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Physiological and Biochemical Consequences of Sleep Deprivation

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology.

By

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Under the mentorship of Dr. Johanne Lewis

ABSTRACT

Sleep is a universal phenomenon in vertebrates and lack of sleep has been linked with various abnormal behaviors (Singh et al 2013). Studies have shown that a strong linkage exists between stress and sleep, or lack thereof. In fact, the Better Sleep Council's 2009 survey revealed that 65% of Americans lose sleep due to elevated stress (Wells and Vaughn 2012). Continual (chronic) elevated stress levels have been linked with serious negative health effects. By using sleep deprivation studies, on a simpler animal model than humans it is our aim to investigate the consequences of sleep deprivation at the physiological and biochemical level in a teleost fish. The stress response in teleost fish has many similarities to that of other terrestrial vertebrates, including humans, so as well as being a simpler model the teleost fish also presents itself as a physiologically relevant model organism. In fish, corticosteroid production occurs via the same pathway as terrestrial vertebrates (called the brain-pituitary-adrenal axis). The increased production of blood corticosteroids in response to stress is one of the most evolutionary conserved organismal responses to stress (Aluru and Vijayan 2009). Chronic elevation of corticosteroids have been linked with increased blood sugar levels, elevated appetite, increased weight gain (due to increased storage of fats) as well as impairment of the immune response, digestive system, reproduction and growth (Wendelaar Bonga 1997). The main objective of our study is to determine if sleep deprivation will result in an increase in stress levels of the fish, which can be measured by changes in the circulating levels of cortisol (corticosteroid).

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Introduction

At colleges across the nation, students often sacrifice sleep to studying, social events, etc. Adequate amounts of sleep are essential to a healthy life (Blagrove & Akehurst, 2001). The recommended amount of sleep for a normal human adult is eight hours in a 24-hour period (Tune, 1968). From 2000-2002, The National Sleep Foundation conducted polls that reported the average duration of sleep for Americans was 6.9-7.0 hours per night. Since 1960, the average sleep duration had fallen by 1.5-2.0 hours and today a large percentage of adults are in bed for only 5-6 hours per night (National Sleep Foundation 2002). A study done on 90,666 college-aged students (average age 22.6 years) found that 75.2% of participants felt tired, dragged out or sleepy during the day for at least 1-5 days of the week. In particular, 44.4% of participants felt fatigue for at least 3-5 days a week (American College Health Association [ACHA], 2012). Studies have shown that a strong linkage exists between stress and sleep, or lack thereof. In fact, the Better Sleep Council's 2009 survey revealed that 65% of Americans lose sleep due to elevated stress (Wells and Vaughn 2012). This lack of sleep inevitably causes additional stress to the individual, making it an interesting topic of study. We began this study with the intention of studying the effects of sleep deprivation in vertebrates.

When vertebrates are stressed, a complex signaling pathway is activated amongst neurons and somatic cells. This pathway is the vertebrate stress response, also known as the "fight or flight" response. Corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) are released from the hypothalamus and work in conjunction to respond to stress. CRH stimulates the release of cortisol, the primary

corticosteroid released for fight or flight. Cortisol also raises glucose concentrations in the bloodstream, which aids in the distribution of oxygen and sugar fuel to tissues to ensure survival. Studies show that it is expressed at highest concentrations in the early morning (Randall, 2010). It targets metabolic actions but also plays a role in ion transport and the immune response by preventing cells from losing sodium and increasing the rate the cells lose potassium. This helps the cells keep pH equilibrium even after a stressful situation has occurred. Negatively, T-cells are prevented from recognizing interleukin signals and histamine secretions are blocked (not allowing for inflammation) when cortisol is present. This combination of immune response alteration can put individuals under chronic stress at risk for major health complications.

In addition, AVP stimulates the release of vasopressin and epinephrine to aid in vasoconstriction and redirection of blood flow. This response raises the organism's blood pressure while also supplying more oxygenated blood to the tissues and organs that need it most. An organism needs to be physically prepared to respond to a stressful situation that can help it have unusual amounts of energy and efficiency. This response is highly effective in vertebrate organisms, but is also a highly conserved signaling pathway. Elevated levels of cortisol and related stress hormones in the bloodstream are a clear indication of the organism being in a stressed state (Peter et al, 1978). Overexposure to stress hormones can have serious implications on the organism's internal homeostasis.

