x 100 x 100 mm in size, and each arm set at exactly 120° apart from each other. The maze was constructed with a plywood base, and with aluminum walls encompassing the perimeter of the maze. Two Y-mazes were constructed to simultaneously run experimental trials. The Y-maze offers a simple two-choice selection exercise.

4.3.3.3 Transfer

Subjects were allowed to habituate to the Y-maze for approximately two hours a day, for five days. Both the S^+ (25.06 Hz) and S^- (65.09 Hz) boxes were placed at adjacent ends of arms in each Y-maze. Placement of the S^+ and S^- were randomized. For each trial,

subjects were randomly placed in one arm of the maze (start). Choice criterion was all four limbs past the center of the maze with the body directly aligned in one arm. When the subject chose the correct arm, a 10-15 mm mealworm larvae was dropped at the end of the arm. Subjects were only reinforced for a correct response. For an incorrect response, the subject was returned to the start arm. Subjects were tested for five trials per testing day. After each trial, the Y-maze was cleaned with 70% ethanol. A 20-minute criterion was set to limit the length of each trial. Tuatara were removed from the apparatus after each trial for an ITI of two minutes. A total of 40 trials (eight blocks of five trials) were completed. Temperature-dependent maze performance was examined over five different temperature ranges: (1) $<17.9^{\circ}$ C, (2) 18.0-18.9°C, (3) 19.0-19.9°C, (4) 20.0-20.9°C, and (5) $>21.0^{\circ}$ C.

4.3.3.4 Extinction Probe

The same six subjects in transfer tests were used. The Extinction trial tests whether subjects have discriminated the S^+ on the basis of a distinctively discriminative light or some unusual basis (e.g., if subjects continued to travel to the S^+ arm reliably when both S^+ and S^- were at 65.09 Hz, the it was not the flicker rate that subjects used to choose path selection). In these trials, the S^+ was increased to 65.09 Hz. Five Extinction probe trials (S^- constant at 65.09 Hz) were completed.

4.3.3 Statistical Analysis

Data were analyzed using SPSS for Windows (v11.5). Dependent measures included latency to choice, percent accuracy and log d (a point estimate of discriminability independent of bias; White & Alsop, 1993). Accuracy was defined as subjects correctly responded at 75% of the criterion acceptance over trials. Statistical analyses incorporated descriptive statistics (n \pm SE, %). ANOVAs were used to compare performance at each oscillation speed, time variances in completion of each trial, temperature-dependent performance (*F*), and when the S⁺ was at different locations. Mixed model ANOVAs was used to assess performance indicators (i.e., sex, temperature, egg laying type, and incubation method) with type III tests of fixed effects. Akaike's Information Criterion (AIC_C) was used to gauge the main effect of performance indicators (Burnham & Anderson, 1998). Kruskal-Wallis H chi-squared (2) test comparing accuracy of performance between trials, S⁺ location, sex, natural lay versus induced egg laying, and artificial versus natural incubation method. Analysis using two-tailed *t* tests also included differences between sexes, LNAT versus IND egg laying, and ART versus NAT incubation method. Mann-Whitney *U* nonparametric tests compared extinction trials to each oscillation speed. Post hoc multiple comparison tests were used for latency (Tukey HSD) and accuracy (Bonferroni) nonparametric adjustments. All values, _ = 0.05, are reported to the nearest significance threshold with a minimum value of 0.001. Only mean+1SE was used in graphs for legibility.

4.4 Results

4.4.1 Experiment 1 - Acquisition

A decrease in response latency and increase in accuracy in the open field testing arena shows that subjects were able to learn the acquisition task and discriminate the S⁺ at 2.65-45.61 Hz, but not at 65.09 Hz (Figure 1A and 1B). Subjects were similarly accurate from 2.65-45.61 Hz, but accuracy significantly decreased at 65.09 Hz (Kruskal-Wallis H, $_{-2}^{2}(4) =$ 63.40, *p*<0.001). Latencies were significantly different (*F*_{4,2100} = 88.66, *p*<0.001) with a mean (±SE) difference between 45.61 Hz and 65.09 Hz of 598.24±50.95 s.

Accuracy did not significantly change across successive trials within a session (Kruskal-Wallis H, $_{2}^{2}(3) = 1.28$, p=0.733) nor did latency significantly decrease over successive trials ($F_{2,2103} = 1.14$, p=0.320) for trial 1 (386.11±15.52 s), trial 2 (359.47±16.02 s), and trial 3 (392.80±17.04 s). Accuracy significantly increased across the successive 14

blocks (Kruskal-Wallis H, $_{2}^{2}(20) = 49.53$, p < 0.001). Latency significantly decreased from the first block trial to the last ($F_{20,2085} = 28.63$, p < 0.001). S⁺ location did not affect accuracy (Kruskal-Wallis H, $_{2}^{2}(3) = 1.91$, p < 0.001) or latency ($F_{3,2102} = 1.537$, p = 0.202).



