THE UNIVERSITY OF HULL

The Effects of Sexual Selection on Male Extended Phenotype Polymorphism and Potential Sleep Reductions of Lake Malawi Cichlid *Nyassachromis microcephalus*

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Abstract

Although cichlids are great models for understanding sexual selection their courtship behaviours have not been well documented in regards to any behavioural trade-offs nor has there been researched conducted on their behavioural plasticity in relation to changes in their extended phenotypes. Recent research by Lesku *et al.* (2012) found that Pectoral sandpipers (*Calidris melanotos*) reduce their rest periods in order to increase courtship behaviours during breeding season, prompting further enquiries into how widespread this behavioural trade-off is. Negative frequency dependent intrasexual selection could be facilitating the invasion and maintenance of novel bower shapes in cichlid populations, through reductions in conspecific aggression (Magalhaes *et al.* 2013). We predicted that males would increase nocturnal activity when in the presence of a female and that males would not change their behaviours when their bower sizes were altered, as we would expect behaviours to be related to bower size and shape if negative frequency dependant selection is apparent. We experimentally tested both of these hypotheses on a species of Lake Malawi cichlid (*Nyassachromis* cf. *microcephalus*). We separated four dominant males in adjacent tanks, yet still enabled females and subordinate males to freely move between tanks, this was permissible due to their significantly smaller size compared to dominant males. Dominant male bower sizes were changed midway of a two-week observation period and their behaviours towards conspecifics were observed. Cichlids were micro-tagging during our second study with their movements being recorded over a week. Two tanks with a population of only males, and a population of a two males accompanied with a female were used, thus permitting or restricting courtship behaviours. Behaviours of males were found to not significantly differ when given differing sized bowers, suggesting that behaviours are not plastic responses to bower size and negative frequency dependant selection could be acting upon cichlid bowers. No significant changes in nocturnal activity between

courting and non-courting males were found, suggesting there is no trade-off between rest and courtship behaviours. However sensor recordings were inaccurate and perhaps didn’t reflect true fish activity along with an uncertainty whether courting males truly displayed courtship behaviours. Overall we have found no evidence suggesting that males trade their rest periods for courtship behaviours. Male behaviours were not plastic responses to bower size and our findings along with Magalhaes *et al's.* (2013) work suggests that negative frequency dependant selection could be acting upon cichlids to facilitate the invasion of novel bower shapes.

Table of Contents

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Chapter 1: Introduction

1.1 Sexual selection

Darwin suggested that evolution functions via two processes of selection, natural and sexual selection. Sexual selection was first proposed by Darwin (1859) in order to justify the elaborate characteristics found in the males of many species. Sexual selection in short is defined as "competition of individuals with members of their own sex" which encompasses a number of properties such as mate choice, body size and weaponry (Darwin 1871), with Darwin defining sexual selection in order to explain the evolution of secondary sexual characteristics that differ between sexes. Sexual selection is a well researched area as it is unequivocally considered a major factor in powering the vast divergence and speciation of many polygamous species (Lande 1981; McKaye *et al*. 1990). Both natural and sexual selection result in genetically favoured genes being passed onto future generations in greater proportions than that assumed if genetic selection was random. Natural selection involves genetic variations enabling individuals to become more likely to survive due to an inherited trait, and thus pass on their advantageous traits to offspring. Over time the advantageous trait becomes more common within a population (Darwin 1859). Sexual selection however, arises from variance in mating success with individuals bearing favoured genes being selected for through reproductive efforts, and individuals with sub-optimal genes being less successful in reproducing. Sexual selection is therefore different from natural selection due to the involvement of more favourable genes producing more or better quality offspring, rather than genes being selected for due to increased survival chances from natural selection (Lande 1981). Selective pressures not only influence species at a genetic level through natural and sexual selection but pressures can influence one or more traits at the expense of others, known as a behavioural trade-off. Darwin first noted this in (1859) in *The Origin of Species* 'the whole organism is so tied together that when slight variations in one part occur, and are accumulated through natural selection, other parts become modified'. When selective pressures are strong enough natural and sexual selection can result in the development of new species, known as speciation, through genetic differentiation and mechanisms such as genetic drift.

1.2 Male-male competition

In order for sexual selection to power speciation two main factors have to be present in males and females. These factors take the form of direct competition such as male-male competition and sperm competition as well as mating preferences such as mate choice. These factors can force sexual selection to lead to population divergence when there is a parallel change within a population of both mate preferences and sexual traits on which mating preferences are based (Lande 1981; Panhuis *et al.* 2001).

As males throughout the animal kingdom compete for female access, four main techniques are used in order to gain mate access; firstly to destroy rivals, secondly to outcompete rivals through energetic sexual displays, thirdly to use extended phenotypes as a selective quality, and finally the use of indirect mate choice. The first technique is to outcompete rivals and eliminate them from competition by destroying them or through the war of attrition model, here males will perform elaborate or energetically costly displays for prolonged periods of time against another male (Bishop & Cannings 1978), both of these approaches can lead to the death of an individual. A few examples of such species with fatal male-male competition are; giraffes, *Giraffa camelopardalis* (Simmons and Scheepers 1996), male red deer (Clutton-Brock *et al.* 1982), bees of the genera *Lasioglossum* and *Perdita* (Short and Balaban 1994) as well as the genus *Aotus,* owl monkeys (Fernandez-Duque and Huck 2013) and naked mole rats, *Heterocephalus glaber* (Clutton-Brock *et al.* 2006; Sherman *et al.* 1991). Even if fatality doesn't arise male-male competition has been found to ultimately lead to reductions in survival rates either through direct competition or through the process of producing 'weaponry' or energetic displays used for competition (Darwin 1871; Sinervo 1997). It has been suggested that the production of such elaborate traits may come at a cost. Simmons and Emlen (2006) have shown in *Onthophagus*, a genus of the Horned Beetle that males were found to have enlarged testes when their horns were removed, suggesting that males invest in testes size when growth is not directed towards their horned weaponry, thus evidencing a trade-off between male-male competition weaponry (horn growth) and the production of sperm to outcompete other males sperm (testes size). Even though reductions in survival rates result from energy being directed towards male-male competition traits this competition still occurs widely and is an effective way of gaining a mate and being selected for by a female (Hunt *et al.* 2009). Due to the cost of producing elaborate traits some animals may 'cheat' in their signalling systems (Backwell *et al.*, 2000). An example is the fiddler crab (*Uca annulipes*). Males have enlarged claws that are sexually selected for by females as an honest indicator of male fighting ability. When a male loses their original claw a similar sized but far less robust claw is grown to imitate a good fighting claw. The new claw is a dishonest signal as it is far weaker than the original, less costly to produce and use during sexual displays, yet it is not discriminated against by females when choosing mates (Backwell *et al.*, 2000). The second technique used by males is that of a visual performance displayed and then assessed by females in which males with the most preferable display is chosen and selected for by the female, this can be through females selecting in favour of elaborate male traits such as peacock tail length (Petrie *et al.* 1991). Thirdly the selection of a mate can arise through the selection of a males' extended phenotype. This is part of the individuals phenotype that is extended beyond the body, including any manipulative action or construction behaviour (Schaedelin and Taborsky 2009). The final technique often used for gaining a mate is that of indirect mate choice. Indirect mate choice restricts an individual's set of potential mates often before any direct interactions have occurred, therefore the individual indirectly chooses a mating partner. An example of this is lek size in cichlid fish in which females actively choose males from larger leks to mate with (Young *et al.* 2009). There are many other ways individuals gain mate access and these four techniques are not mutually exclusive. It has been shown that bird plumage for example is not only used for female mate choice preferences but also reduces conspecific aggression from subordinate males, thus reducing the costs of male-male competition (Montgomerie 1986). Male cichlid fish such as *Nyassachromis* cf. *microcephalus* use a series of displays alongside elaborate extended phenotypes to gain access to female mates such as colour hue, swimming patterns, bower shape and size (Young *et al.* 2010).

1.3 Secondary selective traits, sensory bias theory and mate choice

Often males of species that have been thought to have diverged due to sexual selection can be found to provide no resources or parental care for their young (Dawkins 1982), they act purely as a genetic vehicle to pass genes to future generations. Females thus become the sexually selective sex and have a plethora of differing males to choose from, resulting in elaborate sexually selective traits displayed by males in order to compete with each other for access to females. When males do not provide parental care or resource benefits, sexual characteristics that are not directly part of the reproductive system of an animal, called secondary sexual characteristics/traits are often displayed to 'sway' the preferences of the females. In cases of elaborate secondary characteristics being displayed it is suggested that the sole purpose of these displays is to be used for mate choice by females and often females choose whom to mate with solely on the characteristics of these elaborate traits. Female mate choice preferences can potentially lead to rapid speciation and diversification (Darwin 1871; Fisher 1930; Lande 1981).

However male secondary sexual traits can simultaneously put males at higher risk of predation and parasitism therefore generating a trade-off between sexual signal elaboration and natural selection. This trade-off is known as the handicap principle which will be discussed further in this section along with the sexy-son hypothesis, sensory bias theory, honest indicators and fisher's runaway mechanism (Andersson 1994; Heinen-Kay *et al*. 2015; Rosenthal *et al.* 2001; Wagner 1996; Zuk *et al.* 1998).

It was initially unclear why very elaborate secondary characteristics were selectively chosen for by opposing sexes, such as non-edible food displays (Yosef & Pinshow 1989), cichlid bowers (McKaye *et al.* 1990) and overly exaggerated horns and tusks (Goodman 1871). None of these examples provide direct benefits to the female or direct benefits to the survival of young or parents. Although no direct benefits are apparent, the sexy son hypothesis indicates that females do benefit indirectly. This indirect benefit is due to females producing male offspring that inherit the 'sexy' elaborate trait of their fathers and are therefore more likely to attract females due to their elaborate trait and therefore sire more offspring (Gwinner and Schwabl 2005).

Another mechanism for the elaboration of secondary sexual characteristics is that of the sensory bias theory. Here secondary sexual traits are not involved with mating and courtship and that the secondary trait exploits a pre-existing preference by the choosy sex (Boughman 2002). One of the best examples is the guppy *Poecilia reticulata*. The species are attracted to the orange colour of their food. Males have evolved orange spots on their body as secondary sexual traits, which females are attracted due to the colour. It has been found that the brighter the orange spots on males the more attractive they are to females, thus natural selection is acting on females to be attracted to the colour orange in order to find food, and sexual selection is driving the orange colouration of males (Dugatkin 1996).

There are two further theories at to why secondary sexual traits have become so elaborate for female mate choice. Sexual traits can either evolve as an honest indicator of male breeding value for offspring quality, or evolve through Fisher's runaway mechanism (Fisher 1930). The Fisher's runaway mechanism (Fisher 1930) suggests that female mating preferences and secondary sexual characteristics of males co-evolve as more discriminating females mate with more elaborate displaying males. Fisher's runaway mechanism can be explained easily using a simple genetic approach with female preference and male traits being genetically based in a haploid organism.

Here we have a choosy and a non-choosy female and an elaborate and a normal male, with choosy females only mating with males with elaborate displays and non-choosy females choosing elaborate and normal males in a ratio of 50:50 as expected under random mating. After 1 generation of mating 66% of all offspring carrying the elaborate gene would also carry the choosy gene and only 33% of offspring carrying the elaborate gene would also carry the female non-choosy gene. All of the non-elaborate genes are carried with non-choosy genes. These genetic elaborate and choosy tendencies are then amplified as there is a relationship between female preferences and male traits (66% of elaborate genes associated with choosey females), so further generations will concentrate elaborate and choosey 'genes' (Sinervo 1997). This is a simplistic view but it displays how subsequently female mate choice and male traits can become linked and result in rapid runaway speciation when these preferences are strong enough (Dominey 1984).

Fisher also developed an alternative 'indicator model' which later extended to include the good genes model and the handicap principle. This model provides an explanation for the evolution of female choice in which the male trait is assumed to be a strong indicator for male genotype superiority (Andersson 1994). The good genes theory proposes that females choose mates based on variations in males indicator traits. These traits indicate males overall genetic quality, which they pass onto both their daughters and sons. Therefore although very similar to the sexy son hypothesis the good genes model assumes a benefit to both sons and daughters (Tomkins *et al.* 2004). For example Watson and Simmons (2010) found that female dung beetles, *Onthophagus taurus*, choose their mates based on the size of the male, with larger males indicating better genetic quality. Garcia-Gonzalez and Simmons (2011) then found that female dung beetles who mated with better quality males tended to produce offspring that survived until reproductive age, whereas females that mated with males of a poorer genetic quality produced offspring with a reduced survival rate.

As male traits become increasingly elaborate they can often become a hindrance to survival. Zahavi's (1975) handicap principle suggests that reliable signalling must have associated costs. Here males experience a trade-off between reproductive attractiveness to females and their ability to conduct behaviours that increase their survival rates. Costly 'handicap' traits act as indicators to females of the genetic quality of the male, as males of poorer quality would not be able to 'carry' such costs and still survive, whereas males of a better quality can carry traits with larger costs, indicating a better quality (Sinervo 1997; Zahavi 1977). A widely used example of the handicap principle in a species is that of male peacocks (*Pavo cristatus*) of which peacock tails are thought to be difficult to grow and maintain as well as being useless for everything apart from female mate choice (Luxen and Buunk, 2006; Petrie *et al.* 1991; Zahavi, 1975). In line with the handicap principle, peacock tail length has been shown to be a true indicator of male fitness. Petrie (1994) hypothesised that females that were randomly mated with males would produce better quality offspring when males with greater and more elaborate trains fathered them. This is due to these males being able to survive diverting energy and resources to growing and maintaining elaborate trains. This prediction was found to be true; offspring had increased growth and survival rates compared with offspring from fathers with less elaborate trains (Petrie 1994). Females were therefore found to use the elaboration of male trains as a true male quality indicator (Petrie 1994). Interestingly more recent studies have shown that train elaboration can be broken down into different components of elaboration which all indicate different male qualities such as immunocompetence (Loyau *et al.* 2005; Møller and Petrie 2002).

1.4 Extended phenotypes

Indicators for a superior genotype are not solely displayed through direct interactions between males through male-male competition or physical displays. Some species use external sites as sexually selected traits in which an individuals phenotype is expressed beyond the body and assessed by females (Schaedelin and Taborsky 2009). These traits are commonly known as extended phenotypes and are often secondary sexual characteristics used for mate choice. Unlike bodily ornaments, extended phenotypes should provide a more reliable signal to females of the males' quality as extended phenotypes of competing males are produced/constructed in the same environment. This isn't always true for bodily ornaments as males could have produced such ornaments due to excellent environmental conditions rather than superior male quality (Schaedelin and Taborsky 2009). However there are some examples of males producing unreliable signals from extended phenotypes such as the bower building cichlid fish of lake Malawi, who have been shown to use rocks as platforms to build bowers upon rather than sand (Martin 2010). Other examples of extended phenotypes are bird bowers (Uy & Borgia, 2000) as well as lizard caches in desert Shrikes (Yosef and Pinshow 1989) and territory size and quality in many species of bullfrog (Howard 1978). Extended phenotypes are often more attractive to females if they are more elaborate (Petrie *et al.* 1991) or in more competitive environments, such as bowers in larger and thus more competitive cichlid leks (Genner *et al.* 2008; Young *et al*. 2009). Such elaborate traits provide females with indicators of male quality, yet these traits are often traded-off against other beneficial reproductive or general survival behaviours. Bower building cichlids have reductions in foraging times and increases in competition for females by having bowers that are located in larger leks, which provide indirect mate choice benefits as males encounter more females in these larger leks (Young *et al*. 2009).

1.5 Sleep, breeding season and sexual selection

A relatively recent paper by Lesku *et al.* (2012) describes the trade-off between reproductive success and sleep durations in pectoral sandpipers (*Calidris melanotos*). To the best of my knowledge the study by Lesku *et al.* (2012) is the only study to research sleep during breeding season and the potential trade-offs courtship has upon the durations an individual is awake or asleep for. Lesku *et al.* (2012) suggested that male *C. melanotos* could have the adaptive ability to reduce the amount of time spent sleeping when social demands favoured wakefulness. Lesku *et al.* (2012) investigated whether male-male competition during breeding season constrained sleep times for male *C. melanotos*, resulting in a trade-off between sleep duration and reproductive success. The males who sire the most offspring will be those males who can court the most females, and are the most active, and by being the most active they are therefore likely to sleep the least. Lesku *et al.* (2012) found that the male *C. melanotos* that slept the least were indeed the most active, and that they sired the greatest number of young. Sexual selection is therefore selecting for males that are more active. However there is still variation in sleepy and active males, as sexual selection has not resulted in a loss of males with longer sleep durations. It is also unknown whether sleep duration is a heritable trait in *C. melanotos*. Siegel (2012) has suggested that perhaps the reason why sexual selection has not eliminated sleepier males is due to natural selection preventing the most active males from surviving to the following breeding season resulting in a fatal trade-off between reproductive success and the male survival. For males, breeding season is an energetically costly time and it is likely that active males deplete most of their energy reserves. Therefore variation in activity rates of males is still apparent due to natural selection favouring sleepier males and sexual selection favouring more active males. Potentially male *Calidris melanotos* are embarking on slightly different R and K selection strategies. With some males favouring to expel lots of energy to sire many offspring but in turn reduce their own survival rate. Others choose to expel little energy and produce fewer offspring but have a greater survival rate (Pianka 1970). However the heritability of sleep durations along with survival rates needs to be researched to determine if different strategies are being used. Are highly active males just as active year after year? If so is sexual selection strongly selecting for these active males? Further work also needs to confirm the survival rate of males who sire more young and are more active during breeding season.

One potential problem with the study by Lesku *et al*. (2012) is that the pectoral sandpiper is an artic seabird and during breeding season this species is exposed to constant daylight, thus courtship can occur 24 hours a day. This means that the results may be specific to species that are able to actively court all day due to constant daylight. When males are sleeping, other males can be actively courting females, whereas during light-dark cycles many species only court during the day or night, therefore all males will attempt to court females at the same time. In most species, light limits the amount of time males can court for, where as for *C. melanotos*, sleep is the factor limiting the amount of courtship males undertake.

A study by Steinmeyer *et al*. (2010) looked at the affect sex, age and the environment had upon the sleep behaviour of the blue tit, *Cyanistes caeruleus*. Here it was discovered that females slept for 15 minutes longer than males during the winter period and this gap in sleep duration increased during early spring leading up to breeding season. Steinmeyer *et al. (*2010) suggested that sexual selection was acting upon male sleep durations and that potentially, males who woke up earliest would sire the most young. This would agree with Lesku *et al.* (2012) findings in *C. melanotos* that the more active males, who slept the least, sired the most young. Poesel *et al*. (2006) and Kempenaers *et al*. (2010) found that males of the songbirds Chaffinch (*Fringilla coelebs*), Eurasian blue tit, (*Cyanistes caeruleus*), Great tit (*Parus major*), Common blackbird (*Turdus merula*) and European robin (*Erithacus rubecula*) who started singing earlier in the day during breeding season sired the most young, with artificial light causing songbirds to sing earlier in Kempenaers *et al*. (2010) research. Although sleep was not tested for by Poesel *et al*. (2006) and Kempenaers *et al*. (2010) it is possible that males who started singing earlier in the morning have reduced sleep durations compared to males who start singer later.

In a later study Steinmeyer *et al.* (2013) hypothesised that male blue tit*s* would reduce sleep durations in order to increase the amount of time available to court females. This would result in the males who slept the least siring the most young, as suggested in his previous paper (Steinmeyer *et al*. 2010) and as found in the artic seabird, *C. melanotos* (Lesku *et al*. 2012). However Steinmeyer *et al.* (2013) found a reduction in sleep duration was not associated with increases in the number of young sired. It was found that the link between sleep duration and reproductive success was actually who slept earlier from the day before and for longer were most likely to sire more offspring, in conflict with the findings in *C. melanotos* by Lesku *et al*. (2012). However Blue tit males observed by Steinmeyer *et al*. (2013) were observed outside the natural breeding season due to the inability to assess male sleep duration as males do not sleep in nest boxes in these periods. Thus the awakening times of females and males may be regulated differently in early spring when observations took place than during breeding season.

Pectoral sandpipers are known to have very active behaviours during breeding season in order to gain access to females, such as intense male-male competition as described by Lesku *et al*. (2012). These significantly higher activity levels during breeding compared to non-breeding season could be an indicator of potential sleep loss, as a significant increase in activity is likely to cause a decrease in the time available to rest/sleep. Rose (1981) discovered that *Sceloporus virgatus,* an iguanid lizard were active for 97% of the entire day (24hour cycle) during breeding season. During non-breeding season this percentage of time spent active was severely reduced, the amount of activity during non-breeding season being dependent on age with younger males maximum activity being 57% and older males only 29%. Asplund (1981) found variations in activity rates in northern Swedish mallards in which daily activity rates during breeding season can reach up to 90% of the time. The same can be said for observations by Dwyer (1975) on Breeding Gadwalls (*Anas strepera*) in which males' activity rates soared during breeding season. Although none of these studies researched sleep times directly it is possible that such vast increases in activity rates would result in decreases in rest periods and thus subsequently sleep times. Potentially, sexual selection could be selecting for individuals who are more active and thus potentially sleep less during breeding season as these individuals could be producing more offspring. More research is needed in order to better understand the ecological constraints on sleep and to assess the costs associated with sleep deprivation and suppression. We can then better understand the costs and benefits of sleep in different species potentially leading us to discovering more functions of sleep.

1.6 Sleep in fish

Fish are a group of animals that could potentially change their sleep and rest durations dramatically during the breeding season, because their behavioural activity patterns are very flexible (Ali 1992). Fish, especially freshwater fish, have been found to be capable of changing their circadian rhythms from diurnal to nocturnal in very short periods of time (Reebs 2002). The intrinsic system of the circadian clock regulates sleep within animals, unless a specific influence changes their sleeping pattern. Within marine and freshwater fish, such changes can arise from events such as migration, spawning or parental phases. In freshwater fish the circadian clock is also suggested to be used to anticipate daily events such as food availability, night and daytimes with foraging success and predation risk having large influences on an individuals activity pattern (Reebs 2002). This change in activity pattern in freshwater fish is observed in yellow perch (*Perca flavescens*), minnows, many centrarchids and bullheads during the spawning season when male-male competition is rife (Baade & Fredrich 1998; Baras 1995; Brown *et al.* 1970; Cooke & Bunt 1999; Harden Jones 1956; Helfman 1981, 1986; Nash 1982). There is little literature on sleep in fish and none of these examples of changes in activity patterns tested whether there was a trade-off between sleep duration and reproductive success, nor was it researched if sleep was directly affected by events such as migration, spawning or parental phase. However, vast changes in activity levels during such events do suggest there are potential constraints upon fish's sleep times during these events. However the large increases in activity during breeding season may not result in decreases in sleep times as individuals could have simply slept at differing times of the day around major spawning events.

