

The Influence of Melatonin on the Reproductive Behavior
of the Green Frog, *Rana clamitans*

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ABSTRACT

Melatonin, a hormone secreted by the pineal gland, has been found to be an inhibitor of reproductive activity in many small mammals. Since the pineal gland is indirectly light-sensitive, it is hypothesized that melatonin levels fluctuate with amount of daylight present and therefore contribute to seasonal breeding cycles. Past research has experimentally varied melatonin levels in small rodents by removing the pineal gland or injecting melatonin to study its effects on reproductive behavior. In this study melatonin levels were manipulated by injections into the green frog, *Rana clamitans* to study this reproductive behavior variability. Parameters measured were skin pigmentation, release calls in amplexus, and gonadal atrophy according to the level of melatonin present. Findings include increased skin lightening in male subjects injected with melatonin and atrophy of the oviducts by injected females. The results show that melatonin affects pigmentation of the frog and also plays a role in affecting reproductive physiology.

The Influence of Melatonin on Reproductive Behavior

BACKGROUND & SIGNIFICANCE

It is easy to notice that in many natural populations, reproductive behavior (such as mating and breeding) follows a seasonal schedule. This schedule most often allows for the young to develop in the most optimal environment of temperature, light, and moisture. Aquatic environments, after seeming fairly dormant in the winter, are active with the chirping of birds, the buzzing of insects, and the croaking of frogs with the onset of spring and summer. All of these sounds are one way to tell that reproductive behavior is occurring. How do the animals know that the environments are becoming more optimal for their young to survive? What environmental cues are telling them this? How are their bodies processing these cues so that they can modify their behavior? The key to this knowledge often lies in the amount of daylight present (Relkin, 1983).

Photoperiodic species detect the amount and length of light present through special receptors and convert these signals into physiological changes (Relkin, 1983). This process is complex, but it is controlled by an endocrine gland in the brain called the pineal gland. The pineal gland is reputed to be the neuroendocrine transducer which converts photoperiodic information into a hormonal response which has profound effects on the organism (Matthews et al., 1982). In general the mode of action of pineal photoreception resembles that of ocular photoreception in that onset and offset of light are followed by inhibitory and excitatory changes in neuronal activity (Dodt, 1987). The hormone released by the pineal and responsible for these changes is melatonin. According to Ralph (1985), radioimmunoassays show that melatonin is present in the blood of several species of vertebrates. There tends to be a marked daily variance with higher levels at night and lower levels during the day. The diurnal range is commonly remarkable, often being 3- to 4-fold. This apparently universal and persistent phenomenon suggests some very basic role for circulating melatonin (Ralph, 1976). It has been shown by numerous experiments that the pattern of the daily cycle of melatonin is closely linked with the phasing of photoperiods (Ralph, 1983).

Researchers have shown, often using hamsters or rats, that higher melatonin levels inhibit the frequency of mating as well as the size and activity of the gonads (Reiter et al., 1968; Wurtman, Axelrod, & Chu, 1963). Many studies have repeated these results, concluding that shorter days of less light (and higher melatonin levels) lead to a marked decrease in reproduction. Therefore, as the days begin to have more light in the spring, reproduction begins to increase as melatonin levels drop.

In Wurtman, Axelrod, and Chu's study (1963), rats were injected daily with microgram amounts of melatonin. They found that ovary weight decreased by as

much as 30 mg. Even when only 1 μ g of melatonin was injected (an extremely small amount), a significant decrease was shown. The melatonin did not, however, alter body or uterine weight. Wurtman relates the pineal's function to amount of light present, and mentions briefly the relationship of seasonal breeding to this change in ovarian weight.