Figure 1. Acquisition of discrimination task in testing arena: Decrease in latency and increase in accuracy over consecutive block trials for 2.65-45.61 Hz, but high latency and low accuracy for 65.09 Hz and Extinction trials; A) Latency over block of three trials for each oscillation speed and B) accuracy over block of three trials for each oscillation speed; N = number of trials.

4.4.1.1 Extinction

Subjects did not meet criterion for response latency and errors. With similar accuracy and latency responses between 65.09 Hz and the Extinction trials, this suggests that subjects are unable to discriminate between the S^+ and S^- at 65.09 Hz or Extinction, and the CFF

threshold is between 45.61-65.09 Hz (Table 2). Accuracy was overall significantly less
(Kruskal-Wallis H, $^{2}(5) = 87.41$, $p < 0.001$) when comparing the extinction phase to 2.65 Hz
(Mann-Whitney, $U = 1720.50$, $p < 0.001$), 14.08 Hz (Mann-Whitney, $U = 6538.50$, $p < 0.001$),
25.06 Hz (Mann-Whitney, $U = 2415.00$, $p < 0.001$), and 45.61 Hz (Mann-Whitney, $U =$
1591.50, $p < 0.001$). However, accuracy during the Extinction phase was not significantly
different than 65.09 Hz (Mann-Whitney, $U = 751.50$, $p=0.523$). Similarly, latency was
significantly different during the Extinction phase ($F_{5,2121} = 81.08, p < 0.001$). Latency during
Extinction trials was significantly higher when compared to 2.65 Hz ($t(274) = -18.55$,
p < 0.001), 14.08 Hz ($t(1090) = -4.87$, $p < 0.001$), 25.06 Hz ($t(448) = -16.69$, $p < 0.001$), and
45.61 Hz ($t(292) = -8.33$, $p < 0.001$). Latency of Extinction trials were not significantly
different from latency at 65.09 Hz ($t(97) = -0.70$, $p=0.757$). Therefore, subjects
demonstrated learning and discrimination of visual stimuli in an open field in Experiment 1.

Hz	Mean (<u>+</u> se) Latency (s)	Range (s)	% Accuracy	
2.65	199.18 <u>+</u> 10.45	10-1000 (N=255)	80.00 <u>+</u> 5.57%	
14.08	468.51 <u>+</u> 15.11	8-1200 (N=714)	85.99 <u>+</u> 5.25%	
25.06	194.43 <u>+</u> 9.90	8-1200 (N=429)	88.57 <u>+</u> 6.67%	
45.61	333.92 <u>+</u> 21.18	11-1200 (N=273)	85.71 <u>+</u> 7.93%	
65.09	932.17 <u>+</u> 42.59	59-1200 (N=78)	56.41 <u>+</u> 42.06%	
Extinction	996.90 <u>+</u> 81.02	233-1200 (N=21)	52.94 <u>+</u> 4.78%	

Table 2. Summary descriptive statistics for CFF phases showing high latency and low accuracy for 65.09 Hz and Extinction phases; N = the number of trials.

4.4.1.2 Feeding Station Trials

Latency and accuracy performance varied with type of dish. Fewer errors were made on the glass and plastic dishes, and significantly more were made during the friction-pulley trials (Kruskal-Wallis H, $_{2}^{2}(2) = 75.20$, p < 0.001). Subjects also performed significantly

quicker ($F_{2,2103} = 167.470$, p < 0.001) during glass dish trials (160.63 ± 20.25 s) and plastic dish (128.13 ± 5.77 s) than with the friction-pulley dish (481.39 ± 12.01 s).

There were no significant differences in accuracy between sexes (Kruskal-Wallis H, $_{-2}^{2}(1) = 0.004$, p=0.949), birth (Kruskal-Wallis H, $_{-2}^{2}(1) = 0.239$, p=0.625), or incubation type (Kruskal-Wallis H, $_{-2}^{2}(1) = 0.707$, p=0.400). There was also no significant differences in latency as a function of sex (t(2104) = 0.03, p=0.488). However, birth (t(2104) = 4.93, p<0.001) and incubation (t(2104) = -5.57, p<0.001) displayed significant effects. IND juveniles performed faster (261.84 ± 22.20 s) than LNAT juveniles (397.56 ± 10.15 s) as ART juveniles also performed faster (362.31 ± 9.65 s) than NAT juveniles (532.65 ± 32.29).