Due to the small amount of research conducted on sleep in fish our knowledge about the functional benefits and ecological costs of sleep in fish are poorly understood. In mammals and birds EEG methods are used to identify and measure sleep times and patterns (REM or NREM). Fish lack the brain cerebral cortex from which brainwave patterns from an EEG result are detected (Siegel 2008) thus EEGs currently can not be used to define sleep in fish. However a few studies have used behavioural observations to confirm sleep in some species using the behavioural definition of sleep; a state of quiescence in specific posture, with increased threshold to external stimuli and rapid reversibility to a waking state (McNamara *et al.* 2009; Meisel *et al.* 2011; Reebs 2008). An example of sleep-like behaviours being displayed by a fish is that of the coral reef parrotfish (*Chlorurus sordidus*). This fish covers itself in a parasitic repellent mucous cocoon (Winn 1955; Grutter *et al.* 2010) before becoming inactive for large periods of time during the night, which is suggested to resemble a sleep-like behaviour (Dubin & Ruth, 1982). Even though few fish have been researched in order to determine if they sleep or not, many fish species studied so far not only display the behavioural characteristics needed to define sleep but often display physiological features of sleep such as reduced heart and irregular respiration rates (Zhdanova 2009).

Shapiro & Hepburn (1976) found that Mozambique tilapias (*Oreochromis mossambicus*) displayed sleep like behaviours in which individuals would rest at the bottom of their tank with low respiratory rates, they also displayed a significantly reduced response rate to stimuli compared to daytime activity. A study by Titkov (1976) found that brown bullheads, *Ameiurus nebulosus,* had a typical sleep posture combined with a decrease in cardiac and respiratory rhythms as well as reduced sensitivity to stimuli to conclude that brown bullheads do sleep. More recently Zhdanova (2011) unearthed sleep behaviour in zebrafish, *Danio rerio*. A six second period of inactivity was used as a threshold for differentiating between quiet wakefulness and sleep. Yokogawa *et al*. (2007) defined a 6 second interval of inactivity as part of the working definition for zebrafish sleep by pinpointing the overlap in time between decreasing sensitivity to stimuli when inactive and the decrease in percentage of individuals responding to stimuli when active. Zhdanova (2011) recorded periods of up to ten minutes of sleep-like states in which fish would undertake one of two postures, either horizontally near the bottom of the tank or a floating position with the head pointing downwards. This posture was observed together with increases in stimuli needed to move (awaken) an individual, reduced rates of respiration in adults and larval zebrafish whilst sleeping as well as this sleep-like period being conducted predominantly at night (Zhdanova 2009). Compensatory responses have also been found for sleep deprivation in zebrafish, in a sense a temporary trade-off between unknown important functions that require sleep and the need to stay awake and be active. Here individuals will increase their sleep bout durations after periods of sleep deprivation suggesting a homeostatic regulation of sleep in zebrafish (Zhdanova *et al.* 2001; Zhdanova and Reebs 2006).

The cognitive performance of zebrafish deteriorates when sleep deprived suggesting an importance of sleep in the functioning of cognitive performances, however it remains to be determined what the effects of sleep deprivation has on specific cognitive performances. Sleep deprivation may therefore affect multiple combinations of cognitive performances such as memory formation, recall, attention level or visual sensitivity (Zhdanova *et al*. 2008; Zhdanova 2009). However the homeostatic response zebrafish have when sleep deprived is disrupted under strong environmental factors such as constant bright lights; here fishes have been shown to lack any sleep like behaviours, prolonged inactivity periods or increased arousal thresholds, nor is there a compensatory response to sleep deprivation once light conditions have returned to normal (Yokogawa *et al*. 2007). It has been suggested that the lack in sleep behaviours in response to the strong environmental disturbance of constant bright lighting is due to zebrafish's diurnal adaptation to avoid predators in which fish will trade sleep durations in order to avoid predators as well as finding prey (Zhdanova 2009). Other diurnal fish have been shown to also suppress rest like behaviours due to lighting (Shapiro and Hepburn 1976; Tobler and Borbély 1985).

Although behavioural studies have shown sleep states in some fish species, hypotheses have been proposed in which fish do suppress sleep either entirely or almost so, due to the lack in need of the potential benefits associated with sleep. If sleep provides no benefits then there is no need to trade active beneficial behaviours for sleep (Reebs 2008). For example bluefish, *Pomatomus saltatrix,* and Atlantic mackerel, *Scomber scombrus,* do not stop swimming when observed in aquaria and remain responsive to disturbances throughout the night (Olla and Studholme 1972). Thus the behavioural observation needed for the classification of sleep in which the organism must 'be in a state of quiescence for a prolonged period of time with reduced responsiveness to stimuli' is not met. These fish have seemingly evolved the ability to suppress sleep, or more likely certain behavioural aspects associated with sleep. Many coral reef associated species display behavioural aspects that prevent sleep from being defined as they feed nocturnally and reside in schools of fish during the day that are very alert to predators (Reebs 2008). The nocturnal Californian horn shark, *Heterodontus francisci,* has also been found to be alert to disturbances during the day when supposedly inactive hiding in dark caves (Reebs 2008). Reebs (2008) suggested that animals may need to sleep in order to consolidate memories as it might not be possible for the brain to consolidate memories whilst awake and still 'taking in' new stimuli and information to process. This theory is supported by many studies suggesting that memory consolidation is an important function of sleep (Fishbein 1971; Fishbein and Gutwein 1977; Fishbein and Gutwein 1980; Greenberg *et al.* 1983; Hennevin, *et al.* 1995; Lesku *et al*. 2006; Peigneux *et al.* 2004; Plihal and Born 1997; Plihal and Born 1999; Siegel 2001; Smith 1995; Smith 1996). Hence the suggestion that the brain needs to reduce sensory inputs through sleep in order to consolidate memories. Kavanau (1998) and Reebs (2008) also postulated that many pelagic fish perhaps do not need to sleep in the traditional sense in order to consolidate memories due to having far fewer sensory inputs from the pelagic zone environment than most land living species. However this does not mean that pelagic fish do not need to sleep as there are many other functions of sleep and it is very unlikely that simply the reduction in the need for memory consolidation in certain fish species would result in a complete lack of sleep. Perhaps fish do not need to sleep as much or as intensely when compared to other animals, or perhaps sleep in fish is functionally different to other animals thus one of sleeps primary functions may not be that of memory consolidation. The function of sleep is still being debated and it is highly unlikely that memory consolidation is the only role of sleep in fish or any other animal. Multiple hypotheses have been proposed on the functions of sleep, few examples are that of memory consolidation (Kavanau 1996) and processing (Turner *et al*, 2007), development of the brain in juveniles (Van Someren *et al*. 2011), energy conservation (Zepelin and Rechtschaffen 1974), recalibration of thermoregulatory systems (Siegel 2005a), motor inhibition in infants (Roffwarg *et al.* 1966), neurotoxic waste removal of the brain (Xie *et al.* 2013), involvement in the immune system (Zager *et al.* 2007) and parasite resistance (Preston *et al*. 2009), and repairing of tissues (Gümüştekín *et al*. 2004). As animals and likely fish have multiple needs for sleep (Reebs 2008) then constraints of sleep duration or intensity in fish will likely result in different deleterious effects compared to other animals. These different deleterious effects could unveil the unknown functions sleep has within fish, or even unmask deprivation effects that also occur in mammals and birds but have not yet been found.

1.7 Sexual selection and its role in sympatric speciation

Speciation is thought to arise predominantly through large-scale geographic isolation where populations of species are separated from one another. This geographic isolation restricts gene flow between populations, isolating each population's gene pools, which over time will start to differ perhaps due to different selective pressures, genetic drift or differing genetic mutations in each population. These differences may eventually result in phenotypic or genetic divergence and ultimately resulting in speciation known as allopatric speciation. Allopatric speciation was first recognised as an important mechanism for speciation by Ernst Mayr (1942) when the 'modern evolutionary synthesis' was developed between 1936-1947. It was long thought that speciation could not occur if multiple species could interbreed as Mayr, (1947) argued that gene flow was an inevitable result of sympatry. However more recently evidence supporting sympatric speciation as a mechanism for the development of new species has been published (Schluter and Rambaut 1996; Taylor and McPhail 2000). This idea that a physical barrier must be present in order to reduce gene flow and therefore facilitate the conditions needed for speciation is now widely disputed with research supporting the idea that speciation without geographic isolation does occur and perhaps more frequently than first thought (Berlocher and Feder 2002; Bush 1994; Hadid *et al.* 2013, 2014; Jiggins 2006; Savolainen *et al*. 2006).

Smith, (1966) proposed the most persuasive hypothesis on how sympatric speciation may occur. He argued against Mayr's (1963) comprehensive summary of how sympatric speciation was not possible due to a lack of known mechanisms in line with current genetics that could support sympatric speciation. Smith, (1966) used research from Thoday and his colleagues (Millicent and Thoday 1961; Thoday and Boam 1959; Thoday and Gibson 1959) to suggest that disruptive selection, in which selection favours the extremes at the expense of average phenotypes, can produce a stable polymorphism in sympatric environments. Smith argued that a single species inhabiting two sub-environments could have different alleles favouring one of the two environmental niches, therefore stable polymorphisms could be possible. This diverging selection between two niches could then eventually cause reproductive isolation and furthermore speciation (Smith 1966). Mechanisms such as assortative mating and intraspecific competition have since been evidenced to be likely causes for restricting gene glow and driving speciation in species such as African cichlids (Seehausen & Schluter, 2004; Dijkstra *et al*., 2005).

If male morphs differ within a species and females have different mating preferences then sexual selection can occur from assortative mating. It was suggested by Lande (1981) that the divergence of female mate choice preferences for male traits might have caused strong premating isolation of specific male traits and subsequently result in the divergence of such males traits in order to ‘meet the demands’ of female preferences. It has also been noted that changes in male traits can occur with very little resultant genetic differences between males. This means that speciation by sexual selection can potentially occur with relatively little genetic change from the divergence of female preferences, these preferences can diverge in a relatively short period of time when compared to speciation from other pathways such as allopatric speciation (Uy & Borgia

2000). Uy & Borgia (2000) hypothesised that species that have recently diverged due to sexual selection have few genetic or morphological changes, yet the male traits and female preferences that have been developed are vastly different to sister species. Uy & Borgia (2000) tested for the genetic differences between the allopatric populations of the Vogelkop bowerbird, *Amblyornis inornatus*. The results confirmed that the displays of males and female mate choice preferences were vastly different between the two populations of Vogelkop bowerbird, but genetically the two populations were very similar. These results suggested that populations had likely diverged through sexual selection due to the small genetic differences between populations rather than large differences seen from speciation by natural selection. These results strongly suggest that the differences between populations have occurred after the divergence of female mate choice not before. This idea is applicable to sympatric speciation in which sexual selection through female mate choice and male traits could be the driving force for speciation in many species especially that of the polygamous cichlid species of African lakes (Salzburger *et al*. 2002; van Alphen & Seehausen 1998).

1.8 Haplochromine cichlids

A well-known example of rapid sympatric speciation is that of haplochromine cichlid species in the East African Great Lakes. In roughly the last 10 million years, over 2000 species of cichlids have diversified in east African lakes (Zachos and Habel 2011) in which thousands of species of cichlids have evolved in close proximity (Kocher 2004; Zachos and Habel 2011), and many have argued the importance of sexual and natural selection in the divergence of these cichlids inhabiting these great African lakes (Couldridge and Alexander 2002; Dominey 1984; Maan *et al*. 2004; Salzburger *et al.* 2002, 2006; Seehausen 2006; Seehausen *et al.* 2008; Stauffer *et al.* 1995; Stauffer *et al.* 2005; Wagner *et al.* 2012).

This diversification is centred on three of the largest east African great lakes; Lake Victoria, Lake Tanganyika and Lake Malawi or known locally as Lake Nyassa (as described by my Tanzanian uncle). It has been suggested that the rapid cichlid speciation has been conducted via multiple pathways (Kocher 2004) with ecological speciation driving initial divergences between species and forming the basis of reproductive isolation and preventing gene flow between populations. It should be noted that ecological speciation, that being speciation facilitated by reproductive barriers appearing between populations resulting in the use of different environments, should not be confused with adaptive speciation, in which new environmental niches are made available and species diversify accordingly (van Doorn *et al* 2004). During ecological speciation, divergences from disruptive selection can not result entirely from sexual selection and can also occur in allopatry (Rundle and Schluter 2004). Albertson *et al.* (1999) & Zachos & Habel (2011) have shown that the initial divergence of cichlids was likely facilitated by the availability of many ecological niches through adaptive radiation and ecological selection. Hence there is a vast range in diets of cichlids from algal scrapers to piscivores and paedophages with diversification of colouration, jaw and tooth morphologies. Kocher (2004) broke cichlid radiations into three historical stages The initial divergence of cichlids is likely to be through ecologically based divergent selection with reproductive isolation being created from populations inhabiting different environmental niches as gene flow between populations reduced due to the partial isolation of the original population (Kocher 2004; McKaye 1983; Salzburger *et al.* 2002; Schluter and Conte 2009; Seehausen 2006; Uy & Borgia 2000). After habitat divergence the morphology between genera diverged specifically in feeding apparatus through divergent natural selection between niches or environments (Kocher 2004). Thirdly cichlids diversified further due to differences in primary colour patterns as a result of sexual selection rather than ecological speciation (Deutsch 1997; Kocher 2004).

Species colouration has been central in explaining the reproductive success of cichlids living in sympatry and has been confirmed as one of the stages of visual assessment by females with male nuptial colouration being the focus of sexual selection in great African lake cichlids (Schliewen *et al*. 2001; Smith and van Staaden 2009; van Alphen and Seehausen 1998). Pre or post mating isolation mechanisms to prevent hybridisation must be present in order to prevent the dilution of gene complexes that have evolved due to different cichlids diverse ecological adaptations. Pre-mating isolation is likely to be the mechanism to prevent cross breeding with many species using body colouration for assortative mating (Maan *et al.* 2004). Maan *et al*. (2004) hypothesised that *Pundamilia nyererei*, a cichlid of lake Victoria, select for males depending on their colouration. It was found that females would choose males depending on the redness of males with males of a brighter red hue being selected for by females. A similar experiment has been conducted in a species of lake Malawi cichlids *Labeotropheus fuelleborni.* This experiment by Pauers *et al.* (2004) showed that male nuptial colouration is under directional sexual selection as females selected for males with greater colouration saturation as well as higher colour contrast on the flanks of males. Male colour is not the only pre-mating indicator for species recognition (Seehausen 1997; Seehausen *et al.,* 2008; van Alphen & Seehausen 1998) as multiple cues are likely necessary for female cichlid assessments (Fernald 1984; Magalhaes *et al.* 2013; Miranda *et al*. 2005; Young *et al.* 2010). The assessment of male colour by females helps prevent possible hybridisation of emerging species, known as reinforcement, and helps increase the reproductive success of females (Deutsch 1997), theoretically leading to reproductive isolation, and integral part of speciation (Kocher 2004). The extreme diversity in species colour as well as the pattern of colour diversity within cichlids is consistent with Fisher's runaway model in which male traits (colour) and female preferences are strongly linked and evolve together (Couldridge and Alexander 2002; Deutsch 1997). Along with modern divergences in colouration, cichlids have been found to build over ten different basic sand bower forms and some species use rock as a platform for bowers (McKaye *et al.* 1990). The variance in bower forms suggests that sexual selection has potentially played a part in the evolutionary origin of sympatric and parapatric populations and radiation of cichlids.

It should be noted that Adaptive radiation of cichlids has not purely occurred during the initial stages of cichlid diversification. Anthropogenic effects have also facilitated potential cichlid speciation through damaging effects upon cichlid lakes (Seehausen 1997). Population constraints and reductions in gene flow (as seen in lake Victoria by Seehausen 2006) along with the exposure of cichlids to non-native predators could have lead to even more diversification of cichlids (Seehausen 2006). Multiple cues are likely necessary for female cichlid assessments (Fernald 1984; Magalhaes *et al.* 2013; Miranda *et al*. 2005; Young *et al.* 2010), environmental disruptions to any of these different cues could result in biodiversity crashes in cichlid populations. An environmental disruption in the case of man made pollution and resultant eutrophication was confirmed by Seehausen *et al*. (2008) as the cause behind the collapse of cichlid diversity during the anthropogenic eutrophication of lake Victoria. Eutrophication was caused by man made pollution of lake Victoria in which algal blooms created steepening light gradients. These light gradients changed previously habitable areas of the lake, containing species of cichlids, into non-viable locations for reproduction. Here an increase in turbidity of the lake water from anthropogenic causes interfered with mate choice of cichlids due to a reduction in the efficiency of the recognition of species through visual assessment of colour. Due to the inability to recognise species by colour there were vast increases in hybridisation of cichlids in lake Victoria. Turbidity thus prevented the mechanisms used to sustain reproductive isolation and in turn turbidity destroyed the mechanisms of diversification which maintained cichlid diversity (Seehausen,1997; Seehausen *et al*. 2008).

1.9 Bower building cichlids

Extended phenotypes in cichlids are termed "bowers" which resemble sandcastles that vary in shape and size from species to species. Bowers not only vary in shape and size between genus but also at species, population and individual levels (Stauffer *et al.* 1995). For example the bowers of *Copadichromis* in lake Malawi have a volcano-like shape with a flat platform, *Tramitichromis* construct volcano bowers with uneven platforms and *Lethrinops lethrinus* create a large sand pile in a circle around a sunken crater (Kocher 2004). The selection of males along with extended phenotypes (bowers) has resulted in the mass speciation of cichlidae in the great African lakes through diversification through sexual selection resulting in multiple polymorphic bower shapes and sizes. Here we see hundreds of differing cichlid species with many living in sympatry. This rapid speciation has prompted research into sexual selection and the role of extended phenotypes in sexual selection (McKaye *et al.* 1990). The diversity of these cichlids' bowers reflects the diversity in social courtship signals displayed by cichlids and for this reason bower building cichlids are a good model for studying sexual selection in relation to bower shapes and sizes as well as the evolution of their courtship behaviours.

The shape and size of these cichlid bowers has been well researched and studies have confirmed that these extended phenotypes obey Fisher's honest indicator model as bower size is a 'good indicator' for male quality and higher quality males should be able to acquire, maintain and actively defend larger bowers that provide increased reproduction success (Martin and Genner 2009; Kidd *et al.* 2006; Martin 2010; McKaye *et al.* 1990; Young *et al*. 2010; Schaedelin and Taborsky 2009). Bower sites built by male cichlids provide no direct benefit to females so determining the categories a female assesses the site by is complex and often poorly understood. For example it was long thought that female cichlids preferred bowers based purely on the height of the bower (McKaye *et al.* 1990). However Schaedelin and Taborsky (2006) found that when bowers were destroyed males would build upon the diameter of the bower before adding height/depth to it, with males holding larger diameter bowers engaging more frequently in advanced stages of courtship (Young *et al*. 2010). This suggests that the diameter of a bower potentially has as much of, or even a greater preference from females rather than overall height of bower. However a paper by Young *et al.* (2010) suggests that both bower height and bower diameter are selected for by cichlid females with each dimension being selected for at different stages of female assessment. Increases in bower height resulted in more females following males to their bowers and increases in platform dimension resulted in females engaging in premating circling more so with males of larger platform diameters. Kellogg *et al.* (2000) also examined male bower characteristics in relation to the different stages of cichlid courtship and mating success. Males of the study species *Lethrinops parvidens* who had taller bowers were found to attract more females to their bower, in agreement with a study by McKaye *et al.* (1990) who also found bower height to be positively correlated with female visits. However neither of these studies found that bower height increased the mating success of males. In addition to female mate choice studies Lisy (2006) researched the heritability of bower building and hypothesised that Lake Malawi cichlid *Apetra cryptopharynx* (*Tramitichromis*) bower building behaviours were innate and not learned. Lisy (2006) separated fry from their mothers and found that 4th and 5th generation young that were raised in isolation from their parents still constructed bowers of the same ‘cone’ shape of *A. cryptopharynx* providing evidence that bower shape is a heritable trait.

Mate choice for bower building lake Malawi cichlids such as *N.* cf. *microcephalus* is apparent in two forms, direct and indirect mate choice (Wiley & Poston, 1996). Direct mate choice involves the direct assessment and discrimination among males based on their phenotypic traits. For example *N.* cf. *microcephalus* females have been found to use bower size (McKaye *et al.* 1990) and shape (Young *et al.* 2010) to discriminate between males as these dimensions are indicators for male quality. Indirect mate choice occurs when a female's choice is reduced without individual discrimination between males. For example, Young *et al.* (2009) found that females often visit males in larger leks, more so than males in a smaller lek. A male's ability to create and defend a bower on a larger lek in this instance acts as a form of indirect choice.

We know that bower size and shape is assessed by females (Magalhaes *et al.* 2013; Young *et al*. 2009) but bower size and shape does not directly influence courtship success, nor does lek size. Therefore we expect another male trait is likely the final determining factor in direct female mate choice. Males with novel bower shapes and reduced conspecific aggression might be able to build bowers more centrally within the lek as they are not attacked as frequently by conspecifics. This central location of bowers within a lek has been suggested to increase female visits through indirect mate choice (Genner *et al.* 2008; Kellogg *et al.* 2000). This central location gives protection against heterospecific aggression and thus reduces stressful aggressive interactions perhaps making the breeding season much less energetically costly to males (Young *et al*. 2009). Potentially, this central location within leks without conspecific aggression may increase survival rates of males after breeding season. However a review by Kotiaho & Puurtinen (2007) describes how often research does not quantify the benefits from indirect mate choice properly. We may not have seen an increase in number of mates, yet the speed at which a male finds a female mate in larger leks may be significantly quicker than in smaller leks, thus costly courtship displays need not be signalled as often. Therefore perhaps the position within a lek, especially larger leks, may not provide direct increases to reproductive success of males, but instead increase male survival rates perhaps through reductions in heterospecific aggressive interactions, therefore males able to construct bowers more centrally within larger leks could sire more offspring. This needs to be researched further in the potential reproductive benefits of holding bowers in large leks and the position within the lek.