The stress response in teleost fish has many similarities to that of other terrestrial vertebrates, including humans, so as well as being a simpler systematic

model the teleost fish also presents itself as a physiologically relevant model organism. In fish, corticosteroid production occurs via the same pathway as terrestrial vertebrates (called the brain-pituitary-adrenal axis). Simply, the production of corticosteroids by the adrenal glands (or interrenal cells in fish) of the kidney is regulated by the hypothalamus and pituitary glands (Wendelaar Bonga 1997). The increased production of blood corticosteroids in response to stress is one of the most evolutionarily conserved organismal responses to stress in vertebrates such as the teleost fish (Alura and Viajayan, 2009).

As previously mentioned, cortisol being expressed at such high levels over a chronic period of time has been shown to lead to many health complications. The fight-or-flight stress response can be activated long-term and subsequent overexposure to cortisol and related stress hormones can disrupt almost all body processes. Individuals under these circumstances are at elevated risk for serious health problems such as: anxiety, depression, heart disease, sleep problems, weight gain and memory loss (Charlesworth et al. 2004). The hippocampus region of the brain contains many cortisol receptors and undergoes atrophy when overexpression of cortisol occurs. Studies have reported that the elderly experiencing increased levels of cortisol have displayed significant memory loss resulting from hippocampus damage. Cortisol has also been shown to block the secretion of corticotripin-releasing hormone, thus ceasing glucocorticoid secretion. Chronic levels of intense stress may alter the feedback balance resulting in failure of feedback inhibition to process and the production of cortisol to occur. Continued elevated stress levels have been linked with serious negative health effects, all of

which have been suggested to be linked with the chronic elevation of stress hormones such as catecholamines (epinephrine and norepinephrine) and glucocorticoids (corticosteroids such as cortisol).

By using sleep deprivation studies on a simpler animal model than humans it is our aim to investigate the consequences of sleep deprivation at the physiological and biochemical levels in a teleost fish. While human or mammal sample would have been ideal, using fish as the model system was the easiest and safest organism available for the purposes of this study. Using human or mammal organisms would have been unethical with the nature of experimentation because of the serious negative health effects that elevated levels of the highly conserved stress hormones have on vertebrates.

Sleep cycles in lower vertebrates and non-vertebrates are assessed on behavioral guidelines (Campbells and Tobler, 1984). For the purposes of this study, sleep cycles in teleost fish will be defined as prolonged periods of immobility ranging from 7 seconds to several minutes. These cycles are typically found to occur at night hours when environmental activity is low and lights are at low levels (Fryer, 1975). Sleeping adult zebrafish will float either horizontally or with head slightly inclined upwards, with slight eye or fin movement. Oftentimes, the fish will have decreasing buoyancy and will begin to float towards the bottom of the tank. Fish are still able to detect even mild stimulation such as electrical impulses, light detection or noise during these sleep cycles (Zhdanova et al., 2001). A study on states of rest in zebrafish that showed they experience the same restorative functions during sleep as humans and mammals (Zhdanova, 2006). Additional

studies on zebrafish showed anxiety-related behavior in response to sleep-waking (Singh et. al., 2013). Extrinsic factors have been found affect the secretion of cortisol in fish bloodstream, including the tank light intensity over long periods of time (Rotllant et al. 2003).

We believe acute photoperiod exposure in teleost fish will result in significantly elevated cortisol levels compared to control photoperiod-exposed fish. In addition we believe chronic photoperiod exposure will lead to acclimation of photoperiod over time, resulting in cortisol levels similar to control photoperiod-exposed fish. For each photoperiod exposure (acute and chronic), a trial with a control photoperiod tank and an experimental photoperiod tank will be run separately. Blood plasma will be sampled within the hour after the trial period has run.