Accuracy was influenced by temperature with a decrease in errors with an increase in ambient temperature (Kruskal-Wallis H, $_{2}^{2}(4) = 18.25$, p < 0.001). Latency also significantly decreased ($F_{4,2100} = 9.04$, p < 0.001) when the ambient temperature warmed from $<15.9^{\circ}$ C to $>22.0^{\circ}$ C (551.97 ± 91.63 s versus 210.49 ± 15.32 s).

AIC_C was used to gauge the importance and usefulness of sex, egg laying, incubation, and temperature as performance indicators for latency with individual tuatara as the random effect. The AIC_C predicts that the most to least important indicator performance is test temperature, followed by incubation method, egg laying, and sex. The best model showed that all inclusive attributes were plausible performance indicators (Table 3).

Effect	ΔAIC_{C}	Source	df	F	<i>p</i> value
H _o	170.87	H _o	1,2105	1653.14	< 0.001
S	163.17	S	1,2104	0.01	= 0.974
Е	138.22	Е	1,2104	24.31	< 0.001
Ι	131.36	Ι	1,2104	31.05	< 0.001
Т	84.44	Т	4,2100	9.04	< 0.001
E + I	104.80	E I	1,2103 1,2103	18.15 24.87	< 0.001 < 0.001
E + T	49.82	Е	1,2099	26.32	< 0.001
		Т	4,2099	9.54	< 0.001
E + S	125.45	Е	1,2103	29.25	< 0.001
		S	1,2103	4.90	= 0.027
S + I	123.53	S	1,2103	0.14	= 0.713
		Ι	1,2103	31.70	< 0.001
S + T	76.75	S	1,2099	0.01	= 0.978
		Т	4,2099	9.04	< 0.001
I + T	42.28	Ι	1,2099	33.75	< 0.001
		Т	4,2099	9.75	< 0.001
E + I + S	91.55	E I S	1,2102 1,2102 1,2102	23.45 25.36 5.39	< 0.001 < 0.001 = 0.020
E + T + S	36.28	E T S	1,2098 4,2098 1,2098	32.06 9.75 5.68	< 0.001 < 0.001 = 0.017
E + I + T	14.12	E I T	1,2098 1,2098 4,2098	19.72 27.13 10.13	< 0.001 < 0.001 < 0.001
I + T + S	34.39	I T S	1,2098 4,2098 1,2098	33.95 9.76 0.21	< 0.001 < 0.001 = 0.647
E + I + T + S	0	E I T S	1,2097 1,2097 4,2097 1,2097	25.88 27.76 10.38 6.32	< 0.001 < 0.001 < 0.001 = 0.012

Table 3. Type III tests of fixed effects for examining the main effects of performance indicators during feeding station trials showing the best model using all performance indicators; E = egg laying, S = sex, I = incubation method, T = temperature.

4.4.2 Experiment 2 - 65.09 vs. 25.06 Hz

Preliminary data shows that tuatara can discriminate on the basis of CFF rates. There was a distinct discrimination between 25.06 Hz and 65.09 Hz (Figure 2A and 2B). Subjects were more accurate at 25.06 Hz than 65.09 Hz (Kruskal-Wallis H, $_2^2(1) = 34.41$, p < 0.001). Individuals correctly identified the S⁺ averaging 90.38% at 25.06 Hz. However, subjects correctly responded to the S⁺ averaging 48.07% at 65.09 Hz. Accuracy was less in Trial 1 (65.09Hz) than in trials 2 and 3 (25.06 Hz). Accuracy between trials 1 and 2 was significantly different (Kruskal-Wallis H, $_2^2(1) = 18.66$, p < 0.001), but accuracy between trials 2 and 3 was not significantly different (Kruskal-Wallis H, $_2^2(1) = 18.66$, p < 0.001), but accuracy between trials 2 and 3 was not significantly different (Kruskal-Wallis H, $_2^2(1) = 0.51$, p = 0.475). Latency was significantly less (t(154) = -18.44, p < 0.001) at 25.06 Hz (243.88±24.23 s) than 65.09 Hz (1071.88±41.06 s). There was an overall significant difference in latency between the trials ($F_{2,153} = 169.33$, p < 0.001) with a mean (±SE) difference between trials 1 and 2 of 840.79±51.97 s and between trials 2 and 3 of 25.58±51.97 s. Log d (5.819) and log bias (0.034) were calculated for 25.06 Hz, showing discriminatory stimulus control and no bias in discrimination. The log d (-0.133) and log bias (-0.067) values calculated for 65.09 Hz revealed a lack of discriminability between the S⁺ and S⁻ with no bias.