1.10 Importance of aggression in bower building cichlids

Male-male competition through aggression occurs in Malawi bower building cichlid breeding leks such as *N.* cf. *microcephalus* and these aggression levels have been closely linked to bower shape and size. Field experiments have shown increases in received aggression for males holding shorter bowers (Martin 2010; Martin & Genner 2009) and reduced conspecific aggression towards males defending bowers with altered shapes (Magalhaes *et al.* 2013). Martin (2010) concluded from a study that bower size in male *N.* cf. *microcephalus* was sometimes an unreliable signal of male quality as some individuals would use rock bowers to increase initial bower size. Yet there was no difference in aggression levels between rock or sand building cichlids, suggesting that bower size might not be recognised by neighbouring males, and that males assess male quality differently to females. However this contradicts Martin and Genner (2009) who found that males holding artificially taller bowers received significantly less aggression than males holding smaller bowers. Magalhaes *et al*. (2013) also found opposing results, it was discovered that bowers with altered shapes resulted in reduced aggression counts to holding males. This suggests that males do assess rival bowers in terms of their quality and elaboration, and then tune their aggression levels accordingly. However Magalhaes *et al.* (2013) did not differentiate between received and initiated aggression. Without clarity in the separation of these two types of aggression it is impossible to determine whether dominant males reduced their own aggression levels when holding novel bower shapes or that rival males reduced their aggression levels towards males with irregular bower shapes. Further studies confirming whether males assess their own bower size or whether males assess rival bowers and change their behaviours accordingly is vital in understanding the role of bowers within cichlid leks and the potential benefits they possess to males.

1.11 Negative frequency dependent selection

Negative frequency dependent selection is a mechanism that has been proposed to maintain genetic polymorphisms in nature (Futuyma 2013), it has been suggested to be facilitating the establishment and maintenance of novel bower shapes in bower building cichlids (Magalhaes *et al.* 2013). Negative frequency dependent selection is defined when the fitness of an allele is dependent on its frequency within a population, with selection favouring rare alleles (Gray and McKinnon 2007). An example of negative frequency dependent selection is the maintenance of two female morphs in the dimorphic damselfly *Ischnura senegalensis*that being a male-like andromorph and a female-like gynomorph (Takahashi *et al.* 2010). In this study it was shown that the fitness of the two morphs was inverse to their frequency. Negative frequency dependent selection occurred due to males concentrating most of their mating harassment behaviours upon the most common female morph. In doing so the reproductive success of harassed females was reduced. Therefore the common morph suffers from increased male harassment and reduced reproductive success, with rare morphs obtaining far fewer reproductive disadvantages from males. In this case evidence suggests that negative frequency dependent selection is maintaining genetic polymorphism in this damselfly species through the reduction of reproductive success of the common female morph, facilitating an increase in rare morph frequencies within populations.

Negative frequency dependent selection has also been hypothesised to lead to colour polymorphism maintenance within populations through natural and sexual selection. An example of negative frequency dependent selection through predation has been shown by Olendorf *et al.* (2006) in which the frequencies of different male colour patterns of male guppies (*Poecilia reticulata*) were manipulated. It was found that rare phenotypes had significantly greater survival rates compared to common colour patterns suggesting that negative frequency dependent selection maintained colour polymorphism. Why these rare phenotypes had increased survival rates is unknown with Olendorf *et al.* (2006) suggesting that the increases in survival rates are due to predators of *P. reticulata* being visual hunters, as they search for guppies by creating a 'search image' for their prey based upon the common colouration of guppies. Therefore rare colour guppies are less likely to be detected by predators. This image search has been shown to create negative frequency predation in which predators prey upon common prey morphs and therefore help maintain polymorphism in prey (Punzalan *et al.* 2005). Negative frequency dependent selection in colour polymorphism has also been suggested to arise through sexual selection. In some cases females will switch preference to whichever morph is uncommon. This has been observed in guppies as female preference was shown to select against previous mates (Eakley and Houde 2004) and for novel colour patterns (Hughes *et al.* 1999).

Negative frequency dependent selection has recently been suggested to be acting upon bower building males of lake Malawi cichlids (Magalhaes *et al* 2013). Bowers seem to be important in preventing hybridisation of many cichlid species living in sympatry as bower shapes and sizes are species specific (Kocher 2004). Magalhaes *et al.* (2013) suggested that negative frequency dependent intrasexual selection could be facilitating the invasion and maintenance of novel bower shapes in cichlid populations. Magalhaes *et al.* (2013) discovered that a species of lake Malawi cichlid (possibly *Nyassachromis* cf. *microcephalus*), do not receive any detrimental affects in reproductive success when they are given novel bower shapes. This suggests that novel male extended phenotypes in populations of *N. microcephalus* can be preserved and can be important in maintaining polymorphic bower building cichlids. More so, males holding novel bower shapes were involved in fewer aggressive interactions and this may enable the formation and establishment of novel bower shapes within populations. The construction and maintenance of novel bowers within a lek could be easier with fewer conspecific aggressive interactions suggesting a benefit to possessing a novel extended phenotype. This suggests that negative frequency dependent selection may help maintain novel bower shapes as novel males (who produce novel bower shapes) may gain some advantages by being involved in few aggressive interactions. If these novel shapes are inherited by offspring then alternate bower shapes could be maintained until female preference for such a shape develops. When female mating preference links to a specific bower shape then Fisherian runaway speciation could occur (Fisher 1930).

1.12 Summary of chapters

This thesis delves into current gaps in the knowledge of sexual selection and behavioural trade-offs. Chapter 2 explores whether males can assess neighbouring males changing bower size and change their behaviours accordingly, hypothesising that males can assess neighbouring males bower sizes but in turn do not assess their own. Chapter 3's topic is centralised around cichlid speciation and the behavioural plasticity of male bower building cichlids in relation to their activity levels when courting females, hypothesising that dominant males with the ability to court females would increase the activity counts during both the day and night, highlighting potential decreases in rest/sleep behaviours when males are able to court females.

It is important for us to better our understanding on the topic focus of chapter 2, that being whether males can assess their own bower size as this will help further evidence the suggestion that negative frequency dependant selection is a mechanism that has driven cichlid speciation. If negative frequency dependant selection is driving speciation then males should not be able to change their behaviours when they produce a new bower of differing size and shape. Neighbouring males who assess the new bower should reduce their aggression towards such a male, as suggested but unconfirmed by Magalhaes *et al.* (2013). Chapter 3 investigates trade-offs of the same cichlid species. This chapter revolves around potential trade-offs of sleep duration during breeding season. Little research has been conducted on sleep in fish but recent studies in Pectoral sandpipers (*C. melanotos*) has promoted the idea that potentially animals trade sleep durations for active courtship behaviours during breeding season in order to sire more offspring (Lesku *et al*. 2012). Chapter 3 looks into this suggestion using bower-building cichlids that conduct very active and presumably energetically costly behaviours in order to gain mates. The findings can be added to the very small pool of research regarding fish sleep and rest durations as well as reductions of sleep during breeding season of all animals not only fish.

Chapter 2: Are Male *Nyassachromis microcephalus* able to change behaviours in response to artificial changes in bower size?

2.1 Introduction

Sympatric speciation, or speciation with gene flow, is interesting because the idea that populations could diverge in the face of gene flow is counterintuitive. Speciation via sexual selection through divergent female preference on male traits is one possible mechanism, but it is still not fully understood how polymorphisms of male traits are maintained in populations. These male traits need to be sustained within a population until female preference for such a trait emerges and becomes linked to it (van Doorn *et al.* 2004). This link between a male trait and female preference can then lead to runaway sexual selection (Fisher 1930), and ultimately speciation. Understanding this specific process could be key to a better understanding of sympatric speciation as a whole.

A suggested mechanism for the maintenance of polymorphic male traits is that of negative frequency dependant selection (Mikami *et al.* 2004; Seehausen and Schluter 2004). Males with a rare phenotype have an advantage because they receive less aggression from competing males (Dijkstra *et al*. 2006), this would likely benefit males and subsequently increase their access to females as they could control more demanding yet sought after territories such as being part of a larger lek (Young *et al.* 2009). This rare phenotype increases in the population until the advantage conferred by rarity disappears, and the alternative phenotype becomes advantageous again.

Cichlids are ideal study species due to clear polymorphism of male traits as many species of cichlids build extended phenotypes in the form of sand or rock bowers. Cichlids have also been used in previous studies in regards to mate choice and aggression (Genner *et al.* 2008; Martin and Genner 2009; Martin 2010; McKaye *et al.* 1990; Stauffer *et al.* 2005; Young *et al.* 2009; Young *et al.* 2010). Cichlids were also used in the study that prompted further research in identifying that negative frequency dependent selection could be a mechanism for sympatric speciation (Magalhaes *et al.* 2013).

Negative frequency dependent selection has recently been suggested to be a possible mechanism for the sympatric speciation of bower-building lake Malawi cichlids as males seemingly do not receive reductions in mating success when given altered bower shapes (Magalhaes *et al.* 2013), despite being involved in significantly fewer aggressive interactions than males holding regular shaped bower (Magalhaes *et al.* 2013). This reduction in aggressive interactions with other males could be facilitating bower shape polymorphism in populations of cichlids. In order to understand whether negative frequency dependant selection could be a mechanism for cichlid speciation, clarity is needed on the plasticity of bower owner behaviours. It is important to understand whether males can assess their own bower and change their behaviours accordingly when holding different bower sizes. If changing bower shape has some cognitive or physiological effect on the owner, it may be this, which leads to an advantage via reduced aggression, rather than the change of phenotype itself.

In order to better understand if male cichlid behaviours are plastic and independent of their bower size we assessed six different behaviours of dominant male cichlids, *Nyassachromis* cf. *microcephalus*. Three behaviours were reproductive behaviours (two separate stages of courtship & number of different mates), two were aggressive behaviours (aggression towards non-territory holding males & received aggression) and finally a bower building behaviour was also measured. We used laboratory experiments and provided fish with artificial bowers to test between these hypotheses;

1) Male *N.* cf. *microcephalus* cannot assess their own bower size and cannot change their behaviours accordingly.
2) Male *N.* cf. *microcephalus* can assess neighboring males' bowers resulting in variances in received aggression counts of males holding differing bower sizes.
3) Courtship stage 2 counts (male and female circling) will not significantly differ when the same male is holding a small or large bower as bower size should not effect courtship success rate

2.2 Methods

2.2.1 Study species

Nyassachromis cf. *microcephalus* (Figure 1) is a bower building cichlid fish indigenous to Lake Malawi and is part of the *Copadichromis-Nyassachromis-Mchenga* species complex. This complex contains over sixty species and its range expands over most of lake Malawi (Konings 2007). *N.* cf. *microcephalus* females and non-dominant males are grey in colour; conversely breeding dominant males have a shiny blue hue to their heads and sides with fin colour morphing into a vivid yellow and black. Generally in cichlids dominant breeding males are regarded as being much larger than subordinate males. This is due to larger diameter bowers being correlated with male body size as larger males have been suggested to hold larger bowers (Schaedelin & Taborsky 2006) and larger males are able to survive high energy expenditure required to defend and maintain larger bowers (McKaye *et al.* 1990). However large males are not always the most dominant, the colouration of a males seems to be a more accurate signal of male dominance (pers. obs).

Figure 1. Picture of *Nyassachromis* cf. *microcephalus* from Konings, A. (2007). *Malawi Cichlids in their Natural Habitat.* (4th Edition). Cichlid Press.

2.2.2 Husbandry

This research was conducted at the University of Hull using a stock of captive bred *N.* cf. *microcephalus*. Fish to be used for the experiments were separated from the main stock into a group of thirty-two mixed female and male individuals, four dominant males, eight subordinate males and twenty females. A total of 32 individuals were used as this number reflected a similar density of fish per tank compared to the fish's original stock tanks. Four dominant males were used due to space confinement and study duration, however this experiment could have been conducted with any number of dominant males as males were separated and therefore their aggressive behaviours displayed to one another were restricted. Eight subordinate males were used to ensure that dominant males became territorial as males in the wild often chase subordinate males from their bowers (Martin and Genner 2009). Twenty females were used in order to entice males to display courtship behaviours. Following talks with the head aquarist at the university along with personal observations we concluded that holding tanks with more females present would result in an increased frequency of courtship behaviours being displayed by males. We also found that males being held without a female present did not change into their typical vivid yellow and black body colours, which are associated with dominance and courtship. Therefore the ratio of twenty females and four dominant males would be more than sufficient to ensure courtship behaviours were displayed. The cichlids were fed once a day with alternating diets of floating and sinking pellets. The light duration was kept constant throughout the experiments to ensure the time of day does not affect *N.* cf. *microcephalus* behaviour as it is known that the time of day can influence activity levels of some cichlids during breeding season (Reebs 2002). This light duration was set from 12 midday - 12 midnight to ensure that we could move and catch fish soon after the lights turn on at 12 midday as the fish were very unresponsive to the movements of nets within the water at this time (pers. obs). Two-week periods were given to newly introduced fish to acclimatise to the tank conditions before experiments were conducted so that their new tank environment including light cycles did not influence their behaviours. This acclimatization was carried out in holding tanks below the experimental tank, on the same circulatory system and light regime. Dominant males were identified as having blue head and body colouration and only dominant males that were over 20% heavier than the largest subordinate male were used. Although not always true the size of an individual has been found to be a fairly good indicator for dominance (Allee *et al.* 1948; Frey and Miller 1972; Symons 1968) with increases in weight indicating greater dominance in cichlids (Barlow 2002; Barlow and Ballin 1976).

2.2.3 Experimental design

A single tank of dimensions 360cm x 75cm x 40cm (figure 3) was used and partitioned into five equal smaller sections. Separations were created using plastic gratings that were cut in such way to enable subordinate males and female *N.* cf. *microcephalus* to pass through, but not larger dominant males. The furthest right section was used purely to contain the water pumps that pumped water from an equally large sump underneath. The other four sections were randomly assigned as control or experimental sections. The control area contained a large bower of dimensions of 52cm x 44cm, 15cm (figure 2) which was never changed. Each experimental partition contained a randomly assigned large bower with dimensions of 52cm x 44cm, 15cm high, with a 19cm x 19cm platform width, or a smaller bower with dimensions of 41cm x 38cm, 9cm high, with a 20cm x 20cm platform width. All bowers were artificially constructed from concrete with at least 2cm of sand residing along the entirety of the tank floors, this enabled males to construct extensions to their bowers. The large bower size is of similar height to the mean height bowers built in lake Malawi (Martin 2010), with restrictions to base and platform diameter due to tank section size. The smaller bower size is comparable to the smaller bowers in lake Malawi (McKaye *et al.* 1990).

We wanted to test whether dominant male bower holding fish altered their behaviour if the dimensions of their bower changed. Each trial consisted of four males (three residing in the experimental section and one male within the control section) each in a separate section containing an artificial bower, and allowed to establish territory for 24 hours before observation. The control bower (large) was randomly assigned a section within the tank; the three other sections contained randomly assigned large or small bowers Eight subordinate non-bower owning males and twenty females were also added, these had access to all sections. This maximised the possibility that dominant males would actively defend their bowers and ensure all males had the opportunity to mate.

Each trial lasted for two weeks with two clear weeklong experimental phases. The duration of a week was used to ensure that if male behaviours were affected by bower placement or removal then enough time post bower change would be available for males to ‘settle’ again, and then resume what we would expect to be behaviours unaffected by the bower change. Each trial was carried out as follows:

Week 1: the behaviours of the dominant fish were recorded using four wall mounted Swann 720p NVR CCTV bullet cameras from 18:00 until 18.30 for seven consecutive days. Animals were fed at 14:00 everyday as to ensure that feeding did not interfere and alter the behaviours of interest in this study. This observation room was locked and remote observations were used via CCTV, as fish would behave erratically when people were present (pers. obs.).

Transition: After one week the bowers were all replaced with opposite bower sizes (large to small, small to large) apart from the control, in which the large bower was removed and replaced back into the same section. Bower change was performed on day 8 at 12 p.m. At this time the lights will have just turned on. The sudden brightness of the lights causes light shock in *N.* cf. *microcephalus* and causes the fish to be immobile for a period of a few minutes. Thus the fish at this time of the day were not very active and did not try to jump out of the tanks when the bowers were handled.

Week 2: The CCTV cameras were used to observe the males at 18:00 until 18:30 for another 7 days after the transition of bowers. The experiment was repeated four times, obtaining data from 12 dominant experimental males being observed (six large to small bowers, six small to large) and four control males. Females and subordinate males were not moved from the experimental tank for, thus ensuring the same females and males are courting and competing with different dominant males.

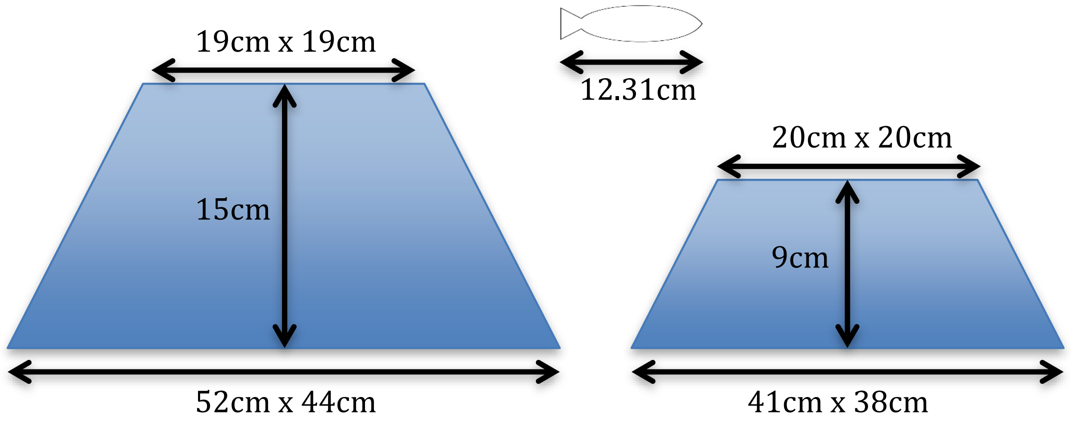

Figure 2. Large and small bower sizes compared with mean average dominant male size, note summit of the bowers are circular when viewed from above.

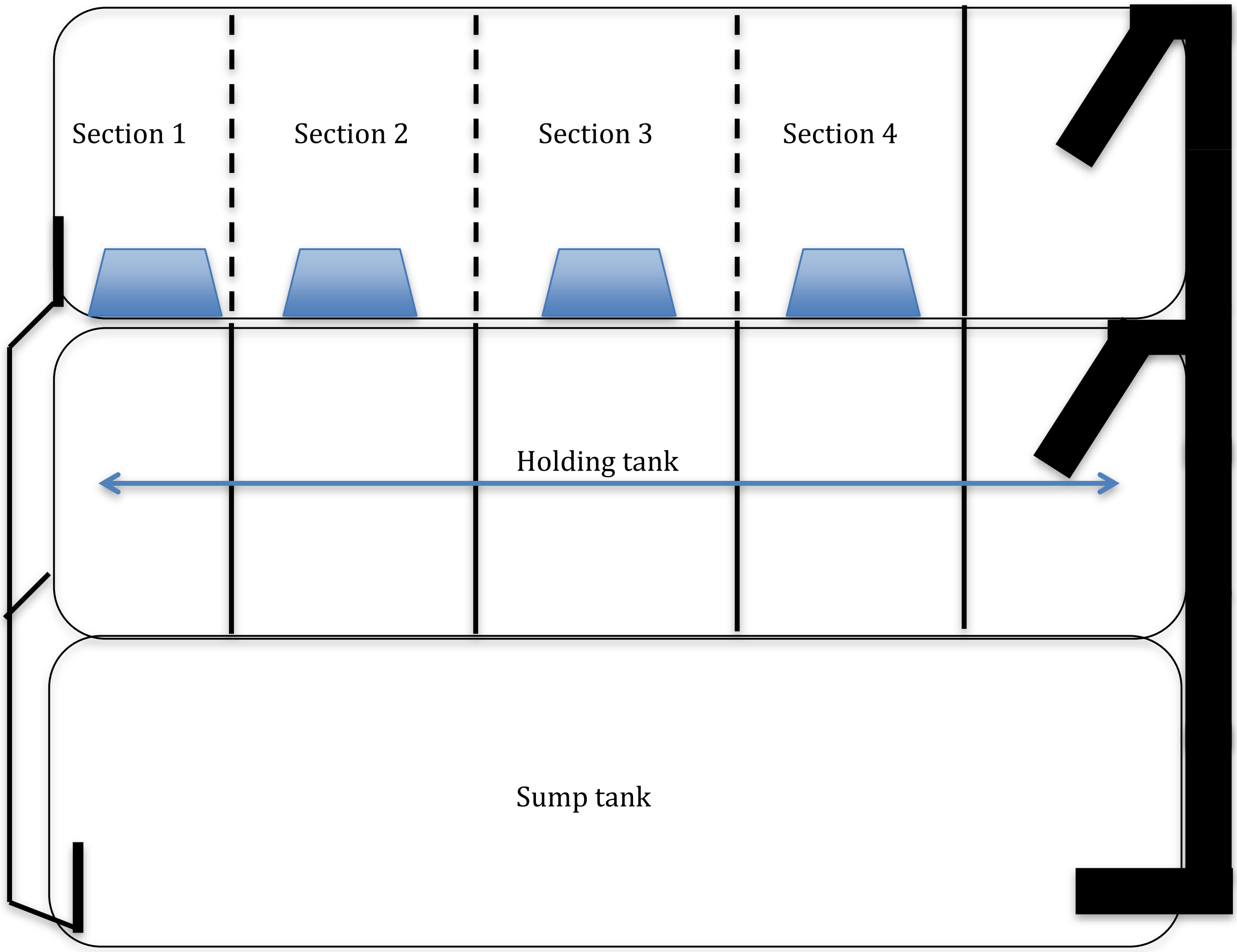

Figure 3. Drawing of the experimental and control tank set up. The large tank is partitioned into five smaller sections using plastic transparent grates to allow subordinate and female fishes through. Three of the sections contain a randomly assigned sized bower while the control section contains a large bower. The section containing the control bower (large – large bower change) was randomised throughout the experiment, with subsequent experimental bowers placed in the remaining sections. Experimental sections consisted of one small bower and two large bowers, which were then changed for week two observations, thus two small bowers and one large bower. These bower sizes are then kept the same from week two to the new week one for the next experimental group. The furthest right section of the experimental tanks was left empty and contains the sump pump, The furthest left section contained a small drain

back to the holding tanks. The tanks below are the holding tanks used for the pre-experiment acclimation of *N.* cf. *microcephalus* to the specific light period.