Methods

Aquarium Setup and Fish Husbandry:

This experiment required extensive initial setup before any live fish could be introduced to the tank systems. Two 50-gallon tanks were housed in Biological Sciences Building Freshwater Aquarium Room in Statesboro, Georgia. One tank was designated as Control Tank 1 and the other was labeled Experimental Tank 2. Daily water tests for ammonia, nitrate, nitrite and pH were conducted for four weeks in order to build up a strong Nitric cycle. Adult goldfish of both sexes were obtained from the supplier Foster and Smith Company. Common goldfish (*Carassius auratus*) were used in this experiment and required specific levels of ammonia, nitrate,

nitrite and pH in their tanks for a healthy and suitable environment. For ammonia, we were looking for levels no higher than .25 ppm. For nitrate, we were looking for levels no higher than 40 ppm. For nitrite, we were looking for levels no higher than 10 ppm. For pH, our optimal range was 7.9-8.3. In order to maintain water chemistry within desired levels, a 25-50% water change was performed daily until a stable system was set up for about two weeks. Then, 50% water changes were performed about every 3 days even when the goldfish were introduced to the tanks. Tank water in aquariums was filtered with an electronic filtration system using glass wool and charcoal.

After four weeks, one medium goldfish was added to each tank to start naturally adding ammonia waste to the tank systems. After two successful weeks of acclimation with these two fish, six additional large sized goldfish were added to each tank. Fish used in experimentation had mean weight of 36.28 ± 2.17 g (range=32.58) and mean length of $10.56 \pm .221$ cm (range=4.38). Fish were held under ambient room temperature and photoperiod (12hr:12hr light to dark) and fed a diet of commercial goldfish pellets to satiation two to three times per week.

Preliminary observations:

Before experimentation of trials began, observations of fish behavior and sleep cycles were recorded. Observations took place during the appropriate times based on the light timer so as not to disrupt the light pattern the fish were already accustomed to. Behaviors of “awake” fish included active and frequent locomotion,

sucking mouth motions every two to four seconds and quick response to movement or neighboring fish. Fish were observed as most active when the tank lights were on for the photoperiod. Once tank lights went off for the dark portion of the photoperiod, fish were observed for over an hour to record behavioral responses. Within ten minutes of darkness, fish became less mobile and migrated towards corners of the tanks. They had slower response times to neighboring fish or any movement outside of the tank. After about 20-30 minutes of darkness, fish were observed to become completely immobile with little to no fin movement for seven-15 seconds at a time. In between these rest intervals, fish would slightly move their fins to reposition or bob slightly up and return back down before entering their next sleep cycle.

Chronic Sleep Deprivation Experiment:

The chronic trial of experimentation took place in December 2013 for four consecutive weeks. Both tanks were completely blacked out so that the only light the fish were exposed to were the aquarium lights positioned above each tank. These lights were controlled with an automatic timer, which was set control 12 hours lights on (7:30 a.m.)/12 hours lights off (7:30 p.m.). The experimental photoperiod was set at 21 hours lights on (7:30 a.m.)/three hours lights off (4:30 a.m.). All water changes and feedings for both tanks during this trial was done during the lights on portion of the photoperiod. A total of 12 fish were used in the chronic experiment: control (n=6) and chronic sleep deprivation (n=6).

Acute Sleep Deprivation Experiment:

The acute trial of experimentation took place in February 2014 and lasted for a 24-hour period. Again, tanks were blacked out to prevent exposure to room lighting. The control photoperiod was set at 12 hours lights on (7:30 a.m.)/12 hours lights off (7:30 p.m.). The experimental photoperiod was set at 24 hours lights on (7:30 a.m.)/0 hours lights off (7:30 a.m.). Sampling took place following the 24 hour lights on period. A total of 12 fish were used in the chronic experiment: control (n=6) and acute sleep deprivation (n=6).

Sampling Procedure:

The blood and tissue sampling procedure was as follows. Sampling of fish in both the chronic and acute exposures occurred between 7:30 a.m. and 9:30 a.m., immediately following the end of the last photoperiod. Fish were caught from tanks with a large net and then transferred to an anesthetic solution. Fish were anaesthetized with clove oil (40 mg/L⁻¹) added to five-gallon buckets of experimental tank water. One fish from each tank were anaesthetized at the same time. When opercular movement had stopped (five to eight minutes), the fish were removed from the anesthetic solution to begin sampling procedure. Prior to tissue sampling fish were weighed and total length was measured. Blood was drawn by caudal vessel puncture with a 23-gauge needle and 1-mL heparinized syringe. Blood samples were centrifuged to separate plasma and erythrocytes. Following blood sampling, a cervical displacement was performed before dissecting out brain

and liver tissues. All tissues were flash frozen in liquid nitrogen and stored at -80°C until analysis.