Subjects showed no significant difference in accuracy between sexes (Kruskal-Wallis H, $_{-}^{2}(1) = 0.61$, p=0.435) and incubation type (Kruskal-Wallis H, $_{-}^{2}(1) = 1.78$, p=0.182). There was also no significant difference in latency for sex (t(154) = 1.33, p=0.093) or incubation type (t(154) = 1.58, p=0.058). There was no temperature-dependent significance in accuracy (Kruskal-Wallis H, $_{-}^{2}(4) = 4.62$, p=0.329) or latency ($F_{4,151} = 0.43$, p=0.787).



Figure 2. Low latency and high accuracy response in 25.06 Hz trials shows distinct S^+ discrimination as opposed to high latency and low accuracy of 65.09 Hz trials; A) Comparison of latency (mean+SE) for both 65.09 Hz and 25.06 Hz and B) comparison of accuracy (mean+SE) for both 65.09 Hz and 25.06 Hz.

4.4.3 Experiment 3 - Transfer

Subjects successfully transferred the learned visual discrimination task from an opem field arena to a Y-maze (Figures 3A and 3B) by discriminating the S⁺ at 25.06 Hz, but not 65.09 Hz. Accuracy was significantly higher during the 25.06 Hz learned trials than the 65.09 Hz Extinction trials (Kruskal-Wallis H, $_{2}^{2}(1) = 22.90$, *p*<0.001). Latency was significantly lower (*t*(268) = -8.86, *p*<0.001) for the 25.06 Hz trials (181.85±13.98 s) than the

65.09 Hz trials (580.90 ± 61.47 s). A log d analysis for the Y-maze trials revealed a similar trend in discriminability across blocks as observed for percent correct with (Figure 3B). Log bias indicated little response bias, with an overall slight preference for the left arm during 25.06 Hz trials and the right arm during extinction (Figure 3C).



Figure 3. Y-maze transfer performance shows a distinct discrimination of the S⁺ at 25.06 Hz from the S⁻ at 65.09 Hz; A) as a function of latency (mean+SE), B) as a function of accuracy (mean+SE)supported by comparable log d values, and C) with log bias values showing little discriminatory bias; N = number of trials.

Accuracy was not significantly different between sexes (Kruskal-Wallis H, $_{-}^{2}(1) = 1.93$, p=0.165) or birth (Kruskal-Wallis H, $_{-}^{2}(1) = 2.69$, p=0.101). The S⁺ location did not show different response patterns for accuracy (Kruskal-Wallis H, $_{-}^{2}(3) = 0.49$, p=0.921) and latency (t(268) = 0.04, p=0.484). Sex (t(268) = -1.72, p=0.957) and birth (t(268) = -0.29, p=0.614) did not affect the performance in the Y-maze when compared to latency. Maze performance was not temperature-dependent. There was no significance in accuracy (Kruskal-Wallis H, $_{-}^{2}(4) = 8.69$, p=0.069) or latency ($F_{8,261} = 1.79$, p<0.001) performance in different ambient temperature regimes.

There was no significance in accuracy over the five successive trials (Kruskal-Wallis H, $_{-2}^{2}(4) = 1.90$, p=0.754). Accuracy increased over successive block trials (Kruskal-Wallis H, $_{-2}^{2}(7) = 14.13$, p<0.05) and latency also significantly decreased ($F_{8,261} = 31.67$, p<0.001) over successive block trials from block trial 1 to block trial 8 (534.90 ± 63.55 s versus 61.03 ± 11.78 s). However, latency significantly decreased ($F_{4,265} = 5.38$, p<0.001) from trial 1 to trial five (368.02 ± 48.83 s versus 167.54 ± 16.07 s).

AIC_C was also used to gauge the importance and usefulness of sex, egg laying, and temperature as performance indicators for latency with individual tuatara as the random effect during Y-maze trials. All subjects used in Y-maze trials were artificially incubated. The AIC_C predicts that the most important to least important indicator of performance is temperature, followed by egg laying type, then sex (Table 4). Performance indicators for Y-maze trials are comparable to open field trials suggesting that temperature has the biggest influence and sex is not a determinant biological factor in task performance.

Effect	ΔAIC _C	Source	df	F	<i>p</i> value
H _o	72.10	H _o	1,269	198.22	< 0.001
S	60.29	S	1,268	2.97	= 0.086
Е	63.11	Е	1,268	0.09	= 0.769
Т	24.21	Т	4,265	1.88	= 0.115
S + T	11.45	S T	1,264 4,264	3.96 2.13	= 0.048 = 0.077
S + E	49.08	S E	1,267 1,267	4.60 1.71	= 0.033 = 0.192
E + T	15.13	E T	1,264 4,264	0.17 1.89	= 0.680 = 0.112
E + T + S	0	E T S	1,263 4,263 1,263	1.88 2.18 5.67	= 0.171 = 0.072 = 0.018

Table 4. Type III tests of fixed effects for examining the main effects of performance indicators during Y-maze trials showing the best model using all performance indicators; E = egg laying, S = sex, T = temperature.