2.2.4 Behavioural observations

Count data of the following behaviours were recorded from the 30 min recording window taken on days 5-7 and 12-14, using adjustable speed video playback:

- Aggressive display by experimental male towards females and/or males in same tank.
  - Fast paced lunge or chase towards subordinate male or female.
- Received Aggression from neighbouring experimental males.
  - Defined as a charge by a dominant male towards the bower holding male, through the separating grating or glass.
- Courtship
  - Courtship dance performed by the dominant male defined by a series of fast paced movements close to a female, with many changes in direction. This movement usually resembled figure of eight swimming pattern
- Circling.
  - Female and male circle each other on top of bower platform in a tight slow formation.
- Bower building.
  - Dominant male moves the sand with its mouth from tank floor to the bower.
- Number of mates
  - The number of different females a male engaged in circling with.

A mean count of each behaviour per individual over the course of week 1 and week 2 were independently calculated.

2.2.5 Statistical analysis

Behavioural count data was tested for deviations from normality using a Shapiro-Wilk test. Data which was not normally distributed had a constant of 1 added, and was log10 transformed in order to normalise the data in order to fit the assumptions needed for the data to be used in parametric tests, this was the case for all six behaviours. Levene's test of homogeneity of variance, and Box's test for covariance were also carried out using SPSS.

Repeated measures ANOVA were run in SPSS using all behaviours separately as the dependent variables, while the grouping variable was the direction of change in bower size (large to small, small to large, or no change for the control group large to large). For the number of mates, bower building and circling, the assumptions for a repeated measures ANOVA were not met and therefore only significant differences between mean counts of behaviour before and after the bower change could be tested for. Due to the data not meeting all the assumptions for an ANOVA test non-parametric tests (Mann Whitney U test) were run used to compare behaviours when males were upon small and large bower sizes during week 1 and week 2 independently.

2.3 Results

Homogeneity of variance.

There was homogeneity of variances, as assessed by the Levene's test of homogeneity of variance in behaviours of aggression towards conspecifics within dominant males section (AGG) F(2.7,46) P = 0.108, received aggression from neighbouring dominant males (RA) F(1.7,46) P = 1.99, and courtship stage 1 counts (CS1) F(1.7,46) P = 0.200.

Homogeneity of covariance.

Homogeneity of covariance as assessed by Box's test of equality of covariance matrices was true for aggression towards non-territory holding males (M = 6.74, F = 1.07, df1 = 6, P = 0.380), received aggression from neighbouring males (M = 4.74, F = 0.751, df1 = 6, P = 0.609) and courtship stage 1 (M= 1.16, F = 0.183, df1 = 6, P = 0.982).

Repeated measures ANOVA

Repeated measures ANOVAs were computed for: aggression towards conspecifics within dominant males section (AGG), received aggression from neighbouring dominant males (RA), and courtship stage 1 counts (CS1). However because of non-homogeneity of variance for counts for the behaviours of bower building (BB), number of mates (NM) and circling (CS2) the assumptions of a repeated measures ANOVA was not met and therefore we could not report the interaction term of each behaviour but we could report the changes in these behaviours over time (pre and post bower change). There was no statistically significant interaction between the intervention (bower size change) and time (pre/post bower change) on aggression towards non-territory holding males (AGG) F(2,61)= 0.765, P = 0.470, partial η^2 = 0.024. However there was a significant increase in behaviour counts for aggression towards conspecific non-territory males (AGG) from week 1 to week 2 regardless of bower size F(2,61) = 6.166, P = 0.016, partial η^2 = 0.092. There was no statistically significant interaction between the intervention and time on received aggression from neighbouring dominant males (RA) F(2,61) = 0.837, P = 0.438, partial η^2 = 0.027. However a significant decrease in behaviour counts was found for received aggression (RA) from week 1 to week 2 regardless of bower size F(2,61) = 14.09, P = 0.00, partial η^2 = 0.188. There was also no statistically significant interaction between the intervention and time on courtship stage 1 counts (CS1) F(2,61) = 1.216, P = 0.303, partial η^2 = 0.038 nor between week 1 and week 2 counts F(2,61) =

0.063, P = 0.802, partial η^2 = 0.001. For the behaviours of bower building (BB), circling (CS2) and number of mates (NM) only pre and post comparisons could be used from the repeated measures ANOVA analysis. Bower building behaviours (BB) were found to significantly differ from pre to post bower change F(2,61) = 9.15, P = 0.004, partial η^2 = 0.13. Circling (CS2) F(2,61) = 0.741 , P = 0.393, partial η^2 = 0.012 and number of mates (NM) F(2,61) = 0.623, P = 0.433, partial η^2 = 0.01 were both found to not significantly differ from pre to post bower change.

Table 1. Repeated measure ANOVA test for all behaviours with BB, CS2 and NM being greyed out due to the data for these behaviours not meeting the assumptions for the repeated measures ANOVA.

Behaviour	Interaction between bower size change and time		Change in behaviour from week 1 to week 2	
	F	P	F	P
Aggression towards non-territory holding males (AGG)	.765	.470	.166	.016
Received aggression (RA)	.837	.438	14.09	.000
Courtship stage 1 (CS1)	1.216	.303	.063	.802
Bower building (BB)			9.15	.004
Courtship stage 2 (CS2)			.741	.393
Number of mates (NM)			.623	.433

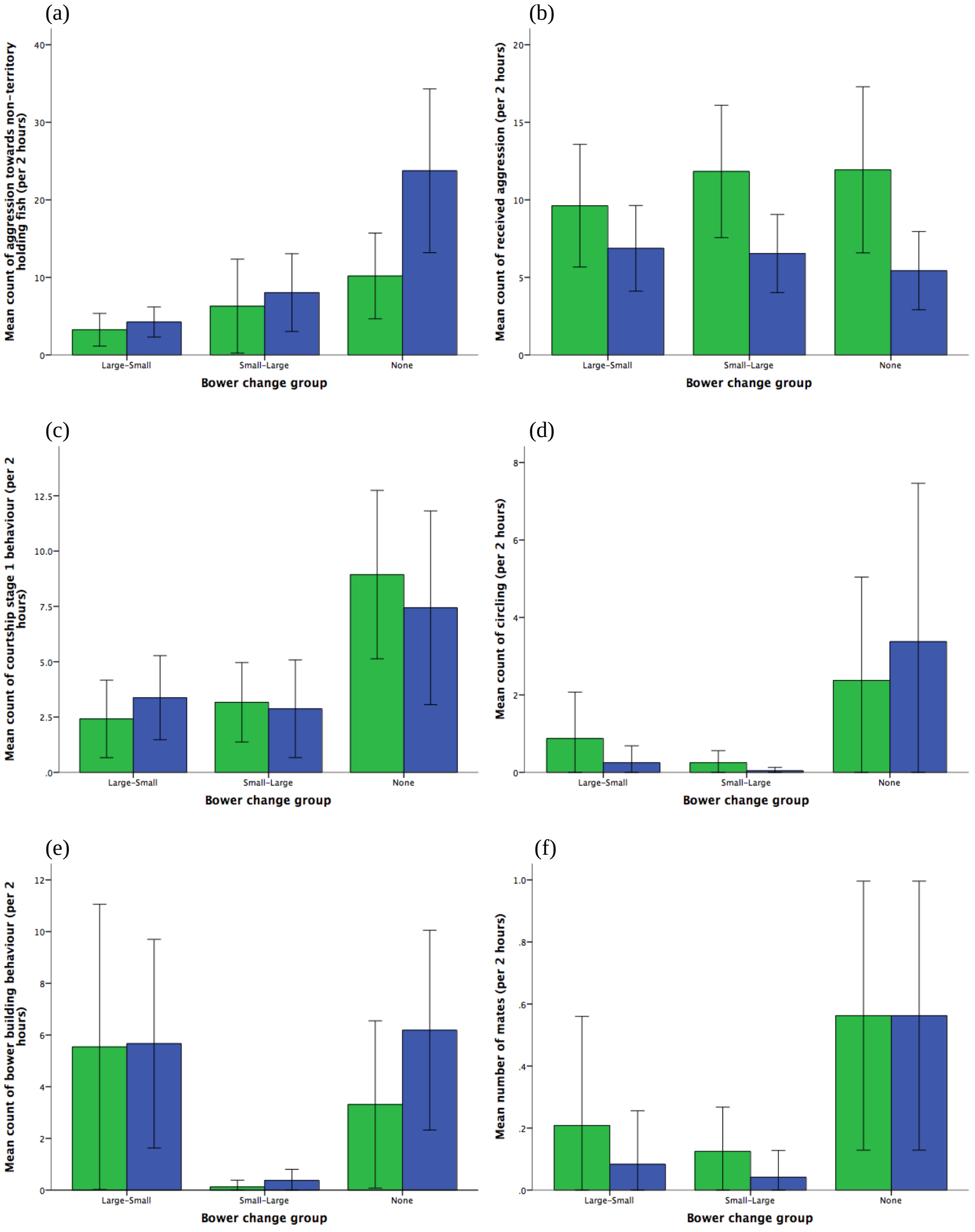

Figure 4. Mean counts of behaviour during week 1 before the size of the bower was changed (shown in green) and during week 2 after the bower size had been changed (shown in blue). Experimental males were given either a small bower that was changed to a large bower, or a large bower that was changed to a small bower. Control males were given a large bower from week and then the same sized bower was removed and

replaced for week 2 observations. Bars represent mean counts of all dominant male behaviours over a total of 2 hours of observations. The whiskers represent 95% confidence intervals. The Y axis is capped at a minimum of zero therefore values of 95% confidence intervals at zero all continued into negative values.

As repeated measures ANOVA data could not be used to compared changes in behaviours due to bower size, a Mann-Whitney U test was used to compare observed behaviour counts between different males holding small or large bowers of the same time period (week 1 or week 2). The assumption of normally distributed data was not met in order to use a parametric independent samples *t*-test therefore a Mann-Whitney U test was used. Significance was not found in any behaviour between bower sizes during week 1 (table 2) or week 2 (table 3) apart from bower building counts (BB). Bower building count observations of individuals from different bower change groups during week 2 were significantly higher in males holding small bowers (M*dn* =0.50) compared to males holding large bowers (M*dn* =0.00), U = 186.500, *z* = -2.486, P = 0.012.

Table 2. Mann-Whitney U test using absolute count data to compare the small and large median bower behaviour counts of each experimental group during the first week of behaviours.

Behaviour	Median		Range		DF	*Z*	U	Exact significance (2-tailed)
	Small (bower)	Large (bower)	Small (bower)	Large (bower)				
Aggression towards conspecifics within section (AGG)	2.00	1.00	0-58	0-20	23	-.581	260.5	.568
Received aggression (RA)	8.00	7.50	0-31	0-40	23	-.630	257.5	.535
Courtship stage 1 (CS1)	.00	.00	0-12	0-17	23	-.461	267.5	.650
Courtship stage 2 (CS2)	.00	.00	0-3	0-11	23	-.072	286.0	.833
Bower building (BB)	.00	.00	0	0-43	23	-1.48	250.0	.109
Number of mates (NM)	.00	.00	0	0-4	23	-.409	277.5	1.000

Table 3. Mann-Whitney U test Week 2 comparing the small and large median bower behaviour counts of each experimental group during the second week of behaviours.

Behaviour	Median		Range*		DF	*Z*	U	Exact significance (2-tailed)
	Small (bower)	Large (bower)	Small (bower)	Large (bower)				
Aggression towards conspecifics within tank (AGG)	3.50	3.50	0-15	38	23	-.471	265.5	.638
Received aggression (RA)	5.00	4.50	0-25	20	23	-.104	283.0	.917
Courtship stage 1 (CS1)	1.50	1.00	0-14	21	23	-.476	265.5	.634
Courtship stage 2 (CS2)	.00	.00	0-5	1	23	-.614	275.5	.539
Bower building (BB)	.50	.00	0-38	4	23	-2.68	179.0	.007
Number of mates (NM)	.00	.00	0-2	1	23	-.030	287.5	.979

* All behaviours were not expressed at some point during the experiment and therefore range is determined from a count of zero up to the given value.

2.4 Discussion

In this study the aggression towards conspecifics within tank section (AGG) and received aggression from neighbouring dominant males (RA) was not significantly different between males holding bowers of different sizes. Aggression towards conspecifics within tank section (AGG) count simply increased in every group (experimental and control) after week 1 observations, regardless of bower size. Therefore, changing the size of a bower for an individual does not affect the initiated aggression levels of the individual. Our research therefore has suggested that the aggressive interactions of dominant males towards other dominant and subordinate males as well as females are not dependent on bower size, as males do not change their aggression levels when given differing bower size nor did males receive any increases or decreases in aggression from neighbouring dominant males depending on their own bower size.

It is important that we note clear behavioural differences seen in Figure 4 between the experimental and control groups. Figures 4 a,c,d, and f show that the control males were consistently the most active group of males. The reasons for such an increase in behaviour is unknown and I can only speculate as to why there is such a difference between the groups. Perhaps the increase in activity alludes to *N.* cf. *microcephalus* needing longer settling periods when their bower sizes are significantly changed (as control males did not receive a bower size change). This would explain the similarities in the expressed pre-post behavioural trends in the control males (Fig 4a,b,e) as control males follow a similar trend to that of the experimental males but with seemingly amplified results. Even though the control male's bower was also removed and replaced, the same concrete bower was used. Although the control male bowers were not significantly changed in size the bower building behaviours of males across both experimental and control groups did result in size changes. Therefore control males shouldn't have been entirely accustom to their 'new' bower when it was replaced after week 1 observations. Further studies could be conducted to clarify if males show similar behavioural activity when their bowers are replaced with similar but not identical bowers.

Conventionally male *N.* cf. *microcephalus* build their own bowers from the sand of the lake bed, or males use rocks as platforms for sand bowers to be built upon (Martin 2010; McKaye *et al.* 1990). Bowers can also be acquired by male *N.* cf. *microcephalus* in breeding leks through male-male competition in which the winning male acquires an already existing bower from the losing male (Konings 2007). This bower can have many previous owners who continuously maintain and adjust the bower to their own requirements. Our research suggests that once a male within a lek acquires a bower the male should not change its aggressive behaviours depending on the size of the bower it has acquired, nor should this male receive any difference in received aggression from other males when acquiring different sized bowers. Therefore McKaye *et al's*. (1990) findings that males upon larger bowers display more frequent aggressive behaviours towards other males is not a plastic response from the holding males due to the size of their bower as our males did not change their behaviours when given differing sized bowers. Perhaps males who are naturally more aggressive build larger bowers.

It is unclear as to whether our results support or contradict the idea that negative frequency dependent selection could drive cichlid speciation (Magalhaes *et al.* 2013). Part of our research supports this theory, as there were no significant changes in behaviours when a male's bower was changed in size. This suggests that fish cannot assess their own bower size and therefore do not change their behaviours according to bower size. Part of our research also contradicts this theory of negative frequency dependant selection acting upon cichlid populations. Our research found that received aggression counts did not significantly alter when bowers were changed in size. If negative frequency dependent selection were apparent in cichlids then we would expect males to be able to assess other males bower sizes and shapes as males would choose not to attack males with novel bower shapes or sizes potentially giving males holding novel bower shapes a fitness advantage over others (Magalhaes *et al.* 2013). However bower shape was not changed in our research and perhaps a simple change in size of bower does not elicit changes in aggression from other males as these changes in size do not differ enough from the norm, it is in fact novel bower shapes that cause the reductions in aggressive behaviours seen in Magalhaes *et al.* (2013) paper and therefore our research if coupled with further research on the affects of bower shape rather than size could support the idea that negative frequency dependant selection facilitates bower building cichlid speciation, but our research alone cannot confirm this.

Received aggression counts did not significantly differ when fish were given a different sized bower, which could suggest that different sized bowers do not elicit differing levels of aggression from other dominant males. A dominance hierarchy could have been established within the first few days of our study as our analysis of received aggression counts reveal a systematic decrease in received aggression counts. Dominance hierarchies are social systems found in many animal species and the ranking of individuals within dominance hierarchies is determined by the direction of agonistic interactions between individuals of which the interactions are determined by permanent (or semi permanent) properties of the animals concerned (Huntingford and Turner 1987). The indication of a possible dominance hierarchy is due to our repeated measures ANOVA test, displaying significant decreases in amount of received aggression by holding males from week 1 to week 2 observations ($P < 0.00$) regardless of their bower size. Overall the amount of received aggression holding males experienced declined throughout the course of the study and this did not depend on the dominant males bower size, perhaps a dominance hierarchy had been established which reduced the number of costly aggressive interactions between males. Therefore perhaps the reason as to why our dominant males did not receive significant changes in received aggression counts is due to a dominance hierarchy being regulated between the four dominant males in our research over the 2-week period. Subsequently reducing the amount of agonistic interactions between males after males have developed a hierarchy.

The lake Malawi haplochromine species flock, of which *N.* cf. *microcephalus* is a part of, are known to establish dominance hierarchies within breeding leks (Martin and Genner 2009; Nelissen 1985). Hierarchies are beneficial to individuals as they reduce aggression levels between individuals in the group, and so enable them to divert energy from male-male competition (and in some species fighting with potential injuries) into reproduction or survival (Huntingford and Turner 1987; Huntingford *et al.* 2012; Neat *et al* 1998; Nursall 1981). This applies to *N.* cf. *microcephalus* too as dominance hierarchies in haplochromine cichlid fishes are known to be regulated through aggressive interactions (Martin and Genner 2009) with most aggression being displayed from the top ranking males (Clement *et al.* 2005). McKaye *et al*. (1990) found that males holding taller bowers were significantly more aggressive than males holding smaller bowers, indicating that individuals holding taller bowers are more dominant. However in our study there is no significant difference in received aggression levels between males holding bowers of different size. This suggests that bowers themselves perhaps do not determine social status of dominant males but are perhaps just indicators of male quality, with dominance hierarchies being constructed through aggressive interactions and determined by male quality, which in the wild we would expect males of greater fitness to be able to construct larger bowers. Nonetheless, the suggestion of dominance hierarchies arising in cichlid leks is suggested in our study with significant reductions in received aggression counts in all bower size categories after week 1 observations. If a hierarchy had not been established then we would expect to see received aggression from neighbouring males counts to stay relatively constant throughout the duration of the study, rather than a significant drop in received aggression counts. This also conforms to an increase (not significant) in the count of aggressive behaviours towards non-territory holding males within the tank sections from week 1 to week 2 observations, as aggression is potentially directed towards subordinate non-territorial males rather than that of dominant neighbouring males.

There is a vast divergence in the shape, size and height in bowers of great African lake cichlids with two significantly different bower types, some species being pit diggers (Depression bowers) such as *Copadichromis virginalis*, others species are castle builders (mound bowers) such as *Mchenga conophoros* (York *et al.* 2015), both these bower types vary between species with some researchers able to identify between cichlid species through the shape of their bowers (McKaye 1990). The mechanisms behind the divergence of these characteristics are generally poorly understood yet they have been suggested to be a driving force of speciation in bower building cichlids (Kidd *et al.* 2006; Magalhaes *et al.* 2013; McKaye *et al.* 1990). Magalhaes *et al.* (2013) hypothesised that bower shape plays an important role in the diversification of cichlid bowers in lake Malawi. It was found that altered bower shapes resulted in fewer conspecific male aggressive interactions without effecting mating success. Magalhaes *et al.* (2013) suggested from this study that the reduction of conspecific male aggression towards males of novel altered bower shapes could cause the mechanism of negative frequency dependent selection to facilitate the invasion of novel phenotypes (bowers) in cichlid populations. Negative frequency dependent selection can occur in systems when the same trait is being selected for by males through aggressive interactions as well as being selected for by female mate choice. Rare phenotypes in this system would receive fewer aggressive interactions from conspecific males and subsequently receive an advantage through reduced aggression. This reduction in aggressive interactions would enable the invasion of the new phenotype (bower) within a population. As the occurrence of the new phenotype increases within the population its advantage from being the rarer form of bower would decrease as males would start receiving more aggression from conspecifics (Magalhaes *et al.* 2013). Eventually female preferences could become associated with the new bower shape, possibly resulting in reproductive isolation of males with that phenotype (bower shape) and possible speciation (Fisher 1930). However it was unclear if the bower shapes altered by Magalhaes *et al.* (2013) for male *N.* cf. *microcephalus* directly influence the behaviour of the new holding male as Magalhaes *et al.* (2013) research found a reduction in overall aggression levels of males holding a novel bower shape but they could not distinguish between initiated and received aggression. Therefore it could be possible that males holding novel phenotypes simply initiated less aggression, reducing the aggression count found in their study. This would imply that fish could assess their own bower shape or size and change their behaviours accordingly. We tested for this idea in our study by observing many different behaviours and our results suggest that males cannot assess their own bower size and change their aggression levels accordingly as our fish did not change any of their observed aggressive or courtship behaviours when given differing sized bowers. This conforms with Magalhaes *et al.* (2013) suggestion that novel bower shapes reduced aggression counts from other conspecifics was not due to holding males becoming less aggressive when their bower shape is changed. Our research further supports, but does not entirely confirm, their theory of negative frequency-dependent selection acting as a likely mechanism for the maintenance of the bower polymorphisms as males holding altered bower shapes receive less aggression from other conspecifics (Van Doorn *et al.* 2004). Van Doorn *et al* (2004) proposed that males will often be able to acquire/build higher quality territories (such as bowers) when being less challenged by other dominant males. Therefore it is likely that males who build altered bower shapes will be able to build better quality bowers and gain a reproductive benefits. However a potential reproductive benefit is untested (Magalhaes *et al.* 2013). Perhaps males with novel bower shapes could build taller bowers as taller bowers have been found to increases courtship success through direct female mate choice as females assess males and their bowers before courtship (McKaye *et al.* 1990). Potentially males could also benefit from having reduced amount of received aggression from other males by being able to construct bowers within larger leks. This has been said to increase reproductive success through increases in indirect female mate choice favouring males within larger lek formations (Young *et al.* 2009). The combination of all these factors in turn could allow males to acquire more female access. Along with improved territory quality, aggressive behaviours towards rival dominant males are energetically costly through chase and flee interactions or through actual physical harm. Therefore males could benefit from receiving fewer aggressive interactions as well as potentially saving energy in defending their bower and perhaps directing their energy towards courtship behaviours. These suggestions into the possible benefits of altered bower shapes and the suggestion that *male N.* cf. *microcephalus* do not change their behaviours according to their own bower shape and size do need to be researched further in order to combine both findings. Eventually a better understanding of the roles bowers play in reproductive efforts and negative frequency dependent selection may demonstrate the relationship between bower evolution and cichlid speciation.