Plasma Cortisol Analysis:

Plasma cortisol levels were analyzed by the Neogen Corporation ELISA Cortisol Protocol (enzyme-linked immunosorbent assay). On day of ELISA testing, plasma samples (100 μ L) were pipetted into glass tubes and 1 mL of ethyl ether was added to each and vortexed for 30 seconds to mix. 600 μ L of organic phase was transferred from each tube into an additional glass tube. Samples were evaporated under a stream of nitrogen gas. The evaporated samples were dissolved in diluted extraction buffer and then further diluted with 100 μ L of diluted extraction buffer. Samples were assayed in duplicate along with a set of standards of known cortisol concentrations ranging from 0 ng/ml to 10 ng/ml. The cortisol enzyme conjugate was diluted and added to each well. The plate was incubated at room temperature for one hour. Concentrated wash buffer was diluted with deionized water. Contents of plate were dumped out and each well was washed three times with 300 μ L of wash buffer. Substrate was added to each well and allowed to incubate for 30 minutes while color developed. 50 μ L of Neogen's Stop Solution was added to each well to stop the enzyme reaction and color development. Absorbance readings of samples and standards were read at 650 nm using the Pro Max software and a spectrophotometer. The extent of color development was inversely proportional to the amount of cortisol that was in the samples and standards.

Data Analysis:

The standard curve was graphed by plotting the percent binding for each standard concentration on the ordinate axis against concentration on the abscissa axis.

Divided the averages of each sample absorbance value by the initial binding value and calculated percentages. Concentration of each sample can be determined by comparing the binding percentage of each sample to the corresponding concentration of Cortisol standard and correcting for the dilution factor (100x).

Standard T-tests were performed to test for statistical differences with $p < 0.05$ determining significance.

Results

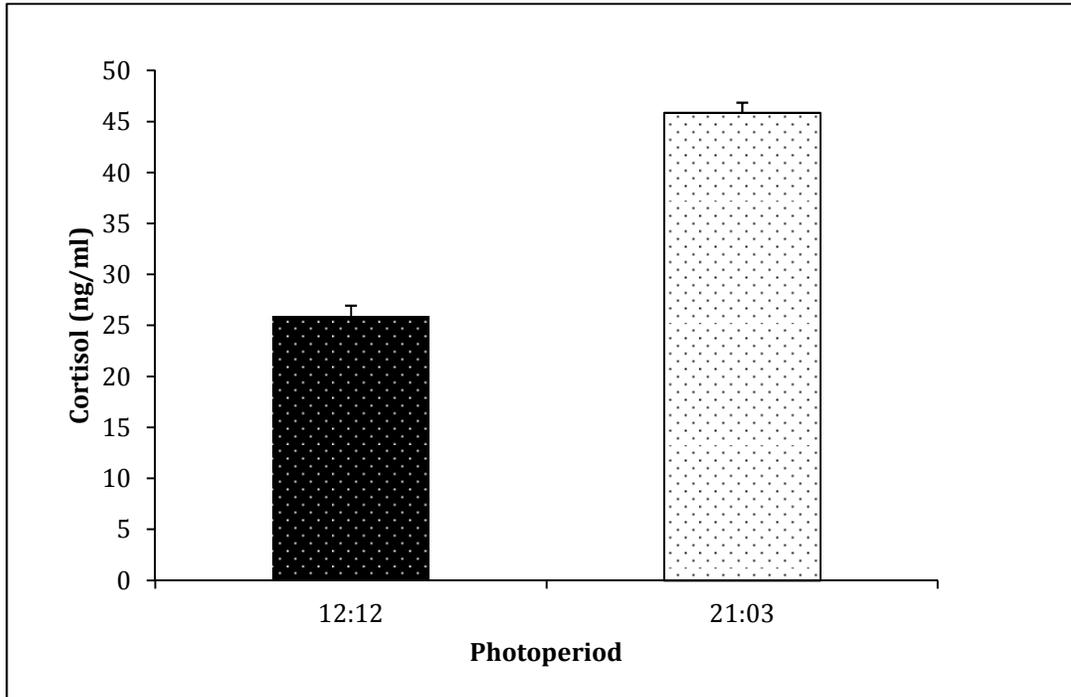


Figure 1. Chronic sleep deprivation trial. Comparison of plasma cortisol levels in goldfish. Data presented as mean \pm SE (n=6). T-test determined no significant difference between groups (p=0.243)