4.5 Discussion

Tuatara are capable of learning a simple operant task, discriminating flicker rates between 2.65-45.61 Hz, and recognizing a successful transfer of learned visual discrimination in an open field to a Y-maze apparatus. Clearly, tuatara are capable of learning and remembering. This study has contributed to our greater understanding of tuatara behavior by demonstrating a method to explore such abilities and by demonstrating that tuatara can learn and remember visual stimuli in the form of CFF rates.

4.5.1 Tuatara Learning Capacity in an Open Field Arena and Y-maze

Acquisition trials from 2.65-45.61 Hz demonstrated that tuatara were able to learn the task by producing low latencies and reduced errors over consecutive trial periods. Tuatara were also able to successfully recognize the transfer of the S^+ to the Y-maze by exhibiting a decrease in latency and errors over consecutive trials. Tuatara performance demonstrated

learning and memory abilities with implications for a higher order cognitive function (Shettleworth, 1998) which supports the hypothesis that animals are capable of developing a "memory strategy" (Forestell & Herman, 1988) for engaging in decision making and problem solving activities. The ability for tuatara to perceive a recognizable stimulus, and respond appropriately may be a function of cognitive strategies used for survivorship.

Task performance in this study was not temperature-dependent. Ambient temperatures ranged from 15.1-23.3°C during acquisition trials and 17.9-21.7°C during Ymaze trials. In New Zealand, the natural climatic range for tuatara has a higher and lower range (Cartland & Grimmond, 1994). Extreme ambient temperatures outside experimental conditions were not tested and a discrepancy in ectotherm performance may occur during trials of extreme temperature differences (Northcutt & Heath, 1973).

There was no difference in performance between sexes. However, Stamps (1977) suggested that reptiles do display dominance hierarchies in social groupings. Dominance hierarchies are mirrored by captive environments where an increase in population density alters the response to ecological factors such as food supply, territory, and niche habitats. Captivity is indicative of island population densities such as Stephens Island where a large tuatara population inhabit a relatively small area (Goetz & Thomas, 1994). However, males and females were not separately tested, and access to food was not a reflection of availability in their natural environment. Sized-based discrepancy may exhibit differences among social groups and level of task performance implicating dominance hierarchies. However, size-based discrepancy performance was not reflected in this study.

IND egg laying and ART incubation individuals performed faster than ones LNAT or NAT individuals during acquisition phases. For tuatara held in captivity, the survival rate is substantially higher than tuatara in the wild. Tuatara in the wild are susceptible to environmental variability. Subjects were ritualistically monitored in captive semi-natural areas. IND and ART individuals may respond and adapt to the task faster than LNAT and NAT individuals because the level of fitness is higher for the former group than the latter. Unfortunately, the six subjects in the Y-maze experiment were all ART individuals and no comparison on Y-maze performance as a function of incubation method could be assessed.

4.5.2 Visual Perception and Critical Flicker-Fusion Rate

Tuatara were able to demonstrate a CFF rate between 2.65-45.61 Hz, but not at 65.09 Hz suggesting that the upper threshold for CFF is between 45.61-65.09 Hz. Low accuracy in the extinction probe and 65.09 Hz trials suggest that tuatara can discriminate on the basis of differences in visual stimuli, vision is important in sensory discrimination, and that the S⁺ provided an aspect of stimulus control. Subjects did approach the S⁻ for incorrect responses. However, if subjects were equally using audition and olfaction as discriminative tools during trials, subjects should have been able to identify food under the pulley-dish. Tuatara may use an integration of all sensory mechanisms in learning tasks, but vision is the dominant sensory mechanism in a behavior such as prey discrimination and results confirm previous observations (Walls, 1981; Meyer-Rochow, 1989; Meyer-Rochow & Teh, 1991). Initially, tuatara had the opportunity to integrate additional sensory mechanisms for discrimination, but CFF discrimination in Y-maze trials have shown that tuatara are able to learn to discriminate a visual stimulus in one context and relatable transfer the learned behavior to another context.

Tuatara CFF rates are comparable to *Anolis spp*. (Jenssen & Swenson, 1974), chickens (Jarvis, Taylor, Prescott, Meeks, & Wathes, 2002), and humans (Sekular & Blake, 2002). Motion detection abilities parallel avian, mammal, and herpetological species that have both rod and cone retinal adaptation (see Table 4 in Chapter 3). Understanding motion detection and discrimination properties in reptiles is not only representative of living species, but elucidates the evolutionary and adaptive importance to cognition and behavioral ecology.