Bower build count is a very useful for understanding the plasticity of *N.* cf. *microcephalus* behaviours. The costs associated with the acquisition, maintenance and defence of bowers in the wild is what limits bower size, hence why bowers are good indicators of male quality and with ample resources and the inability for other neighbouring males to chase holding males away we expected males to continue building bowers regardless of the initial size given. We hypothesised that *N.* cf. *microcephalus* would not be able to assess their own bower size and therefore bower-building behaviours should not change when individuals were given a different sized bower. Our repeated measures

ANOVA showed no significant difference between the experimental groups bower building behaviours nor from week 1 to week 2 observations. However unlike that of which we hypothesised, the Mann Whitney U test showed that males who held a small bower after holding a large bower expressed significantly more bower building behaviours compared to males who held small bowers first as displayed in table 3.

This potentially plastic bower building behaviour has been found in male *N.* cf. *microcephalus* using rock bowers. Martin (2010) found that *male N.* cf. *microcephalus* often use rock bowers as unreliable signals to entice more females into their bower. There were clear advantages for males to use rocks as platforms for their bowers as martin (2010) found these rock bowers were significantly taller than sand bowers. This suggests that males display an unreliable signal of their quality to potential female mates as height of bower has been shown to increase the number of females a male mates with (McKaye *et al.* 1990). The distance travelled by males to transport sand from the lakebed to the top of their bower was found to be significantly shorter in males holding rock bowers and rock bowers were also found to require significantly less maintenance than sand bowers. Thus the energetic costs needed to construct and maintain bowers are significantly reduced in males using rocks as platforms for their bowers compared to sand based bowers. Rock bower holding males did not suffer from any increases in neighbouring male aggression directed towards them. However there were no reproductive benefits to males holding rock bowers. Although males received significantly more visits from females when holding rock bowers females were not found to mate with these males displaying superficial bower heights any more so than males who built 'true' sand bowers of smaller height. Martin's (2010) research suggests that females do not use bower size as a sole indicator for male quality. Multiple sequential assessments of male traits are conducted by females to ensure that females know the true reproductive fitness of the male. This unreliable signal of a rock bower still entices more females to visit 'false' rock bowers than smaller sand bowers but it did not increase the mating success of males holding rock bowers (Martin 2010). This suggests that the benefits of reduced maintenance and construction costs without reproductive benefits still provides a great enough benefit for males to acquire and hold rock bowers. The suggestion that *N.* cf. *microcephalus* benefit from using rock bowers during breeding season potentially could answer why the individuals used in our research are seen to dramatically change bower building behaviours when given a small bower after being given a large one in our research. Males may try to rebuild their bower to their initial 'unreliable' larger bower size, hence why we see a significant increase in bower building behaviours upon the smaller bower after males have held a larger bower compared to males holding a large bower after initially holding a small bower. This however would suggest that males could assess their own bower size and try to build upon it, implying that potentially males may have plastic bower building behaviours that change depending on the size of the bower. Our results also support Martin (2010) research as we did not find any significant changes in the number of courtship behaviours males received either upon artificially large bowers or smaller bowers. These initial larger bowers do not 'fool' females into believing that holding males of larger bowers are of better quality. Therefore the benefits of using rock bowers are likely to be advantageous to males in regards to a shorter construction time compared to sand bowers (Martin 2010; McKaye *et al.* 1990). Thus our research further supports the idea that sequential female assessment is apparent in *N.* cf. *microcephalus,* which prevents males from gaining direct reproductive benefits from unreliable signals.

Our hypothesis that dominant male *N.* cf. *microcephalus* do not change levels of aggression and courtship behaviour according to the bower size they are given, was confirmed by our results. Individuals did not change any of their courtship behaviours nor did they change any aggressive behaviour when individuals were given differing bower sizes. These results imply that these behaviours are not plastic responses to bower size, this suggests that these aggressive behaviours are potentially innate. Lisy, (2006) provides further evidence for bower heritability. Young fry were separated from their mothers soon after hatching to prevent any learned bower building behaviour. 4th and 5th generation fish were then given necessary lab conditions to build bowers, which were constructed. Thus proving that the ability to build bower and its shape is an innate behaviour and is a heritable sexual trait with results from our research suggesting male cichlids potentially have non-plastic behaviourisms as well. This idea that bower shape and size are inherited is necessary to put forward arguments that populations of cichlid fish are likely to speciate due to selection on bower shape with supporting arguments of speciation through bower shape from Magalhaes *et al.* (2013) and Young *et al.* (2010).

2.5 Conclusion

Our results suggest that dominant males do not change their aggressive behaviours depending on the bower size they are given and therefore these behaviours are not a plastic response. Our results also displayed decreases in received aggression counts over time, independent of bower size, reflecting the possible establishment of a dominance hierarchy. Together with research by Magalhaes *et al.* (2013) our research suggests that negative frequency dependent intrasexual selection could be maintaining polymorphism in cichlid species and enabling speciation through the 'good gene model' of male secondary selective traits (Andersson 1994). Secondly heritability of bower shape is necessary if populations evolve due to selection on bower shape, of which is highly suggested in the literature (Lisy 2006; Magalhaes *et al.* 2013; Martin 2010; Martin and Genner 2009; McKaye *et al.* 1990; Young *et al*. 2010). If the mechanism for cichlid speciation were through male behaviours then we would expect male behaviours to be associated with bower size, not a plastic response that can change. Our results suggest that males do not change their behaviour depending on the size of bower given, therefore it is possible that the behaviours that create specific bower sizes could also be heritable as general bower building behaviours and the subsequent shape of a bower have already been found to be innate heritable behaviours (Lisy 2006). However aspects of bower involvement in reproduction are still not entirely understood and the stage at which females choose a mate during courtship is not entirely defined yet. By confirming this stage we could more accurately define the use of bowers in sexual selection as we expect a further male trait to be assessed by females after male bower assessment which determines if a female will mate with a male, and subsequently male extended phenotype quality does not directly effect mating success and courtship levels.

Chapter 3: Is inactivity reduced during breeding in the cichlid *Nyassachromis microcephalus*?

3.1 Introduction

Sleep in general is poorly understood and the greatest volume of research has been conducted on mammals and birds. There has been little consideration as to the possibility of reproductive behaviours such as competition for mates being a constraint on sleep. There is also severe gap in the knowledge of sleep in fish, at over 32,000 species it exhibits the greatest species richness of all vertebrate groups (Froese and Pauly 2015). Research on the potential reduction of sleep during fish's reproductive season to my knowledge is non-existent. Ecological variables have been suggested as the main determinants of sleep duration (Siegel 2009) and researching the effects breeding season has upon individuals may result in some discoveries into the importance of sleep in the same way researching the affects other ecological constraints have resulted in a better understanding of sleep, its architecture (Capellini *et al.* 2009), functions (Siegel 2005a) and a basic genetic understanding of sleep (Duboué *et al.* 2011).

If fish do sleep then it is most likely their activity patterns could act as good indicators for when individuals are most likely to be asleep, whether that be at night, during the day, throughout the day, or not at all. If an animal were observed to be highly active during the day with period of low activity during the night then we would expect to see some sort of rest/sleep like state in these low activity periods. Some fish are very hard to place into a category of activity pattern (Reebs 2002). Many species of fish display activity patterns similar to the four categories of activity patterns that many mammals and birds generally fit into (diurnal, nocturnal, crepuscular, cathemeral). However many activity patterns displayed by fish do not simply fit into one of these categories as variations in activity is much more frequent in fish throughout the year than mammals or birds (Helfman 1986; Zhdanova & Reebs 2005). Zebrafish are the most comprehensively studied fish in regards to sleep and are a diurnal species with polyphasic sleep patterns with their sleep bouts ranging from 5-100 seconds long (Sigurgeirsson *et al.* 2013; Zhdanova *et al.* 2008; Zhdanova 2009). This short sleep bout duration and polyphasic structure could benefit zebrafish perhaps in terms of predator detection. It has been suggested that predation pressures could have major influences of a species sleep architecture with polyphasic sleep being more beneficial to smaller prey who are more susceptible to predation as polyphasic sleep avoids long vulnerable periods of low consciousness states (Ball 1992; Tobler 1989b; Stampi 1992). However a more recent paper by (Capellini *et al.* (2008) argues that energetic constraints determine the sleep architecture of mammalian species with smaller individuals tending to be polyphasic sleepers due to the need to eat more often due to their higher mass specific metabolic rates, rather than due to being more likely to be predated upon during sleep. However this has only been tested for in mammals and may not necessarily be applicable to fish as sleep bouts in mammals generally last from many minutes to hours long, whereas the only fish species with quantified sleep bouts has been found to sleep for seconds at a time (Zhdanova 2011). Potential natural predators of the zebrafish such as the leaf fish, *Taenianotus triacanthus* or species of catfish (Bass and Gerlai 2008) are suggested to be more active during the night (Bass and Gerlai 2008; Shao 2015). Thus it would be beneficial for zebrafish to be more vigilant during the night to increase potential predator detection, which polyphasic sleep enables due to the avoidance of long bouts of sleep unlike that of monophasic sleep.

Cichlids are ideal study species due to their all year round breeding activities in which males will try to court and mate when in the presence of females (pers. obs.) thus breeding and non-breeding conditions for fish could easily be set up. These cichlids are also generally very active fish (pers. obs.) therefore if static sensors are used to determine fish activity then fish should trigger these sensors when awake and active as they move relatively quickly around their tanks. Cichlids courtship behaviours require very active movements (pers. obs) (Martin 2010; Young *et al.* 2010) thus courtship movements should cause high spikes in activity readings. High male activity counts during breeding season, during a period when quiescent behaviour is normally observed (for example during the night) would indicate that males benefit from increasing their daily (24hour) activity levels, most likely an increase in their reproductive success rates. An increase in daily activity would most likely be accompanied with a reduction in sleep duration, as breeding males would be active when non-breeding males would be in a sleep or sleep like state.

The reasoning behind a suggested increase in nocturnal activity in a group of breeding cichlids (a group with a female present) is that during the night some cichlid species have been found to continue courtship behaviours (Reebs & Colgan 1991). Thus activity levels could potentially be higher in cichlid groups of males with the opportunity to mate with a female than the group of males without a female present, and therefore males would not exhibit courtship behaviours. It has also been found in a species of bird (*Calidris melanotos*) (Lesku *et al*. 2012) that sleep is reduced during breeding season as sleep is viewed as a maladaptive behaviour that is not beneficial to reproduction efforts as time could be better spent doing other activities (Siegel 2012).

The activity rates of the cichlid study species *Nyassachromis* cf. *microcephalus* is currently unknown. We know that many cichlids are either diurnal or nocturnal and the suggestion that polyphasic sleep has evolved in zebrafish due to predation pressures could also be applied to the species of *Nyassachromis* cf. *microcephalus*. *N.* cf. *microcephalus* is susceptible to predation by living in a shallow open water environment, which has few hiding spots to avoid predator detection. This species crowd in shallow waters to help avoid potential larger deep dwelling predators such as species of catfish who feed at night (McKaye 1983; McKaye *et al*. 1990). It would be therefore more beneficial for *N.* cf. *microcephalus* to have a polyphasic sleep pattern so that they are alert to predators more often during the night but be mostly active during the day when their predators are less active. Whether *N.* cf*. microcephalus* sleep is concentrated at night like that of *D. rerio* or in over the entirety of the day in a cathemeral activity pattern is yet to be determined. We predict *N.* cf. *microcephalus* to be a diurnal species as potential predation pressures might be at play and all previous research on *N.* cf. *microcephalus* has been conducted during the day, with *N.* cf. *microcephalus* displaying active behaviours such as feeding, male-male competition and courtship displays (Young *et al*. 2009, 2010; Magalhaes *et al.* 2013; Martin 2010). *N.* cf. *microcephalus* have also displayed in personal observations quiescence behaviours first thing in the morning before daytime/light.

If a diurnal activity pattern consisting of low activity levels during the night over an entire day is observed then specific time periods could be used for future research into sleep of *N.* cf. *microcephalus*. A better understanding of *N.* cf. *microcephalus* activity patterns could help pinpoint potential sleep behaviour using behavioural analysis of sleep during periods when individuals are least active, potentially adding a species to a sparse list of fish confirmed to display sleep like behaviours.

From this research into the effects a breeding season environment can have upon male sleep times we predict that males who do not have access to females will display a diurnal activity pattern like many cichlids of lake Malawi (Konings 2007). Secondly we predict that dominant males will display higher activity counts when given access to females, during periods when males who do not have access to females are resting. This research was conducted in order to address and answer the following hypothesis.

1) Dominant male *N.* cf. *microcephalus* have increased day and night activity counts when in the presence of a female compared to that of a dominant male without female access.

3.2 Methods

This experiment was used to test whether dominant males when in the presence of a female, would change their activity levels during the night when compared with males who did not share a tank with females. If activity counts increase overnight then it is likely that sleep durations will decrease. If activity counts did increase in dominant males when accompanied by a female there is a possibility that the dominant males sleep durations would also be reduced due to the presence of a female.

3.2.1 Study species

The study species used in this research is the bower building cichlid fish *Nyassachromis* cf. *microcephalus*. The research was conducted at the University of Hull in a small quiet experimental room away from human disturbances. The experiment used a stock of captive bred *N.* cf. *microcephalus* from the cichlid aquaria at the University of Hull. Over a period eight weeks of testing 48 fish were used, all of which were sexed, weighed and had their standard length measured (from the tip of the head to start of caudal fin). They were individually anesthetized in MS222, Tricaine Methanesulfonate and then micro tagged using passive integrated transponder tags (PIT tags) which were injected into their body cavity. The University's licensed head aquarist conducted this procedure. In chapter 2 we found colour to be a good indicator of male dominance, however with a lack of female presence males will not 'colour up'. The size of an individual has been found to be a good indicator for dominance (Allee *et al.* 1948; Frey and Miller 1972; Symons 1968) with increases in weight indicating greater dominance in cichlids (Barlow 2002; Barlow and Ballin 1976). Thus 16 males that were around 20% larger than other subordinate males in the tank were selected. 24 subordinate males were used and 8 females were used. Once fish were sexed, measured, weighed, anesthetized and PIT tagged fish were then placed into five conjoined holding tanks of 360cm x 75cm x 40cm and given a two week recovering period. This period enabled the insertion hole of the PIT tag to heal before individuals were used for experiments. These holding tanks were not separated and movement was allowed between tanks. Fish in holding tanks were fed once a day with alternating diets of floating and sinking pellets at 1:30pm. The light duration was kept constant throughout the experiments to ensure the duration of the daytime did not affect *N.* cf. *microcephalus* behaviour. The duration of the day time can influence activity levels of some cichlids during breeding season as different behaviours occur during day and night periods such as courtship, male-male competition and the fanning of eggs by females (Reebs 2002). This light duration was set from 12 midday - 12 midnight to ensure that we could move and catch fish soon after the lights turn on at 12 o'clock midday as the fish seemed to be in a state of quiescence of which these fish could be caught with a net without trying to evade capture (pers. obs).

3.2.2 Experimental design

The experimental room contained two glass walled tanks of dimensions 360cm x 75cm x 40cm both of which were separated by a metre in distance to ensure fish did not react to neighbouring tank fishes yet small enough to ensure that males would be in close enough proximity to compete with one another over the female. Two self-timers were used to control the lighting of a single fluorescent tube above each tank. The timing for lighting was kept the same as the holding tank light schedule of 12 midday - 12 midnight. A Wyre Micro Design Ltd multi point decoder system was used with three sensor plates placed within each tank wired up to a ThinkPad laptop with an operating system of windows 2000 running an SPDlogtag V1.1 program to interpret the decoding system. This laptop and decoder system ran continuously through experimental phases and only turned off during water changes between experiments when experimental and control tank fish were changed.

One tank was randomly assigned to be the experimental tank and the other the control tank. Fish to be used for the experiment were taken from the holding tank with one female, one subordinate male and a larger dominant male placed in the experimental tank. Within the control tank was two equally sized subordinate males within 20% of each others body weight and a dominant larger male. Three individuals were used in both the experimental and control tanks instead of two males (control), and a male and a female (experimental) in order to allow for both male-male competition in both tanks. If we had not used three individuals then we would be recording male-male aggressive behaviours in the control tank and only courtship behaviours in the experimental tank, along with the normal daily swimming activity unattached to accompanying males and females. Pilot studies were conducted prior to this study to test whether a single male could be used as a control to assess whether males could be tracked alone. However this pilot study found that male fish became stressed when isolated from other *N.* cf. *microcephalus*, with enlarged activity counts and no signs of sleep. Fish were given 24-hours to acclimatise to the new tank set up before the sensors were turned back on.

From here seven consecutive days of recordings were taken. Unfortunately due to technical faults only the last day (day seven) of recordings could be used for analysis.

After seven days of recordings fish were taken and placed back into the large stock of *N.* cf. *microcephalus* in the cichlid aquarium. The water from the experimental and control tank was then changed and subsequent water quality tests for nitrates, nitrites and suitable PH levels were conducted using an API salt and freshwater testing kit as large changes in these substances can cause erratic behaviour and eventually death of fish species (Camargo et al., 2005; Kroupova et al., 2005). These tanks were then left until day eight before new fish were taken from holding tanks and placed into experimental and control tanks in which tests were repeated until eight sets of day seven control and experimental data were collected.

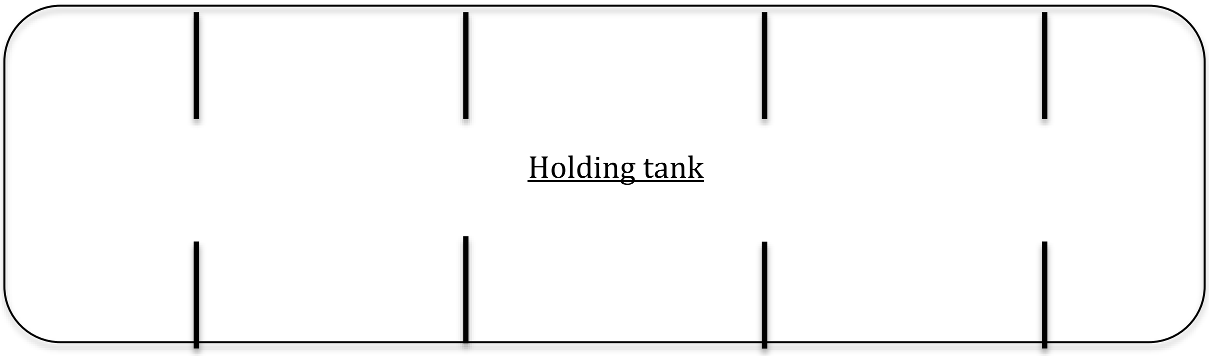

Figure 5. Diagram of holding tank for males and females who have been tagged

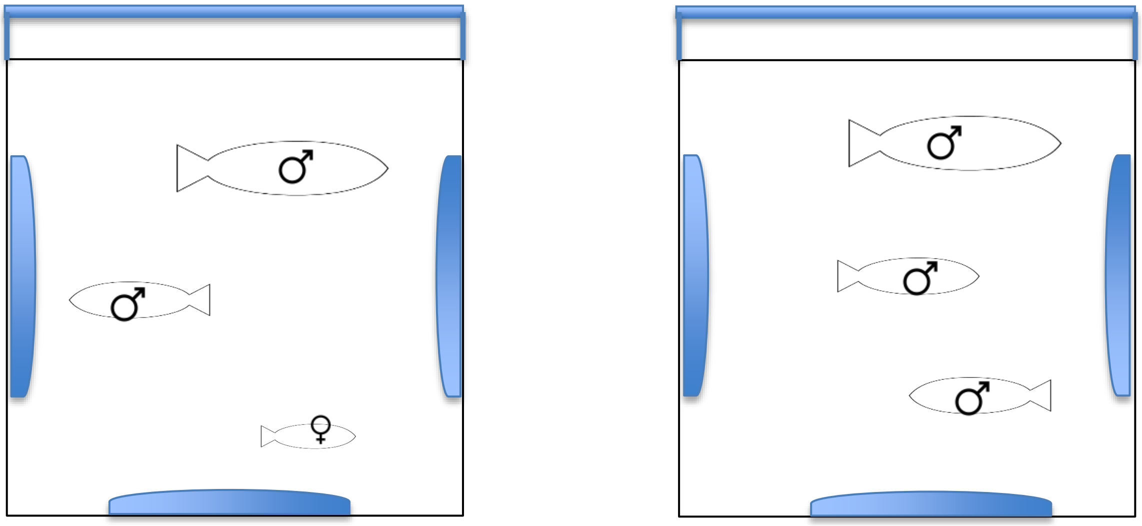

Figure 6. Experimental (left) and control tank (right) set up. Tanks contain one dominant male and two subordinates in the control tank and one dominant male, one subordinate and one female in the experimental tank. Three sensor plates were placed equally on two opposite sides of the tank and another along the tank floor.

3.2.3 Data collection and behavioural observations

We conducted pilot studies using infrared Swann 720p NVR CCTV bullet cameras to determine if using infrared cameras produces images clear enough to observe individuals during periods of no light. However Infrared light has a very poor penetration depth into both water and glass (Mobley 2001) and the images produced from the cameras reaffirmed this as it was only possible to determine an individual's location when 2-3 inches from the tank walls. Thus observation of fish in the middle of the tank was extremely difficult and therefore tracking an individual's movement by watching video recordings was not possible.

In order to assess activity levels *N.* cf. *microcephalus* movements were monitored using three placed sensors. The sensors used were continuous pass-by sensors meaning that for a reading to be counted by the tag reader a PIT tag within the fish must be directly over the sensor (or which ever orientation the sensor is facing) with the sensor continuously reading the PIT tag every one second until the fish moves out for the sensors readable area. These sensors were linked to a Wyre micro design ltd multi point decoder system for 24-hours for seven days per observational period. These sensors then each second searched for PIT tags within range, for every time a PIT tag was found information containing the time of reading, the sensor number, the PIT tag number unique to each individual fish was recorded. This information from the sensors was recorded into an output file. The sensors used in this experiment continuously read tag readings. Using this data the number of times an individual moved from one sensor platform to another was tested for using excel.

The data were separated into recordings of individual fish over each day and night period for day seven (the final day) of each week of recordings. From the row data readings of how many times each fish moved from once sensor plate to another during both day and night periods of day 7 were extracted. This movement from one sensor to another was used to estimate activity levels of individuals with the number of movements each fish made between sensors being used to determine how active each fish was. This experiment was repeated 8 times for both experimental and control tanks.

3.2.4 Statistical analysis

All data were found to have normal distribution ($P > 0.05$) as a Shapiro-Wilks test for normality was computed for each time of day (day and night) in each group of fish (experimental and control). In order to assess differences in the activity levels of dominant males of experimental and control groups an independent samples *t*-test was computed using SPSS 22. The independent samples *t*-test was used to separately compare the total number of times an individual moved over another sensor during the day (12 hours) and during the night (12 hours) between the experimental and control tank individuals per individual type. The independent variable was the group type, with two levels, control and experimental with the dependent variable being activity count. This is a comparison of both dominant males, experimental and control subordinate males and the experimental subordinate male and the second control subordinate male.