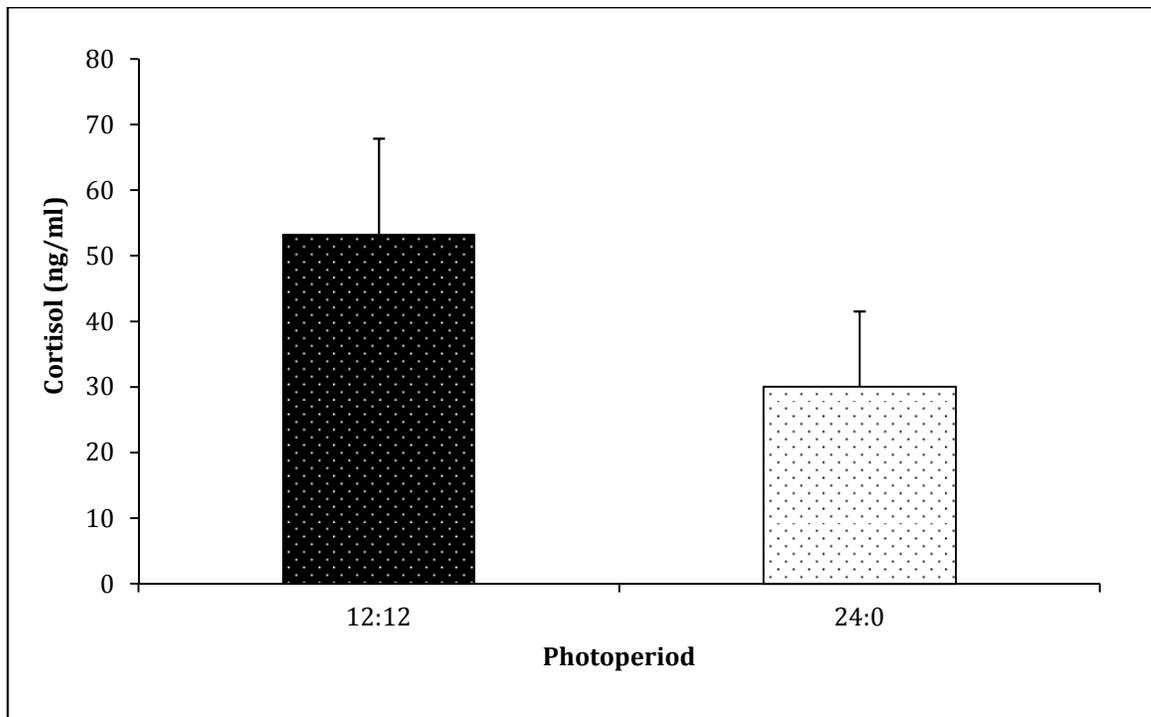


Figure 2. Acute sleep deprivation trial. Comparison of plasma cortisol levels in goldfish. Data presented as mean \pm SE (n=6). T-test determined no significant difference between groups (p=0.098)

In chronic and acute trials, averages for cortisol concentration and standard errors were calculated. The mean cortisol concentration the chronic control group was 53.35 ± 14.50 ng/mL (Figure 1). Exposure to the increased photoperiod for four weeks did not result in a significant increase of cortisol concentrations (30.07 ± 11.46 ng/mL; p = 0.243). The mean cortisol concentration for acute control group was 25.96 ± 4.15 ng/mL (Figure 2). Exposure to the increased photoperiod for 24

hours did not result in a significant increase of cortisol concentration (45.87 ± 11.01 ng/mL: $p = 0.098$).