4.5.3 Implications for Behavioral Ecology

Spatial learning and visual discrimination reflects important ecological and behavioral processes such as optimal foraging strategies (Punzo & Madragon, 2002; Lima & Dill, 1990; Kaufman, Burghardt, & Phillips, 1996; Goldberg, Hart, & Wilson, 1999; Day, Crews, & Wilczynski, 1999; Cooper, 2000), predator-prey interactions (Cooper, 1998; Burghardt, 1964), kinship/individual recognition (Main & Bull, 1996; Aragón et al., 2000), mate selection (López & Martín, 2001), and communication (Bradbury & Vehrencamp, 1998). For example, male tuatara display a head-bobbing movement may initiate a courtship response for mate selection, and serve as an important component for conspecific communication (Gillingham et al., 1995). Dynamic visual modality communication transmits ubiquitous information regarding sexual status (Sanderson, Szymura, & Barton, 1992; Watkins, 1997), competitive status (i.e., "dear enemy" recognition, López, & Martín, 2002), territoriality (Marcellini, 1977), and warning (Martins, 1993).

The receiving organism should recognize and discriminate stimulus patterns from irrelevant images (Peters, Clifford, & Evans, 2002; Fleishman, 1988a; Fleishman, 1988b). However, the properties of environmental "noise" such as windblown vegetation influence the signal structure (Fleishman, 1992). Signals of various sensory modalities evolve with the nature of the environment in which the signal must pass through (Fleishman, 1988a; 1988b). The tuatara's capacity for visual discrimination mechanisms has the potential for individual recognition, reference memory abilities, and implications for social organization.

Juvenile tuatara are susceptible to predation by cannibalistic adults (Walls, 1981). Although tuatara are sit-and-wait predators, juveniles still need to locate a proximal food supply, remember the location, be able to distinctly discriminate between prey item and environment (i.e., various substrates, vegetation, leaf litter), and return to their burrow as quickly as possible to avoid predation by adult tuatara, and other species. However, a comparison between adult and juvenile performance should be tested to identify differences regarding age-based performance and ontogeny (Cooper & Lemos-Espinal, 2001).

4.5.4 Development of a New Operant Procedure

The study generated a reliable method in which to test learning and flicker rate discrimination in tuatara, but the methodology may also be used to comprehensively examine other aspects of visual perception in addition to testing other sensory mechanisms such as audition, chemoreception, and tactility. Tuatara performance is not only representative of the species, but can serve as a model for living amniotes.

Since little was known about tuatara performance in operant tasks and testing involved a new methodology, the protocol was implemented on a trial and error basis until a reliable procedure was established. A more intense examination of sensory perception would be forcing a response to the S^+ where the two opposing stimuli are suspected within the CFF range (i.e., 2.65 Hz versus 14.08 Hz).

The study has helped better understand how tuatara use their environment and what features of their natural environment are important. By using this methodology to answer basic questions regarding visual perception, we have the potential for improving conservation management, animal husbandry, and welfare practices. For example, initial stress in captivity may have promoted poor breeding success (Cree & Daugherty, 1990; Tyrrell & Cree, 1994). Stress-induced effects may also be correlated with high adult mortality, lack of social interaction, improper nutrition, small enclosures/high densities, inappropriate conditions for egg incubation, and juvenile mortality (Goetz & Thomas, 1994; Cree & Daugherty, 1990). Additional stress-related effects may be a function of enclosure size and layout, environmental conditions, physical necessities (i.e., cover, food, and water), pathological conditions, osteological problems, and deformities (Goetz & Thomas, 1994). Tuatara demonstrated acquisition of stimulus control by discriminating flickering lights in an open field testing arena. Tuatara use local visual cues, and adapt to behavioral response strategies when stimuli or reinforcers change. Tuatara were able to transfer that learned discrimination in the context of an open field arena to a Y-maze. By establishing a successful transfer, we have the potential to investigate environmental, light/dark, substrate, or temperature preferences in tuatara which was made possible with a Y-maze. The Y-maze allows the tuatara to select a path signaled by a recognizable discriminative stimulus; tuatara path selection indicates a preference for a variety of environments. We can improve animal welfare practices by selecting conditions representative of natural ecological variables to identify optimal niche habitat by contextual relation to performance in a Y-maze (Patterson-Kane, Harper, & Hunt, 2001).

The findings of this study identify neglected visual cues that may improve captive animal welfare. If captive tuatara can be trained to recognize a stimulus, their behavior can be governed by an element of stimulus control that improves husbandry practices. Sensory mechanisms are overall important, and this study helped to identify how it correlates to feeding behavior, prey selection mechanisms, and optimal habitat for restoration or future translocations. Understanding the tuatara's learning ability and sensory biology has the potential to improve animal welfare, zoo management, captive husbandry, translocation techniques, conservation management plans, and education programs and literature.