A paired *t*-test was used to test our prediction that when males do not have access to females they will display a diurnal activity pattern. A paired *t*-test was used in the same manner as the independent *t*-test but comparing day and night activity count comparisons of the same individual types of the same groups (control or experimental) not across groups like that of the independent samples *t*-test.

In order to assess if *N.* cf. *microcephalus* have rest periods during certain parts of the day the diel activity patterns (cyclic 24-hour activity pattern) of *N*. cf. *microcephalus* was calculated for each hour of day (12 hours) and night (12 hours), that being the number of movements from one sensor to another, and thus our determined activity level. The readings from sensors were categorised and separated by individual, generating a long list of individual recordings in time order and at which sensor the individual was recorded by. Every time an individual changed the sensor it was being read from (thus moved from one sensor to another) a value of 1 was given, indicating movement from one sensor to another. A value of 0 was given if an individual was recorded by the same sensor as the previous time reading thus generating a long list of 0 and 1 values for each specific individual over an entire day. The same was done for every individual. The number of times each individual moved from sensor to sensor (value 1) during each hour of day and night was compared using excel. The activity counts during specific time periods could then be calculated per individual per hour. These results were then analysed through line graphs produced in SPSS 22 of activity count against time period over an entire day for each individual during each week of observations, therefore if *N.* cf. *microcephalus* are a diurnal species we should see decreases in activity counts during the night. If activity levels are seen to differ during specific times of the day then a paired samples *t*-tests comparing the activity count during the rest periods of the day and the active periods of the day can be used to statistically determining if at specific times of the day individuals reduce their activity levels.

3.3 Results

3.3.1 Male activity in the presence of a female.

The independent samples *t*-tests conducted compared combinations of experimental and control group individuals in both day and night lighting durations, the results of which are presented in table 4. The P-values from the six conducted independent samples *t*-tests revealed no significant results. Thus, the presence of a female has no affect on male activity levels during the night.

Table 4. Descriptive statistics for independent samples *t*-test of control and experimental group individuals in both night and daylight hours.

		Activity count						
		Experimental		**Control**				
Test	**Time of day**	**Mean**	**Std. Deviation**	**Mean**	**Std. Deviation**	**Degrees of Freedom**	**T statistic**	**P value**
DOM: DOM	Day	244.75	175.58	187.38	157.68	14	0.71	0.49
SUB: SUB	Day	308.13	200.44	194.25	113.41	14	1.40	0.18
SUB: SUB2	Day	308.13	200.44	256.63	174.75	14	0.55	0.59
DOM: DOM	Night	214.25	148.45	218	151.08	14	0.05	0.96
SUB: SUB	Night	241	155.79	238	109.82	14	0.05	0.97
SUB: SUB2	Night	178.38	144.70	238	109.82	14	0.93	0.37

Mean activity levels (Mean) of day and night periods taken from a 24-hour period of day seven of seven recorded activity day and nights.

Figures 5,6 and 7 represent mean activity levels during the day and night from control and experimental individuals. In all comparison cases of experimental and control individuals of the same dominance type no significant P values were found comparing day and night activity levels separately (table 4). This is observed when comparing the bar charts of figures 5,6 and 7. The figures also suggest there is no significant difference between mean day and night activity counts, thus a further paired *t*-test was conducted (table 4) which compared the activity counts of separate individuals day and night time activity counts to determine if individuals change activity levels depending on the time of day, day (light) or night (dark).

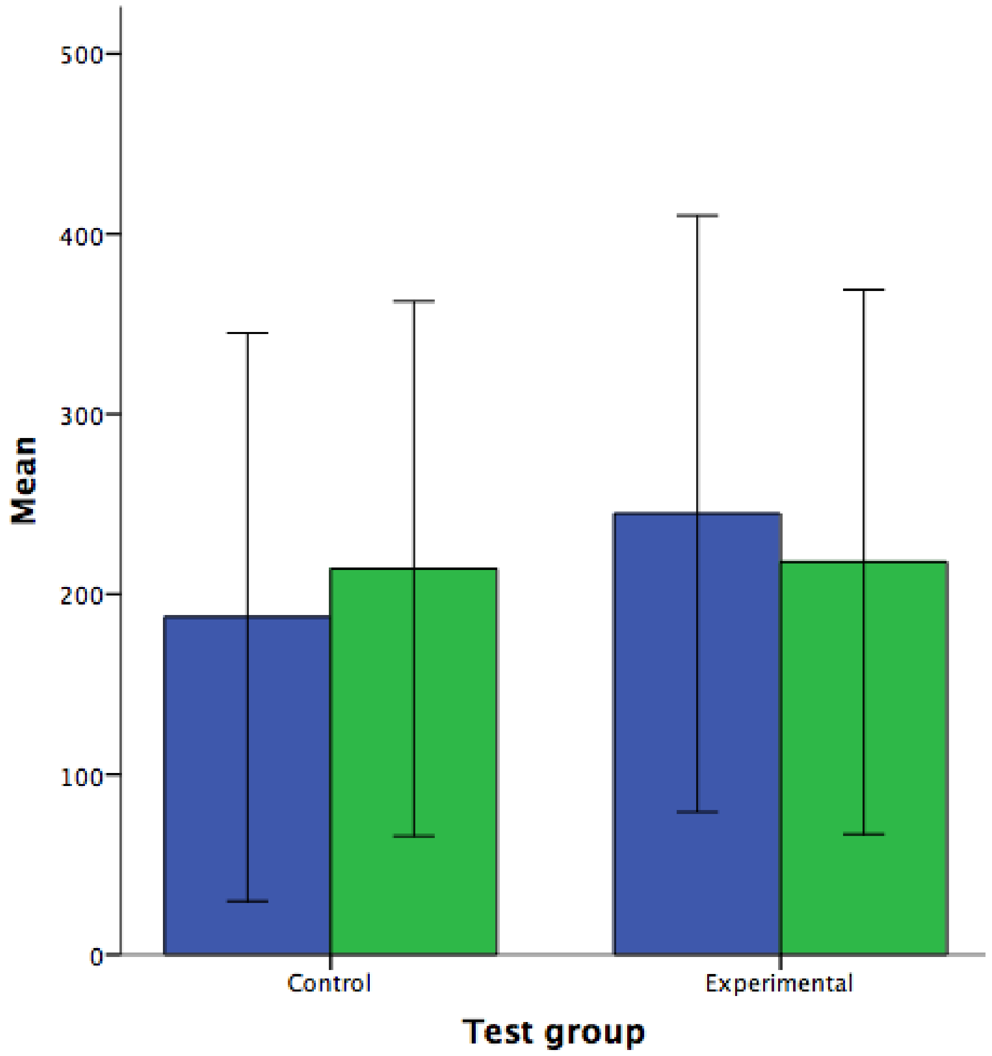

Figure 5. Bar chart depicted from table 4 representing the mean activity count (±1 SD) of experimental dominant males (n=8) and dominant control males (n=8) during both day (blue) and night (green).

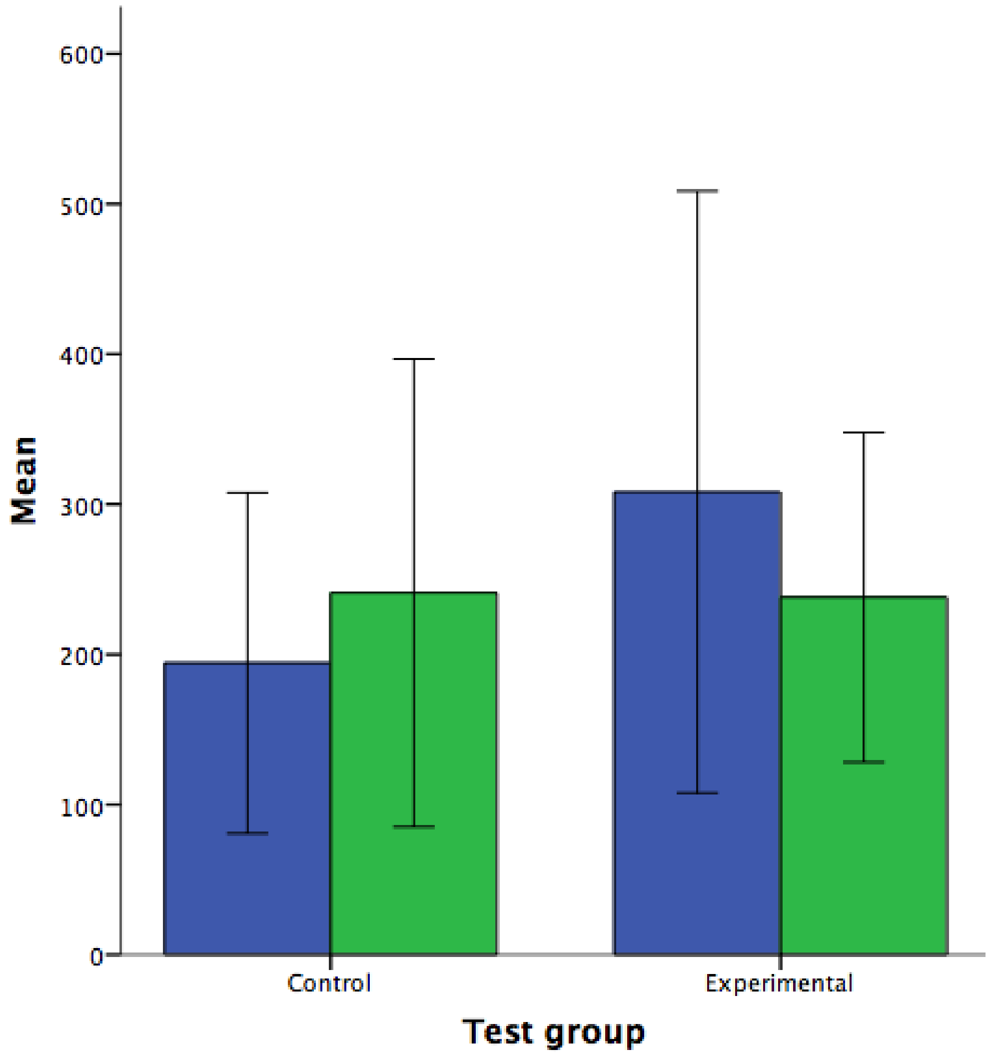

Figure 6. Bar chart depicted from table 4 representing the mean activity count (±1 SD) of experimental subordinate males (n=8) and subordinate control males (n=8) during both night (green) and daylight hours (blue).

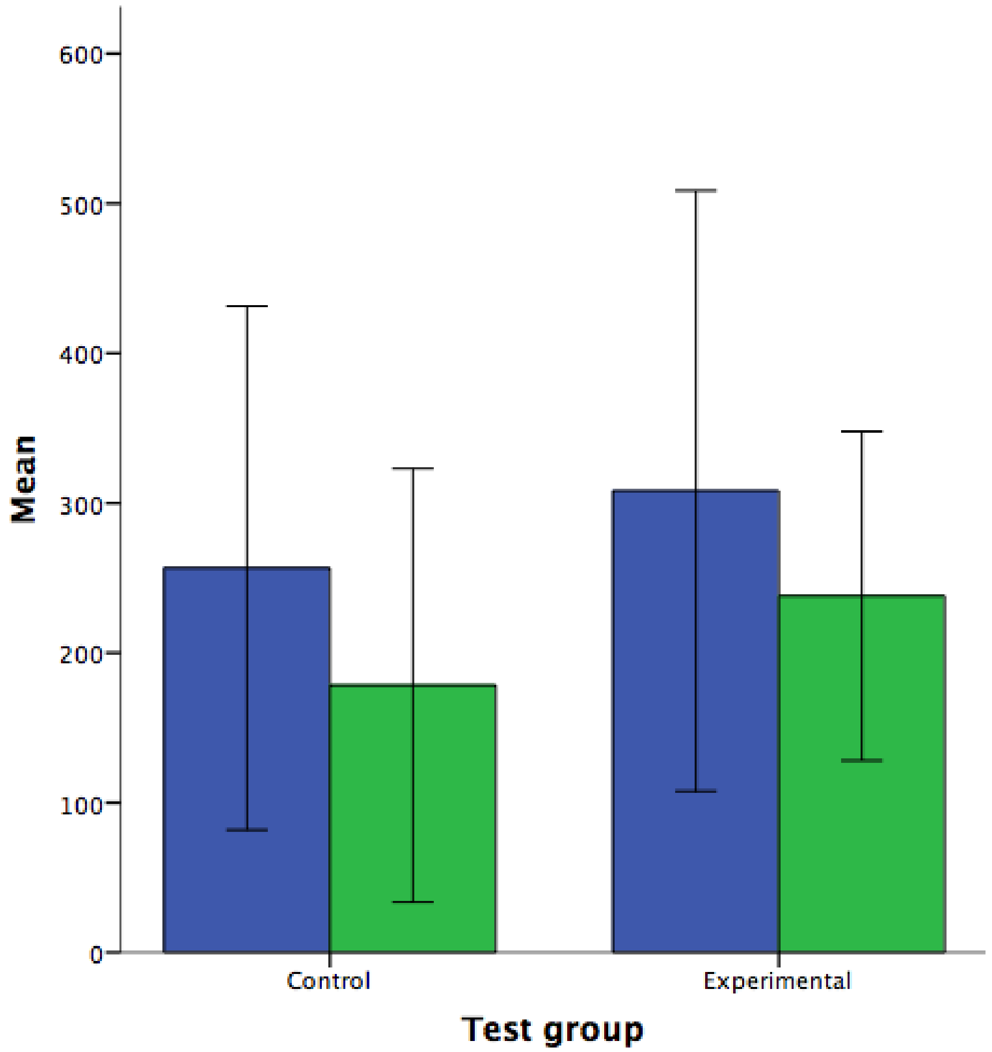

Figure 7. Bar chart depicted from table 4 representing the mean activity count (±1 SD) of experimental subordinate males (n=8) and the second subordinate control males (n=8) during both night (green) and daylight hours (blue).

3.3.2 Day and night activity count comparison

The results from the paired *t*-tests are presented in table 5. The p-values from all pairs were not below the significance threshold of P = 0.05. Thus individuals maintain the same activity levels throughout day and night hours. Figure 8, 9 and 10 visually represents the mean activity count data per hour for the dominant and both subordinate control group males. These control group individuals should not have been affected by courtship and should provide indicative natural activity patterns for *N.* cf. *microcephalus*.

It is clearly shown here there is no significant change in day (12:00 to 00:00) or night time (00:00 to 12:00) activity counts.

Table 5. Descriptive statistics for a paired t-test on day and night activity levels of all individual types in both control and experimental group.

Individual	Group	Time of day	Mean	DF	Std. Deviation	T value	Sig (2-tailed)
Dominant	Experimental	Day	244.75	7	165.58	0.731	0.489
		Night	218	7	151.08		
Subordinate	Experimental	Day	308.13	7	200.44	1.203	0.268
		Night	238	7	109.82		
Female	Experimental	Day	218	7	184.78	0.357	0.732
		Night	198.25	7	138.39		
Dominant	Control	Day	187.38	7	157.68	-0.391	0.708
		Night	214.25	7	148.46		
Subordinate	Control	Day	194.25	7	113.41	-0.849	0.424
		Night	241	7	155.79		
Subordinate 2	Control	Day	256.63	7	174.75	1.187	0.274
		Night	178.38	7	144.70		

Mean activity levels (Mean) values taken from a 24-hour period of day seven of seven recorded activity day and nights.

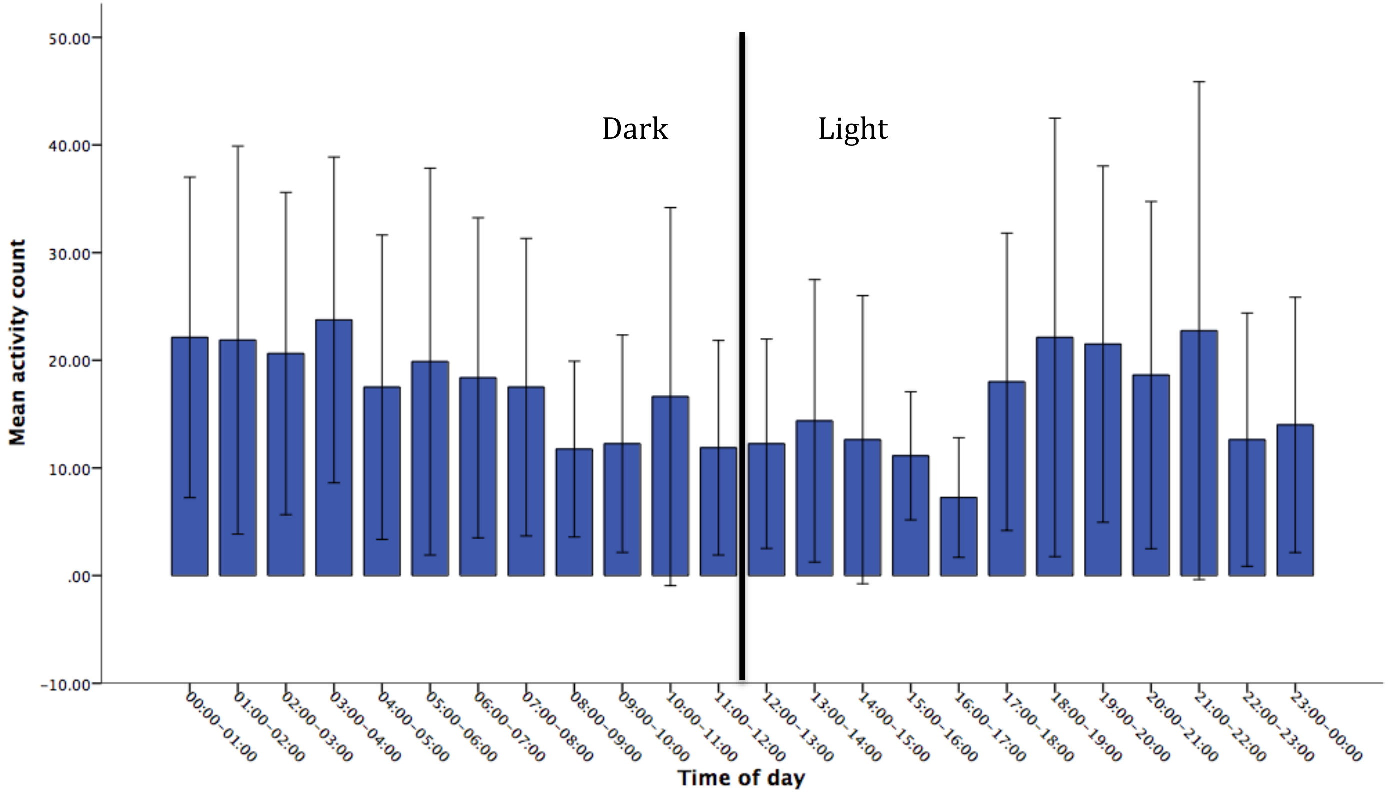

Figure 8. Bar chart displaying mean activity counts per hour of the entire control group of dominant males (n=8) with the error bars displaying 95% confidence. Individuals were exposed to daylight from 12:00 – 00:00, the lights were turned off to imitate night from 00:00 - 12:00

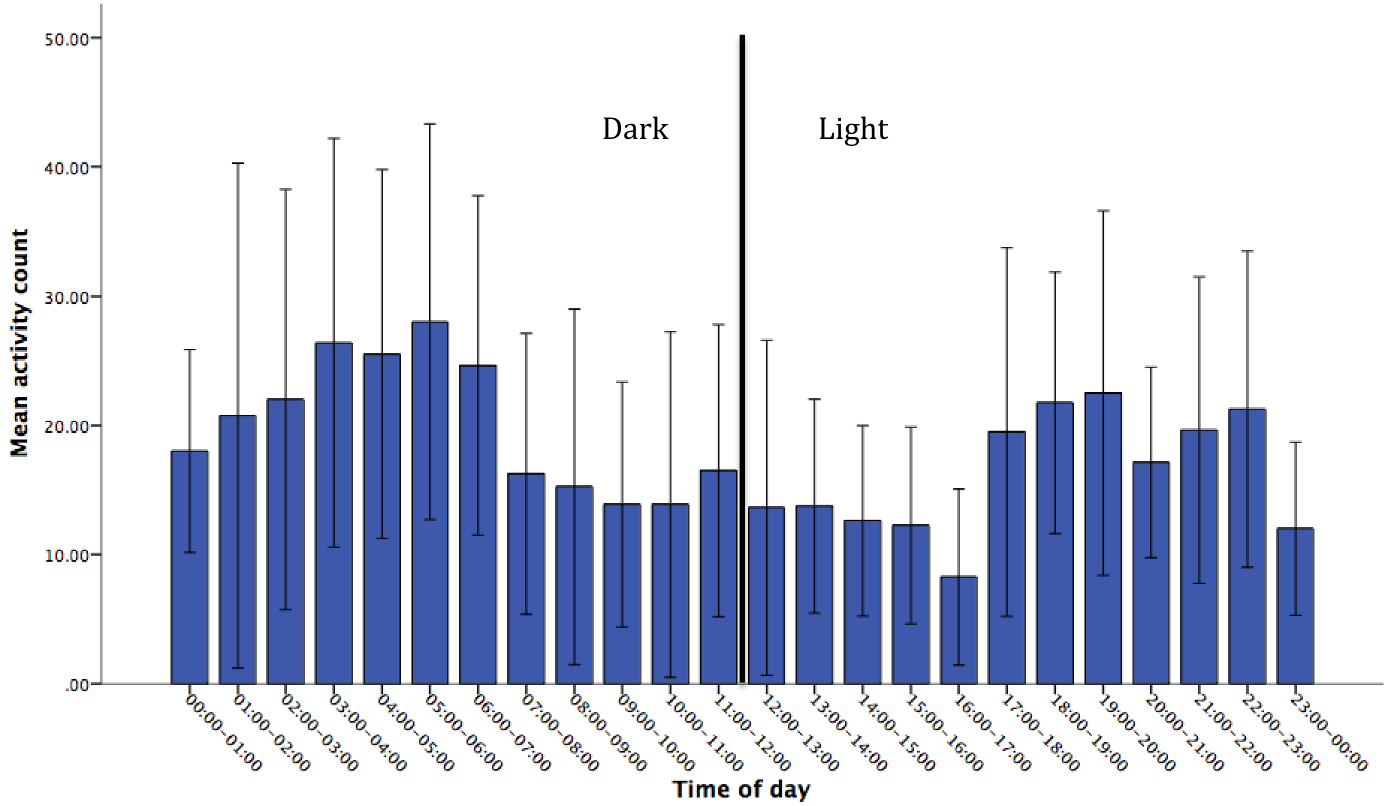

Figure 9. Bar chart displaying mean activity counts per hour of the entire control group's first set of subordinate males (n=8) with the error bars displaying 95% confidence. Individuals were exposed to daylight from 12:00 – 00:00, the lights were turned off to imitate night from 00:00 - 12:00.