Discussion

Sleep is a universal phenomenon in vertebrates and lack of sleep has been linked with various abnormal behaviors, such as stress (Singh et al 2013). Sleep deprivation is a common trend in younger generations with the average adult getting up to two hours less sleep than recommended by the National Sleep Foundation. Lack of sleep often induces emotional and physical stress in individuals and can lead to fight or flight signal pathways being activated. The principal corticosteroid released in the vertebrate stress response is cortisol and overexposure to the hormone can lead to serious health complications (Mommsen et al, 1999). Because the stress response is a highly conserved mechanism in vertebrates, elevated levels of cortisol in the bloodstream are a clear indication of the organism being in a stressed state (Peter et al, 1978). Studies have shown that chronic exposure to stress hormones can lead to anxiety, depression, weight gain, memory loss, high blood pressure and various other serious health complications (Charlesworth et al, 2004). The stress response in teleost fish has many similarities to that of terrestrial vertebrates, such as humans. In fish, corticosteroid production occurs via the brain-pituitary-adrenal axis, which is the same pathway for stress response in terrestrial vertebrates (Wendelaar Bonga, 1997). While humans would

have been the preferred sample, it was unethical to subject humans to the process of our experimentation. The common goldfish presented itself as a simpler systematic model as well as a physiologically relevant organism for our study. Our objective was to study the effects of acute and chronic sleep deprivation at a molecular level in teleost fish.

After completion of experimentation trials, plasma cortisol levels were analyzed using the Neogen Corporation ELISA Cortisol Protocol. Standard T-tests were performed for both trials to test for statistical differences with $p < 0.05$ determining significance. For the purposes of this study, no significant differences were found in the changes of plasma cortisol concentration of sleep-deprived goldfish.

Explanations for why the cortisol level did not fluctuate between control and experimental groups can possibly be attributed to several factors. We chose the length of photoperiods to represent acute and chronic sleep deprivation in college-aged adults (average age 22.6 years). The acute trial was a 24-hour light exposure, meaning to represent a student who stays up all night studying and has been awake for 24 consecutive hours. Our hypothesis suggested that the acute group would have significantly greater differences between control and experimental cortisol concentrations than that of the chronic group. The body is not accustomed to foregoing the restorative properties that sleep provides and could therefore be assumed to induce stress even when acutely deprived from it. However, fish have to be readily able to respond to stress in their natural environment on a more regular basis than the typical human. It is possible that their biochemistry is more

adaptable to acute sleep deprivation because it mirrors situations they might encounter in the wild. Further studies that lengthen the duration of acute photoperiods may produce significant results that are more similar to the environment the fish may encounter in natural habitats. Similarly, chronic sleep deprivation did not produce significant differences between control and experimental groups either. The chronic photoperiod was administered to represent a student who consecutively gets inadequate amounts of sleep over a long period of time (four weeks). It is possible that teleost fish are able to adapt to their stressors and this is one of the coping mechanisms to help defend themselves in the natural environment. While fish do require the restorative functions that rest cycles provide, it may also be plausible that continual or low light exposure in the wild does not stress the fish out to the same extent as overpopulation or electrical impulses under water might have. It is necessary for fish to be aware of their surrounding and neighboring predators in the wild, so low light levels may actually be preferred when fish are in a relaxed state.

While our cortisol concentrations between control and experimental groups were not significantly different in either trial, our concentration numbers were within normal range. Previous studies showed standard cortisol binding percentages for goldfish fell in a 30-90% binding range (Peter et al, 1978). This suggests that the goldfish were releasing cortisol as a response, but most likely due to causes other than sleep deprivation. We chose a sample time in our experiment for both trials when fish cortisol levels are known to be at their highest state.

Diurnal rhythm studies in fish have suggested that cortisol concentrations peak in

the first one to two hours of light onset and again one to two hours into a dark photoperiod (Fryer et al, 1975). Sampling for this study took place at 7:30 a.m. immediately following the light on photoperiod for both trials. This was done in order to ensure samples were being taken at a time in the fish diurnal cycle that would not further stress out the fish and alter concentrations.

In conclusion, human studies show significant differences in circulating cortisol levels in the bloodstream due to stress derived from sleep deprivation (Von Treuer, 1996). Overexposure to stress hormones can lead to health complications in the human body and should therefore be monitored. While terrestrial vertebrates show evidence of sensitive cortisol concentration fluctuations (and other related stress hormones) due to sleep deprivation, lower vertebrates may be more equipped to withstand or acclimate to harsher conditions in the natural environment. Our findings from this study suggest that goldfish subjected to acute and chronic sleep deprivation trials undergo little to no fluctuation in cortisol concentration in response to this study's photoperiods and did not experience harmful exposures to cortisol and stress related hormones in their bloodstream.