4.5.5 Future Directions

Further studies and improvements should examine variations of the S^+ and training regimes. Variations in visual stimulus such as luminescence, color, black and white, and infrared discrimination may further provide insight into the visual ability, acuity, and perception in tuatara. Juvenile tuatara were only used in this study and examining adult performance would provide a more quantitative comparative experiment with implications

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for age-based and ontogenetic differences. The pineal eye has been suspected to play an intricate role in behavioral adaptation, but its sensitivity to visual stimuli and influence on tuatara behavior have yet to be meticulously examined. This study has shown that reptiles can be used as models for studying behavioral theory and learning paradigms. Therefore, examining learning theory such as place versus cue discrimination, reversal learning, use of alternative discrimination tasks (i.e., radial arm maze, alley maze), equivalence classes, effectiveness of reward systems, food preference, and navigational strategies in reptilian models will add further to what is known about cognition in tuatara and other reptiles.

4.5.6 Summary

Tuatara have demonstrated learning and memory abilities, recognition of a successful transfer of the S^+ , and visual discrimination at various flicker rates. The methodology has demonstrated visual discrimination, but can be used to examine audition, chemoreception, and tactual responses in tuatara and other reptiles. The study has implications for species management and provides a better understanding of the tuatara natural sensory world.

4.6 References

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Chapter V

General Discussion

This study generated a reliable psychophysical method to test acquisition of stimulus control and visual sensory discrimination in tuatara. Tuatara have learning and memory capabilities with the ability to formulate higher order cognitive functions. The basis of the study was to help identify basic learning mechanisms and to provide insight into the visual sensory world of the tuatara; the study increases our understanding of tuatara natural history and also produces a baseline study of tuatara behavior. The study not only has a significant scientific contribution, but several applications for species conservation and welfare.

5.1 Summary

Clearly, tuatara are capable of learning and remembering. This study introduced a novel procedure in which to test acquisition of stimulus control and sensory perception in tuatara (*Sphenodon punctatus*). This novel procedure examined sensory perception in tuatara, but can also be used to study the sensory capacity of other reptiles. This method tested visual discrimination of flickering rate in tuatara, and in the process demonstrating that tuatara behavior can come under stimulus control. This study has contributed to our understanding of tuatara behavior by demonstrating a method to explore sensory abilities and by demonstrating that tuatara can learn and remember visual stimuli in the form of critical flicker-fusion (CFF) rates.

Tuatara were able to correctly discriminate visual stimuli were flickering with oscillation speeds between 2.65-45.61 Hz, but not at 65.09 Hz. This suggests that tuatara were capable of responding to a learned stimulus within a perceivable CFF range, and the upper threshold is between 45.61-65.09 Hz. CFF rates and motion detection ability in tuatara are comparable to lizards, avian, and mammalian species (see Table 4 in Chapter 3).

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Tuatara successfully recognized the transfer of the discriminative stimulus to a Ymaze. Maze studies reflect reptilian behavioral ecology, their ability to assess environmental conditions using various visual stimuli, and the evolutionary adaptive importance of learning processes to sensory discrimination. The ability to discriminate between visual stimuli functions as a mechanism for predator-prey interactions, kinship/individual recognition, mate selection, environmental discrimination, optimal foraging strategies, and communication. Discrimination is a function of interpreting multiple sensory cues. Tuatara may use an integration of all sensory mechanisms, but vision is the dominant sensory mechanism in behaviors such as in prey discrimination (Walls, 1981; Meyer-Rochow, 1989; Meyer-Rochow & Teh, 1991). The ability for tuatara to perceive a recognizable stimulus, and respond appropriately may be a function of cognitive strategies used for survivorship. Understanding learning and sensory capacities have several implications for conservation of the species and future investigations in tuatara behavior.

5.2 Conservation

The findings of this study may be used to support previous proposals for conservation management of the tuatara. Initially, stress in captivity may have promoted poor breeding success (Cree & Daugherty, 1990; Tyrrell & Cree, 1994). Stress-induced effects may also be correlated with high adult mortality, lack of social interaction, improper nutrition, small enclosures/high densities, inappropriate conditions for egg incubation, and juvenile mortality (Goetz & Thomas, 1994; Cree & Daugherty, 1990). Additional stress-related effects may be a function of enclosure size, enclosure layout, environmental conditions, physical necessities (i.e., cover, food, and water), pathological conditions, osteological problems, and deformities (Goetz & Thomas, 1994). Correlations between surface activity and growth can be used to gauge the effectiveness of captive management procedures (Goetz & Thomas, 1994). Cameras can be used as a conservation tool to monitor breeding success, revegetation in

natural habitats, and damages to the current ecosystem (Markwell, 1997). The findings of this study identify neglected visual cues that may improve captive animal welfare. Vision is a dynamic sensory cue that has implications for environmental discrimination. If captive tuatara can be trained to recognize a stimulus, their behavior can be governed by an element of stimulus control in improving husbandry practices.