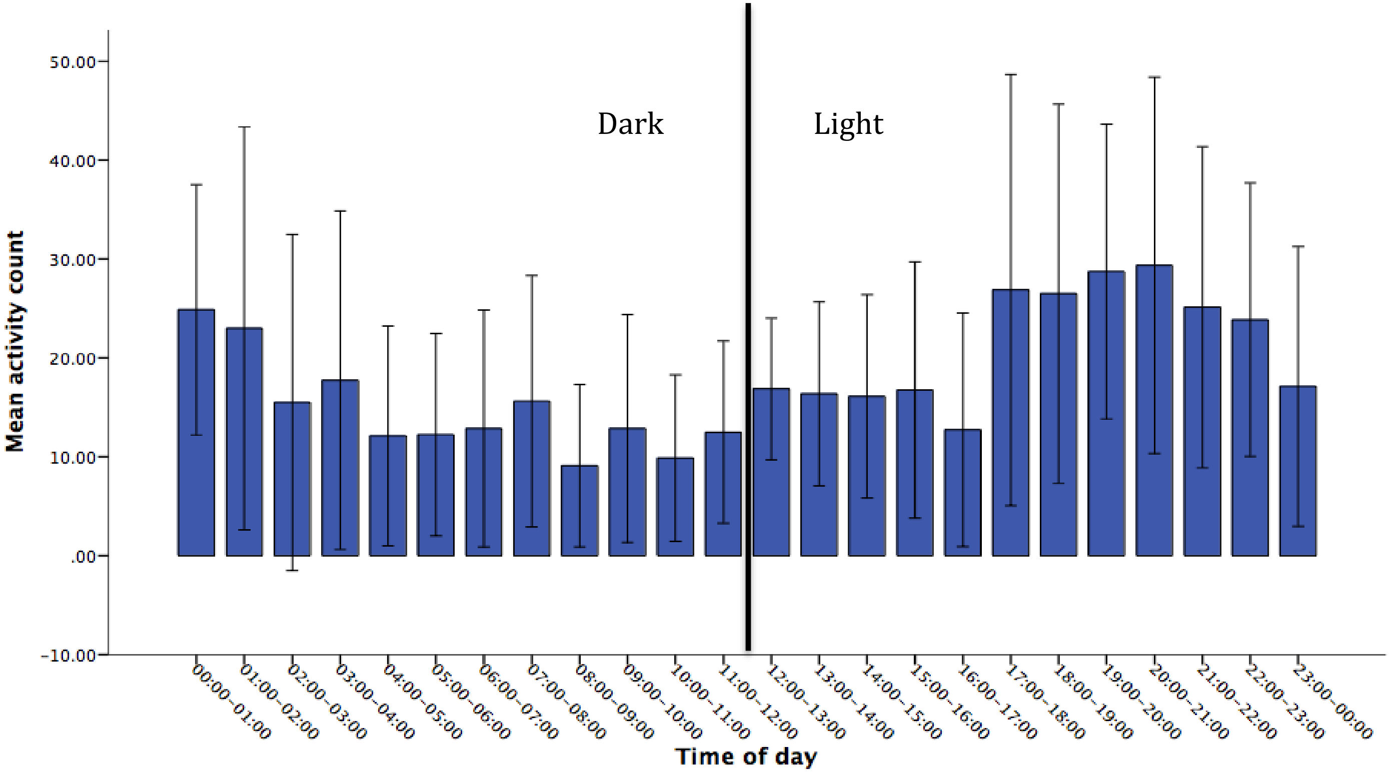

Figure 10. Bar chart displaying mean activity counts per hour of the entire control group's second set of subordinate males (n=8) with the error bars displaying 95% confidence. Individuals were exposed to daylight from 12:00 – 00:00, the lights were turned off to imitate night from 00:00 - 12:00.

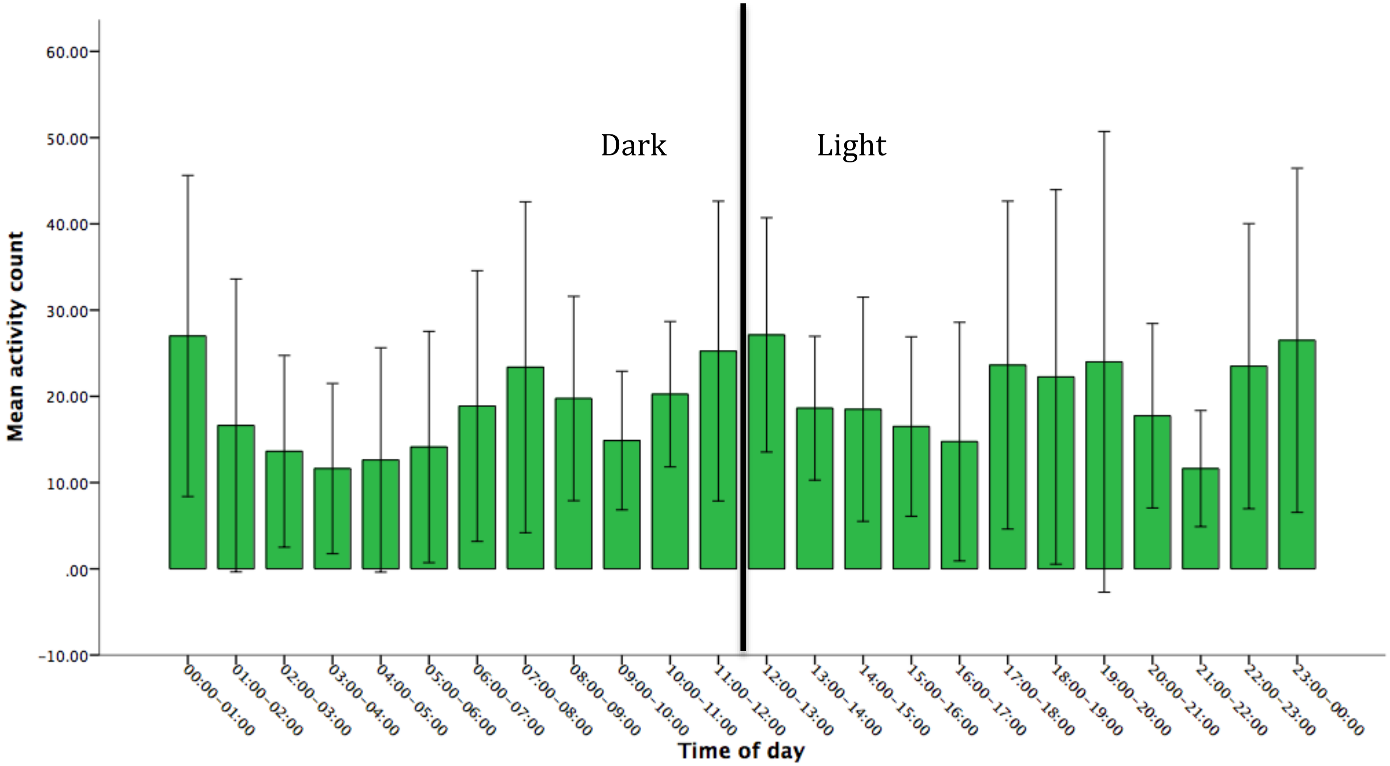

Figure 11. Bar chart displaying mean activity counts per hour of the experimental group's dominant males (n=8) with the error bars displaying 95% confidence. Individuals were exposed to daylight from 12:00 – 00:00, the lights were turned off to imitate night from 00:00 - 12:00.

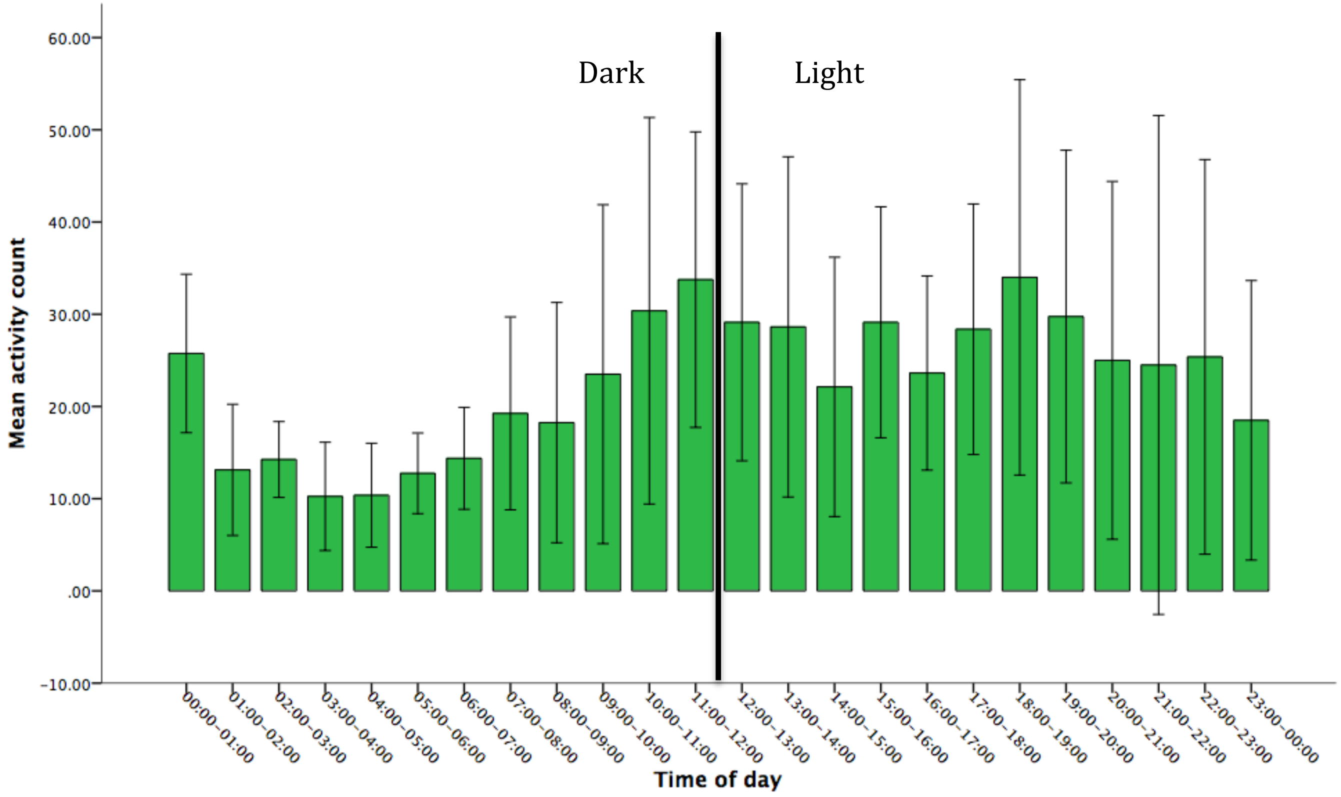

Figure 12. Bar chart displaying mean activity counts per hour of the entire experimental group's subordinate males (n=8) with the error bars displaying 95% confidence. Individuals were exposed to daylight from 12:00 – 00:00, the lights were turned off to imitate night from 00:00 - 12:00.

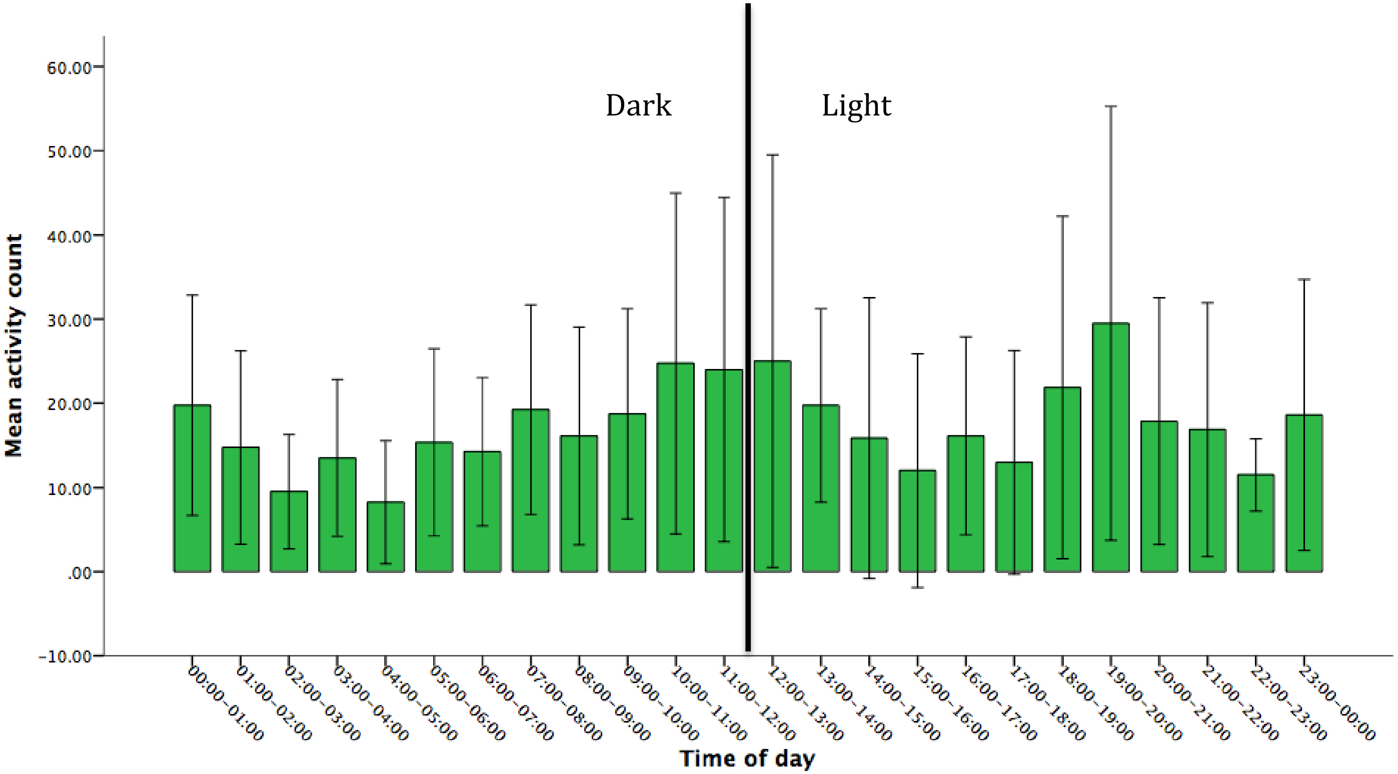

Figure 13. Bar chart displaying mean activity counts per hour of the experimental group's females (n=8) with the error bars displaying 95% confidence. Individuals were exposed to daylight from 12:00 – 00:00, the lights were turned off to imitate night from 00:00 - 12:00.

Table 6. Total duration tagged fish were undetectable by sensors for each 12 hour day and night period.

	Experimental Dominant male		**Experimental Subordinate Male**		**Experimental Female**		**Control Dominant Male**		**Control Subordinate Male**		**Control Subordinate Male 2**	
Week Number	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
3	09:44:16	05:21:30	04:18:09	04:12:54	02:34:12	04:12:09	01:34:18	03:05:45	02:46:05	02:10:16	00:46:43	01:30:00
4	02:56:50	02:03:32	00:00:00	00:21:00	06:34:11	00:46:57	07:03:54	02:31:33	05:28:30	01:16:05	04:10:10	00:39:22
5	04:28:42	09:41:45	01:52:29	04:30:08	02:12:58	09:00:58	00:00:00	00:56:06	01:04:34	03:39:23	03:19:15	06:55:11
6	01:24:36	00:16:57	01:45:43	00:00:00	05:15:25	01:06:27	01:53:14	00:30:16	00:49:49	00:11:00	03:09:16	00:10:38
7	00:23:50	00:31:38	00:30:36	00:30:19	00:27:15	05:52:13	10:13:14	02:29:44	06:27:48	05:05:50	09:50:24	03:44:48
8	00:11:46	00:12:47	04:18:50	00:14:36	02:29:48	00:23:50	00:19:29	01:01:01	04:33:21	05:19:10	00:05:04	05:42:20
9	00:00:00	00:00:00	00:00:00	00:00:00	00:38:34	00:36:27	02:55:07	02:34:43	03:46:50	00:32:57	01:57:34	03:26:17
10	01:59:05	00:00:00	00:00:00	02:42:06	00:00:00	00:28:02	01:58:02	00:30:32	02:59:11	01:52:25	06:08:01	06:37:17

3.4 Discussion

Our hypotheses predicted that *N.* cf. *microcephalus* increase activity levels when males were given the opportunity to court females. We also predicted that *N.* cf. *microcephalus* individuals would have significantly reduced nocturnal activity levels, thus suggesting a diurnal daily activity pattern. Our second prediction was that during control male's periods of reduced activity (during the night as we predicted a diurnal species), the experimental group males with female access would show significantly more activity counts compared to control males as they reduce rest/sleep times in order to court females throughout the night. Our hypotheses and predictions were not supported by our results. *N.* cf. *microcephalus* did not change their activity patterns when given the opportunity to court females thus we conclude that dominant males do not increase nocturnal activity levels in order to court females more. This suggests that the amount of sleep *N.* cf. *microcephalus* males undertook was not reduced due to courting behaviours as activity count was maintained. The activity pattern was also found over a 24-hour period to not significantly differ between light and non-light periods replicating day and night in both the control and experimental group tanks, in fact activity resembled a weak bimodal pattern with activity being high during the middle of the day and the middle of the night in the control fish. This highlights that *N.* cf. *microcephalus* used in this experiment were displaying a weak bimodal activity pattern or even a weak cathemeral (irregular) activity pattern over a 24-hour period with the only similarity between all three groups of males being an increase in activity at 5pm (5hours into the light duration). Diurnal or nocturnal species, unlike species with cathemeral activity patterns, which lack any regular rhythm, have clear rest periods, these periods indicate where sleep behaviours are most likely to occur. Thus future work into determining if *N.*

cf. *microcephalus* do sleep should consider that sleep is likely to occur throughout the day and night and are not concentrated to specific times or light periods.

For the first prediction we tested for *N.* cf. *microcephalus* activity rates in comparison to the time of day, comparing day to night periods of activity. However our results did not support our prediction of a diurnal activity pattern in *N.* cf. *microcephalus* as results did not display significant changes in activity levels from day to night. Interestingly all 6 groups of fish displayed a cathemeral/polyphasic activity pattern, meaning that individuals did not seem to have long periods of highly active or rest like behaviours over an entire 24 hour period. Individuals instead had slight fluctuations in activity count, which perhaps resembles an increase and decrease in rest or potentially sleep, seemingly regardless of daytime. However we can see from analysis of figures 8 – 13 that individuals did peak in activity during the middle of the night and the middle of the day suggesting a weak bimodal activity pattern dissimilar to the common diurnal and nocturnal activity patterns of cichlids. This is unexpected, as we believed that individuals would reduce their activity levels during the night in order to sleep, yet their movement remains in a cathemeral activity pattern.

One of our predictions was that male *N.* cf. *microcephalus* would increase their activity levels during the day and night when in the presence of a female, compared to having no female presence. This is due to males having the opportunity to express courtship behaviours and assert dominance through male-male competition when accompanied in their tanks by a female. Our results did not display significant changes between the activity levels of males when comparing the experimental group to the control group in regards to changes in light periods. This suggests that behaviours associated with courtship in *N.* cf. *microcephalus* such as sharp figure of eight movements by males were not conducted during the night. These figure of eight movements are repeated multiple times and would most certainly be distinct enough to trigger more than a single sensor (pers. obs) and result in clear activity spikes compared with non-courting males. Control group males who had no females to court and therefore should not display courtship behaviours, had relatively similar nocturnal activity levels to that of males who did have access to females. Interestingly experimental subordinate males seemed to be more active than their control counterparts around the light change period, resembling morning (dark – light changeover). Perhaps the subordinate increase was due to dominant males chasing subordinates as males often do when trying to court females (pers. obs.). However without visually observing these night/day time activities we can not rule out that perhaps males within the experimental group reduce behaviours associated with normal nocturnal activity that the control males display, and in turn increase courtship like behaviours. Nor can we confirm whether the activity peak around 5pm for all groups was due to courtship behaviours without visual/video analysis of displayed behaviours.

Our results overall delved into the potential relationship between breeding and non-breeding activity levels in *N.* cf. *microcephalus*. We expected to see changes between these two environments due to males very active courtship behaviours resulting in much higher activity counts especially during the day when courtship has been previously observed in chapter 2, but our results have shown no difference between the experimental and control groups. Our results displayed cathemeral activity levels in both our experimental and control groups, which in turn make us question our results of activity patterns from this experiment as expressed circadian rhythmicity. Literature is replete with observations of fish over 24-hours and cathemeral activity patterns during breeding season is not rare but very few species have a relatively constant activity level with irregular rest periods during non-breeding conditions. However little research has been conducted on activity levels during breeding or parental care phases of fish and even less so on cichlids (Reebs 2002).