Further Directions

This experiment will be further explored to study the brain and liver tissues harvested from both acute and chronic trial samplings. We will investigate the cortisol receptor levels on the tissue samples and assess if sleep deprivation causes significant increase or decrease in receptor numbers in tissue samples between control and experimental groups. A significant finding in receptor

numbers may help explain our current study's plasma cortisol concentrations. In addition, this experiment is under evaluation to be altered and repeated using different methods. It may be more beneficial to sample plasma from fish in control and experimental groups prior and after trial runs. This will let us compare the original concentrations of the fish against concentrations post overexposure to light. Significant differences in these data may show fish do grow accustomed to the photoperiod over time or that they actually respond to the continued stress. Our lab plans to further investigate the sensitive control of stress hormones in teleost fish in future studies.

References

- Aluru, N. and M.M. Vijayan. 2009. Stress transcriptomics in fish: A role for genomic cortisol signaling. *General and Comparative Endocrinology*. 164, 142-140.
- American College Health Association (2012). American College Health Association-National College Health Assessment II: Reference Group Data Report Spring 2012. Retrieved April 5, 2013, from http://www.acha-ncha.org/docs/ACHA-NCHA-II_ReferenceGroup_ExecutiveSummary_Spring2012.pdf
- Blagrove, Mark and Lucy Akehurst. "Personality and the modulation of effects of sleep loss on mood and cognition." *Personality and Individual Differences*, vol. 30 2001: 819-828. University of Wales Swansea, February 25, 2013.
- Campbell, S.S. and Tobler, I. (1984). Animal sleep: a review of sleep duration across phylogeny. *Neuroscience Biobehavioral Review*. 8, 269-300.
- Charlesworth E.A., et al. *Stress Management: A Comprehensive Guide to Wellness*. New York, N.Y.: Ballantine Books; 2004:24.
- Fryer, J.N. 1975. Stress and adrenocorticosteroid dynamics in the goldfish, *Carassius auratus*. *Can. J. Zool.* 53: 1012-1020.
- National Sleep Foundation. *Sleep in America Poll, 2001-2002*. Washington, DC: National Sleep Foundation.
- Mommsen, T.P., Viajayan, M.M. & Moon, T.W. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews of Fish Biology and Fisheries*, 9: 211-268.
- Peter, R. E., A. Hontela, A.F. Cook, and C.R. Paulencu. 1978. Daily cycles in serum cortisol levels in the goldfish: effects of photoperiod, temperature, and sexual condition. *Can. J. Zool.* 56: 2443-2448.
- Randall, Michael. 2010. *The Physiology of Stress: Cortisol an the Hypothalamic-Pituitary-Adrenal Axis*. Dartmouth Undergraduate Journal of Science, Fall 2010.

- Rotllant, J., Tort, L., Montero, D., Pavlidis, M., Martinez, M. & Wendelaar-Bonga, S.E. & Balm, P.H. 2003. Background colour influence on the stress response in cultured red porgy *Pagrus pagrus*. *Aquaculture*, 223: 129-139.
- Singh, A., Subhashini, S., Sharma, S., and B.N. Mallick. 2013. Involvement of the α 1-adrenoreceptor in sleep-waking and sleep-loss induced anxiety behavior in zebrafish. *Neuroscience*. 245: 136-147.
- Tune, G. S. "Sleep and Wakefulness in Normal Human Adults." *British Medical Journal*, vol. 2, 1968; pp. 269-271. PubMed Central. March 5, 2013.
- Von Treuer, Kathryn, Norman, Trevor R., and Stuart M. Armstrong. 1996. "Overnight human plasma melatonin, Cortisol, prolactin, TSH, under conditions of normal sleep, sleep deprivation, and sleep recovery." *Journal of Pineal Research*. 20: 7-14.
- Wells, M. and B.M. Vaughn. 2012. Poor Sleep Challenging the Health of a Nation. *Neurodiagnostic Journal*. 52.23302349
- Wendelaar Bonga S.E. 1997. The Stress Response in Fish. *Physiological Reviews*. 77. 591-616.
- Zhdanova, I.V. (2006). Sleep in zebrafish. *Zebrafish* 3, 215-226.