The study is supportive of the current Department of Conservation (DoC) tuatara recovery plan (Gaze, 2001). Extended knowledge on the tuatara's learning capabilities and sensory biology has the potential to improve zoo management, captive husbandry protocols, translocation techniques, DoC management plans, outreach education programs, educational literature, and animal welfare.

Tuatara demonstrated acquisition of stimulus control by discriminating flickering lights in an open field testing arena. Tuatara were able to transfer that learned discrimination in the context of an open field arena to a Y-maze. By establishing a successful transfer, we have the potential to investigate environmental, light/dark, substrate, or temperature preferences in tuatara made possible with a Y-maze. The Y-maze allows the tuatara to select a path signaled by a recognizable discriminative stimulus. Tuatara path selection indicates a preference for a variety of environments. We can select conditions representative of natural ecological variables to identify optimal niche habitat by contextual relation to performance in a Y-maze, thus improving animal welfare practices (Patterson-Kane, Harper, & Hunt, 2001).

In addition, there are several general conservation benefits for tuatara. First, the study has helped better understand how tuatara use their environment and what features of their natural environment are important to them. This has the potential in identifying optimal habitat for restoration or future translocations. Secondly, there are implications for improved husbandry practices. For example, we can improve housing conditions based on the information acquired in this study. Sensory mechanisms are overall important, and the study helped to identify how it correlates to feeding behavior, and prey selection mechanisms. Finally, any knowledge gained will help improve the delivery of outreach education programs. Outreach programs often take tuatara into foreign surroundings. Information on perception allows educators to create environments that reduce stress to the animals while endorsing an ethical approach and conveying a positive conservation message to the public.

5.3 Future Directions

Psychophysical research on tactility (i.e., vibratory and seismic), chemoreception (i.e., olfaction and gustation), and audition have been underrepresented in herpetological studies. Although the current study provides a basic methodology for investigating visual perception, this procedure is applicable in examining the other sensory mechanisms. Vision has been identified as an important discriminatory component in tuatara ecology. However, studies have suggested that chemosensory perception is stronger than visual perception (Cooper, & Lemos-Espinal, 2001; Font & Desfilis, 2002). Both color and odor are important. Color allows for distal discrimination while odor allows for proximal discrimination (López & Martín, 2001). Integral use of sensory mechanisms is apparent for survival, but sensory dominance may be context-specific.

To date, little is know about the learning capacity and sensory world of the tuatara. A multidisciplinary combination of psychological, biological, and ethological theory provides insight into tuatara behavioral ability. Future directions in tuatara behavior may include investigating reversal learning, equivalence classes, escape/avoidance learning, and performance in other mazes (i.e., Morris water maze, alley maze, and radial arm maze) as a function of stimulus control. Performance over these tasks may provide further implications for conservation management (see section 5.2).

Animals navigate through their environment using various combinations of strategies: dead reckoning, beacons, landmarks, routes, environmental shape, sun compass, and cognitive maps (Shettleworth, 1998). For example, young loggerhead sea turtles (*Caretta*

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caretta) have a guidance system where regional magnetic fields function as navigational markers and elicit changes in swimming direction at geographic boundaries (Lohmann, Cain, Dodge, & Lohmann, 2001; or by olfactory-based orientation, Grassman, Owens, McVey, & Marquez, 1984). The cognitive functions in which tuatara can identify locations and navigate within an area is not well understood. Individuals may use environmental conditions, directional choice, and use of existing pathways to gauge orientation to goals because locomotion is energetically costly (Yeomans, 1995). Alternative mechanisms for acquiring information from the environment may include the use of proximal cues such as olfaction, acoustics, and moisture gradients. Animals may also use Type I (reception of cue emanating from goal location) or Type II (compass orientation) for homing and navigational purposes.

Additionally, the role of the pineal eye in reptiles and lizards has been poorly understood and has been speculated participate in an organism's behavior regarding homing ability and visual discrimination. The pineal eye has been suggested to function by acting as a sky-polarized compass sense for celestial cue (Freake, 2001). By covering the parietal eye, it may disrupt an organism's homing ability; the photoreceptors may be unable to receive and detect e-vector polarized light as a directional cue.

5.4 References

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