Although a cathemeral activity pattern is relatively rare in fish it is displayed by some fish species suggesting that our results displaying *N.* cf. *microcephalus* cathemeral activity pattern is possible. Although cichlids are usually recognised as being diurnal fish (Reebs 2002) there are some cases of cichlids being active throughout the night as well as during the day (Reebs and Colgan 1991). An example of species of cichlid being active throughout the night is that of the female Convict cichlid *Cichlasoma nigrofasciatum* and the female Rainbow cichlid *Herotilapia multispinosa,* who both display a cathemeral activity pattern as both remain active at night during parental care phases in order to fan their eggs (Lavery and Reebs 1994; Reebs & Colgan 1991). As suggested by Ali (1992) fish activity patterns are plastic, these two usually diurnal rainbow and convict cichlids can override their diurnal circadian rhythms that regulate activity levels and potentially reduce sleep durations during parental care phases at night. This is due to females increasing their activity levels at night to ensure the survival of their young through nocturnal maintenance of their eggs. Therefore these females are likely to reduce the amount of time they sleep during the night, as less time is available to sleep, with no known deleterious effects of such a reduction in sleep (Lavery and Reebs 1994; Reebs & Colgan 1991). Little research has been conducted on cichlid activity levels during breeding season and the question remains if other cichlids have the ability to increase activity levels during periods when sleep normally occurs during breeding season. Reebs (1994) also observed nocturnal mate recognition and brood defence behaviours throughout the night in female convict cichlids. Reebs (1994) found that fights would occur between females and some of the largest intruding males during the night, with most males chased away by the protecting females without the need for fighting. This suggests that females stay alert throughout the night to the presence of intruding males. It is therefore likely that females need to be alert to stimuli of intruding males, thus it is more likely that females reduce sleep times as being in a sleep like state increases the threshold needed for stimuli to awaken the individual, which would be disadvantageous to females needing to chase males away during the night. If these behaviours were apparent during the night in our species, females chasing males, or even perhaps nocturnal male-male competition, then these behaviours could be the cause of our observed high levels of night-time activity readings. We would however expect to see a difference between the individuals in the differing groups which present the opportunity for males to court females or not. Our results however do not fit this prediction as the control and experimental groups both displayed similar cathemeral activity patterns, although activity patterns seemed to differ slightly between experimental and control groups during the light change resembling morning. Perhaps males reduce their sleep durations and became more active earlier in the day in order to court females earlier. Nevertheless we would expect activity levels to still be significantly higher in experimental males during the day compared to control males if this was true. Plenty more data analysis needs to be conducted in order to break down *N.* cf. *microcephalus* activity patterns into dawn and dusk activity differences between control and experimental groups. Due to the lack of significant changes in activity patterns these plausible reasons as to why we have found unexpected high nocturnal activity counts from our research are unproven and there may be another reason as to why our results differ from our hypotheses.

During cichlid breeding season shifts from long periods of activity and quiescence to a relatively constant level of activity with shorter rest periods over the entirety of the day is much more common than a complete shift from diurnal to nocturnal or visa versa (Reebs 2002). It would therefore be perfectly reasonable for us to assume that *N.* cf. *microcephalus* within our experiment can potentially change to a cathemeral activity pattern when given the opportunity to court females, as males could have increased nocturnal activity levels through male-male competition and courtship displays over the presence of the single female. However our control group males which do not have access to females, thus courtship behaviours should not be apparent, also display cathemeral activity patterns like that of the experimental group. This result is not explainable if the experimental group activity pattern is a result of night courtship and male-male completion increasing nocturnal activity counts, as there is no female for males to court in the control group. This therefore questions the validity of our results in regards to a comparison of breeding and non-breeding activity levels as activity levels did not differ between groups suggesting that either courtship behaviours did not increase individuals activity counts, or that breeding behaviours were not expressed by individuals. There have however been observations of cichlids that slowly move "promenade" around aquaria tanks during the night in what appears to be quiescent activity (Reebs 2002). This could be an indication that cichlids do not need to stop moving in order to sleep and thus during the night activity levels are still relatively constant due to this slow movement. This could be evident in our experiment as a patrol-like movement around our fairly small tanks could result in all three sensors recording movement in a relatively short duration, thus giving us a superficial reading of active movement and wakefulness during the night. This promenading behaviour could potentially cause an increased activity level in control individuals as they rest whilst experimental males may be performing courtship behaviours at the same time. Both courtship and promenade behaviours would result in lots of sensor readings in a short time period therefore we would not be able to distinguish between from our recording sensors. A visual recording of fish would need to accompany the sensory readings in our experimental design in order to discriminate between courtship and promenades or at least the pattern of sensory recordings associated with a promenade pattern and courtship behaviour.

3.4.1 Potential problems with the experimental design

We expected the experimental and control group activity levels to significantly differ from one another due to courtship behaviours being apparent in the experimental group. We did not anticipate cathemeral activity levels over 24-hour daily cycles. It is possible that this species of cichlid expresses a cathemeral activity pattern. Short personal observations seen in the holding tanks on a daily morning basis (night period for cichlids) as individuals displayed quiescence activity during another experiment (chapter 2) may be misleading in suggesting that individuals are in sleep or rest like states during the night. Our results showed that when lights are turned on for the first time of the day (during observed cichlid quiescence behaviour), after the fishes night period, a phenomenon called 'light shock' occurs. This is a species specific effect in which fish are exposed to bright lights after a long period of darkness and this has been rarely reported. It is poorly understood as to why this sudden light exposure causes some species to sink to the bottom of their tanks. It could be an indicator for sleep but we cannot conclude upon this as no research has confirmed what causes light shock responses. This could in turn effect fishes behaviours, further studies should consider using a gradual light increase in order to prevent this phenomenon. Figures 8 -13 display this constant activity level in control males over periods of 24-hours, 12-hours daylight and 12-hours night, with no light present. Alongside this potential behavioural disruption from lighting our equipment may have not accurately recorded the positions of tagged individuals as reliably as we had expected. It could also be possible that the fish did not habituate to their tank after the transition into the experimental and control tanks perhaps being in a smaller tank with fewer individuals during experiments compared to the larger holding tank causes the fish to behave differently. This is certainly the case when fish are isolated individually as we found during previous pilot studies on *N.* cf. *microcephalus*. If fish did not acclimatise to their new environment once in the experimental or control tank erratic behaviours could occur causing rest periods to be suppressed by heightened activity due to restlessness. However erratic behaviours were not observed in my previous work in chapter 2 when individuals were moved from holding tanks to smaller sections of a larger tank for observations. The noise and light levels in the experimental and control tanks were alike to that of the rooms of the holding tanks of stock *N.* cf. *microcephalus*, thus noise and light levels are unlikely to have disrupted rest or sleep periods of individuals however these factors were not measured during my research.

There is no literature describing the typical activity levels of *N.* cf. *microcephalus* in either breeding season, non-breeding season or their daily wake-rest activity pattern. Therefore we cannot fully understand whether a lack in difference between the experimental and control groups during the night is potentially due to raised nocturnal control group activity through nocturnal promenades through the tank or decreased nocturnal experimental group activity due to a lack in courtship behaviours. The activity levels could superficially be raised during night periods in both groups not only through quiescence behavioural promenades around the tank but also inaccurate recordings of fish tags. Inaccuracies could have occurred as fish may be recorded as moving actively from one sensor to another when the fish were in a rest-like state with little movement being made between two sensors range boundaries. The activity levels could also be misleading as data have shown fish to go 'missing' in the tank in which fish were not recorded by any sensors within the tank for considerable amounts of time (see table 6), with one fish lacking sensor recordings for 10 hours during the 12 hour day time recording period. This period comprised of many 10-50 minute periods where the fish was not recorded by any of the tank sensors. Fish are likely to have found blank spots within the tank and could have rested in such areas in which none of the sensors could read tags from. Therefore recorded data could be missing potentially large periods of sleep-like behaviours of individuals. It is more likely that resting behaviours would have taken place in any potential blank spots as it is unlikely that courtship behaviours or male-male competition could have occurred in such blank spots. This is because such courtship or aggressive interactions use large areas to display such behaviours. Therefore very large blanks spots would be needed to mask such courting or aggressive behaviours.

The precision of the sensors used could have caused inaccuracies in the data output by the SPD log tag reader. The sensors used were continuous pass-by sensors and the problem with using pass-by sensors is that fish could potentially be moving above the same sensor. This movement above the same sensor would output datum displaying continuous readings from the same sensor. A continuous recording of a fish from the same sensor would suggest that the fish is resting or in a state of quiescence rather than the fish actively moving in a small area above the sensor. Due to the size of these sensors each sensor could read only one third of the tank thus we could not record any fine movements or specific movements in certain directions. These sensors were large enough to cover most of a tank wall yet there seemed to be blank spots within the tank as our results displayed individuals not appearing on any of the sensor readings within the tank. In order for future research to resolve these issues in regards to sensor readings a smaller sensor could be used, or perhaps a different type of sensor could be more suitable for this experiment. A cord antenna system could be used to record tagged fish movements in this experimental environment or the use of smaller sensors could be used to help increase the accuracy of recordings when using tag reads to determine an individual's movement around the tank. A cord antenna system capable of reading passive integrated transponder (PIT) tags would be ideal for this experimental design. This system would detect tagged fish within the tank by creating a loop of cable (antenna) around an area of the fish tank. When a tagged fish then passes through this loop a reading is generated. By creating several loops around the tank you could determine fish movement from one loop to another which depending on how many loops you create around the tank could be much more precise in recording the movement of fish around the tank than that of larger circular pass-by sensors currently used in this experiment. The potential problems with using this this cord antenna system would be that these sensors would only be able to read tagged fish movements in either a horizontal or vertical plane depending on how the sensor is looped around the tank. However courtship behaviours are lunges and figure of eight movements conducted on a predominantly horizontal plane so this restriction in movement detection would be unlikely to produce inaccurate results in regards to individuals' movements around the tank. However by using smaller sensors finer movements around the tank could be recorded. We could discriminate between slow movements around the tank, such as the potential night time promenades that cichlids may undergo during quiescence behaviour (Reebs 2002) and large lunges or courtship dances which cover large areas of the tank in very short periods of time (pers. obs). This altogether would help reduce issues with the recording of tagged fish movements where fish were perhaps being read multiple times by the same sensor resulting in potentially misleading results of little or no activity when movement is occurring.

Courtship has been displayed by *N.* cf. *microcephalus* in the aquaria at the university of Hull and fry have subsequently hatched in tanks relatively similar to those in our experiment, yet our experimental group males potentially may not have performed any courtship behaviours at all during the observational seven-day period. If this was the case that males did not display courtship behaviours then it would resolve our questions as to why both the control and experimental groups in our experiment have relatively no change in activity levels compared to each other. We did observe in the experimental, control, and holding tanks that males 'colour up' to display dominance, with this colour becoming more vivid during exposure to females, however it perhaps does not necessarily mean courtship behaviours will ensue. A seven-day period in previous experiment (chapter 2) had been enough time for males to display courtship behaviours (pers. obs) and thus this length of time is not an issue in regards to a potential lack of courtship behaviour. Perhaps the presence of a courtship-breeding site (bower) that was present in my experiment from chapter 2 acts as a catalyst for male courtship with behaviours being apparent after a single day in their new environment. In their natural environment male *N.* cf. *microcephalus* move sediment into large piles called bowers upon the lake bed (McKaye *et al.* 1990). These sand bowers have been confirmed in many papers to be used as a sexually selective trait for females to assess males upon (Genner *et al.* 2008; McKaye *et al.* 1990; Stauffer *et al.* 1995; Young *et al.* 2009, 2010). Bowers vary in size and shape and both of these dimensions are unique to each bower building cichlid found in lake Malawi, of which *N*. cf. *microcephalus'* are indigenous to. Our holding tanks of *N.* cf. *microcephalus* did not contain bowers yet individuals did still spawn, but the amount of time taken for males to start courtship behaviours in this environment is unknown. It is perhaps this courtship site (bower) that is vital to initiating courtship behaviours within a short time frame such as our experimental seven-day period. The previous experiment in chapter 2 displayed courtship behaviours from the first day of observations. Therefore the absence of bowers in our experimental group may have resulted in a lack of displayed courtship behaviours and would therefore have an activity level similar to that of the control group. This similarity in activity levels was found in our results with no significant difference between activity levels between the control and experimental groups. A potential problem for future research into the activity levels of bower building cichlids using a similar tank set up in order to record cichlid movements would be that placing a bower within the tank to initiate courtship would be problematic. A pass-by sensor could not be used as these sensors need to be placed on the floor of the tank, a bower would then have to be place on top of the sensor likely blocking the sensor from reading of tagged fish (see figure 6 for sensor placement).

3.4.2 Activity patterns and sleep of Fish

As mentioned in section 3.1 many activity patterns displayed by fish do not simply fit into one of the standard four activity categories (diurnal, nocturnal, crepuscular, cathemeral) as variations in activity is much more frequent in fish throughout the year than mammals or birds (Helfman 1986; Zhdanova & Reebs 2005). Sánchez-Vázquez *et al*. (1996) tested for dual phasing of fish, in which some species have expressed more than one type of activity pattern at certain times, on a seasonal basis, depending on water temperature (Fraser *et al.* 1993) photoperiod (Muller 1978) and light intensity (Eriksson 1978). It was discovered in goldfish, *Carassius auratus*, that some individuals become nocturnal and others diurnal when given a self-feeding mechanism ruling out food availability determining their activity patterns. This suggests that this species have a remarkable flexibility in their activity patterns and that circadian rhythmicity did not dictate when these fish fed or not. It has also been demonstrated in a number of experiments of multiple fish species that feeding at specific times of the day can result in significant activity pattern shifts. Here activity patterns were based around feed times with the individual becoming more active during the times when food was presented (Chen *et al.* 2002; Gee *et al.* 1994; Lague & Reebs 2000; Sánchez-Vázquez *et al.* 1997; Sánchez-Vázquez, *et al.* 2001; Spieler *et al.* 1978a, 1978b). Igo & Tabata (1996) observed multiple sleep patterns in the goldfish *Carassius auratus,* of which 10% of individuals were shown to display cathemeral sleep patterns and McCauley *et al*. (2012) has noted 26 cathemeral species of fish with activity being observed during both the day and night suggesting cathemeral activity patterns in fish are not uncommon. Overall the activity patterns of fish could potentially change very easily and perhaps the quiescence morning behaviours seen in holding tanks of *N.* cf. *microcephalus* (with perhaps diurnal activity patterns) suggesting this species displays quiescence behaviour during the night may in fact be misleading. Our results suggested that this quiescence nocturnal activity is not expressed in the experimental and control groups who have no reduction in activity levels at the same time these holding males were observed displaying brief reduced activity levels.

N. cf. *microcephalus* could potentially be a cathemeral species and thus have no clear periods where rest or sleep takes place, the species could also have a bimodal activity pattern, that is species specific, which emerged in our results with slightly increased activity during the middle of the day and night. This activity would create a pattern opposite to that of a crepuscular animal, perhaps some predators of *N.* cf. *microcephalus* hunt during dawn and dusk and these cichlids rest during these periods to reduce detection. *N.* cf. *microcephalus* could also have very short sleep bouts like that of *Danio rerio* of which sleep periods can be as short as 5 seconds (Zhdanova *et al*. 2008). If this were the case that *N.* cf. *microcephalus* had such short sleep durations then our equipment would not be able to decipher between sleep and a period of little movement. Sensors were large enough for fish to move around on without passing into another sensors range, thus detecting a fish on the same sensor for more than 5 seconds does not mean that the our fish was inactive or in a period of quiescence. With no previous studies on *N.* cf. *microcephalus* activity patterns we cannot assume any particular activity pattern and *N.* cf. *microcephalus* could potentially have any of the types of activity patterns found in animals, but our research indicates a cathemeral activity pattern could be present.

3.5 Conclusions and future work

Although there are potential problems with our experimental equipment our results indicate that *N.* cf. *microcephalus* seem to have a cathemeral activity pattern and that the opportunity to court females has no effect upon male activity patterns during the day or night. Unfortunately individuals were seen to 'disappear' from monitor readings for significant periods of time throughout testing and the only conclusion that can be drawn as to why this happened is that individuals found 'blank spots' within the tank in which monitors could not read the tagged fish. What the fish were doing during their time in blank spots is not known. They could have been potentially sleeping, as these blank spots in the tank are likely to be in the corners of the tank where the sensors could not reach. It is however very unlikely that courtship behaviour could occur in such small spaces. Only periods of quiescence behaviour or that active swimming into the tank wall could occur, which has been seen during feeding times (pers. obs), thus we cannot conclusively say that 'missing' fish were active or inactive. Possibly dominant males did not express courtship behaviour when in the presence of a female, perhaps a lack of cues, such as a bower, contributed to a lack of courtship behaviours and that the time needed for courtship to be displayed, with a lack of a bower, was longer than the seven day observation period for this experiment.

In order for future research to take place it would be very beneficial for a study to confirm our suggestion that *N.* cf. *microcephalus* have cathemeral activity patterns, when not courting. This cathemeral activity pattern was unexpected since these cichlids have been suggested to be predominantly active during the day (Young *et al*. 2009, 2010; Magalhaes *et al.* 2013; Martin 2010), this would provide a good benchmark for future work into the effects courtship has on activity levels of dominant males in this species. Once the activity pattern of *N.* cf. *microcephalus* is confirmed whether that is cathemeral or diurnal then a potential avenue into sleep research in *N.* cf. *microcephalus* should be considered through the use of behavioural observations to determine if *N.* cf. *microcephalus* do indeed sleep. If our results of cathemeral activity was falsely produced from faulty equipment then determining sleep periods of a diurnal species would likely be easier than assessing sleep behaviours of a potentially cathemeral polyphasic species as determining periods when fish are most likely to be in sleep like states will be easier. Future research into activity of this species could result in the discovery of definite sleep periods in another species of fish adding to the very small database of sleep confirmed in fish solely consisting of zebrafish, *Danio rerio.*

Chapter 4: Discussion

This thesis investigated behavioural trade-offs of the species *N.* cf. *microcephalus* in regards to the plasticity of their behaviours when building bowers & their aggressive behaviours as well as when courting females in regards to their sleep behaviour. A previous study by Magalhaes *et al*, (2013) suggests that negative frequency dependant selection could be facilitating the invasion and maintenance of novel bower shapes and sizes in populations of cichlids, which in turn could be a stepping stone to speciation through sexual selection if female preferences link to these novel bowers. However males would need to see a benefit from novel bowers and it was suggested that males received fewer aggressive interactions from neighbouring males when holding novel bowers, however this could have been due to males initiating fewer aggressive interactions. This however would suggest that males change their behaviours when given differing bowers, we therefore tested for this by changing bower size and expected male behaviours not to be a plastic response to changes in bowers. A study by Lesku *et al*. (2012) prompted questions into the behavioural plasticity of sleep, and in particular changes in sleep durations when males are courting females. Lesku found that Pectoral sandpipers have reduced sleep durations during breeding season, males would trade sleep for courtship behaviours. We tested for this in our population of *N.* cf. *microcephalus* in regards to activity patterns when males are given the opportunity to court females.

Both the studies in this thesis indicated that these fish displayed a lack of behavioural plasticity in the behaviours we examined. We found no signs of any trade-offs between sleep and courtship behaviours, or between reproductive and aggressive behaviours. When males' bower sizes where changed experimentally, we observed no trade-offs between the observed aggressive and courtship related behaviours however increases in bower building behaviours were displayed. This increase in bower building behaviour did not constrict the frequency of any other observed behaviour, therefore there was no observed behavioural trade-off. The importance of these findings is recognised when combined with Magalhaes *et al's.* (2013) research with both studies complementing one another in regards to indicating that negative frequency dependant intrasexual selection could be maintaining bower polymorphism in cichlid species, and thus enabling speciation. In chapter 2 male *N.* cf*. microcephalus* did not reduce their rest periods when given the opportunity to express courtship behaviours. Males did not trade any possible sleep related benefits (currently unknown in fish) in order to increase courtship behaviours and gain the expected advantage of having more successful mating interactions.

Both chapters allude to *N.* cf. *microcephalus* having strict non-plastic behaviours. Chapter 2 discusses the importance of non-plastic behaviours in which any behavioural plasticity would undermine the possibility of negative frequency dependant selection being a possible mechanism for cichlid speciation. However our results do suggest that males can change their bower building behaviour depending on changes in their displayed external phenotype. Here males were found to quickly respond to negative changes in their bower size by drastically increasing their bower building behaviours but only when previously holding a large bower. This could be an evolutionary trait as cichlid bowers are sometimes destroyed or damaged from snail tracks and heterospecific species burrowing in the sand for food (Domino Joyce, personal correspondence). Bowers are produced at shallow depths due to increased clarity of water and therefore could be prone to further destruction by lake currents, drought or flooding as well as anthropogenic effects such as wakes from boats. Therefore cichlids may have developed a response mechanism in which they assess their current bower size against previous sizes and rapidly respond in order to rebuild their bowers. These results compliment McKaye *et al.* (1990) & Schaedelin and Taborsky's (2006) research that when male bowers were completely destroyed they be rapidly rebuilt. However this contradicts the theory that bowers are honest indicators of male fitness. Perhaps males assess neighbouring bower sizes and prevent males from re-building bowers higher than pre-destruction by increasing their aggressive interactions. This suggestion is supported by McKaye *et al's.* (1990) findings that males of taller bowers receive more aggression from neighbouring males.

Although our work suggests that males increase their bower building behaviours when their bowers are decreased in size, it is widely thought that increases in behavioural frequencies or male traits always come at a cost. However this cost towards male cichlids is unknown. We found neither changes in reproductive or courtship behaviours, nor changes in interactions with other conspecific males when bower-building behaviours increased. Future research could address whether increases in bower building behaviours come at the expense of sleep durations instead of direct courtship behaviours as we hypothesised in chapter 3. Sleep is a largely exclusive state that prevents an animals from performing many other behaviours, therefore sleep may be in conflict with other behaviours (Lima *et al.* 2005). It is for this reason that Roth *et al.* (2010) considered sleep to be a possible fitness trade-off.

We initially set out to test whether sleep was affected by courtship behaviours in fish that were given territories and access to females, compared with those that weren't. However we lacked any literature confirming the activity patterns of *N.* cf. *microcephalus* apart from a general consensus that research had been conducted during the day on these fish in which they displayed active wakefulness behaviours (Young *et al*. 2009, 2010; Magalhaes *et al.* 2013; Martin 2010). From our analysis of activity patterns we can suggest that it could be possible for *N.* cf. *microcephalus* to have cathemeral activity patterns with short polyphasic sleep periods. If true rest periods would frequently occur during periods when bower-building behaviours would also occur. If we expect bower building behaviours to take place purely during the day then bower building and sleep could be involved in a behavioural trade-off.

Before our study the behavioural plasticity of *N.* cf. *microcephalus* was undetermined. It was unknown how easily individuals could trade behaviours in order to carry out, as perceived, more important behaviours such as aggressive, courtship and resting behaviours. Our results allude to a lack of behavioural trade-offs, which in turn should prompt further research delving into the possibilities of other potential trade-offs between sleep and other behaviours, such as bower building. In particular research should be conducted on the effects of bower building behaviours on rest durations and sleep in *N.* cf. *microcephalus* should be clearly defined and ideally be quantifiable through periods of inactivity, such as that found in *Danio rerio* in which Yokogawa *et al.* (2007) & Zhdanova and Reebs (2006) used a 6 second motionless period as a threshold for sleep behaviours. If a timed duration could be concluded upon for sleep in *N.* cf. *microcephalus* then it would be far easier to further research behavioural trade-offs as sleep durations could be detected during the night when video recording systems cannot accurately identify fish. Our research also supports Magalhaes *et al.* (2013) suggestion that negative frequency dependant selection could be driving cichlid speciation. Overall our research has helped create a better understanding of behavioural plasticity of cichlids and further supports the current drive in research to create a better understanding of cichlid speciation and speciation more general.

5.0 References

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