The melatonin levels in previous studies have been regulated by either injecting extra melatonin (Wurtman et al., 1963) or removing the melatonin altogether via a pinealectomy (Walker, McCamant, & Timiras, 1982). This study will examine the effects of melatonin levels via injections in the green frog, *Rana Clamitans*. Amphibians were chosen as the subjects for two reasons: (1) Pineal organs of fish, amphibians, and reptiles are directly photosensitive whereas light sensitivity of the pineal organs in birds and mammals is restricted to indirect activation by light stimulation of their lateral eyes (Dodt, 1987). Using frogs allows for a more direct conclusion that daylength is directly affecting the pineal gland, and therefore, melatonin. (2) Few studies in amphibians have examined the effect of pinealectomy or melatonin levels. Consequently, little evidence is available on the role of the pineal system in their circadian organization (Reiter, 1984). Knowledge of the effect of melatonin levels on the amphibians is still very untapped, so this study will examine these effects on three different variables.

One variable examined will be the pigmentation of the frog. Melatonin has been shown to be the most effective skin lightening agent in amphibians. This compound produces changes in pigmentation by causing the aggregation of melanin granules within the amphibian melanocyte (Axelrod, Quay, & Baker, 1965). When the granules clump together more, this leaves more room in the melanocyte for light to pass through, revealing a lighter pigmentation. Research has shown that amphibian larvae and tadpoles placed in the dark (where melatonin concentration is high) become very light in color, and then darken again when brought back into the light (Bagnara, 1960). The pigmentation of the *Rana Clamitans* will be observed constantly to monitor how well injections of melatonin are taking effect in the system. This will be done using a greenish brown color scale of varying hues and comparing the lightness of the skin of the frog against it.

The second variable to be studied is the effect of melatonin levels on amplexic clasping. Frogs mate in a very unique way: the typical mating posture is one where the male clasps on to the back of the female and holds on to her with his front legs, an event called amplexus. Sperm and eggs are then released simultaneously into the water, where the eggs are externally fertilized (Tynning, 1990). A female who is receptive to this reproductive behavior will allow herself to be clasped. A female who is unreceptive to reproductive behavior will give a specific croak, or release call. The clasping male normally releases his grip in response to this call (Tynning, 1990). Conversely, Tynning also notes that males who are themselves clasped by other males will not want to be clasped during the

breeding season, a time where they would much rather gear their behaviors towards mating with females. Thus, males give release calls more often in the breeding season when they are primed for reproductive behavior. It is possible that the levels of melatonin in the frog determine how receptive it is to clasping. The purpose of this part of the study is to show that increased melatonin levels increase the number of release calls by female *Rana Clamitans*, showing that they are not as responsive to reproductive behavior. The study also hopes to show that increased levels of melatonin in male frogs will decrease the number of release calls, indicating that they will allow their reproductive time to be replaced by other clasping male frogs. These release calls will be measured as the frogs respond to a manual clasp by the experimenter. This will demonstrate how shorter photoperiods reduce the tendency to mate.

As the second variable deals with reproduction on a behavioral level, the third variable examines the physiological level. Is it possible that higher melatonin levels also affect the reproductive organs? A wealth of studies on small mammals have shown that higher levels of melatonin cause an atrophy of the gonads and testes, i.e. a decrease in the weight of the gonad (Matthews et al., 1982; Wurtman et al., 1963; Reiter et al., 1968; Reiter et al., 1976). Melatonin will be injected into the frogs to show that increased levels lead to this atrophy, a factor that would greatly reduce the amount of reproduction that takes place when amount of light is low. The gonads as well as the sperm and eggs present in the gonads will be examined. In all of these experiments, it is assumed that all of the subjects have some amount of melatonin in them naturally. The natural level begins to rise at dusk, so the injections will be given at this time to ensure that the level is increased at a more natural time of the day. If the injections were given in the morning, the frog would experience two peaks in its melatonin level: one in the morning and the natural one at night. This may confound the results. The purpose of the study as a whole is to show that melatonin has an inhibitory effect on the physiology of the reproductive system of *Rana Clamitans* as well as the reproductive behavior. This will give insight into how seasonal breeding of amphibians is determined by length of day.

METHODS AND EXPERIMENTAL DESIGN

A sample of 40 *Rana Clamitans* were obtained from May 28 to May 30, 1992 from two vernal ponds in an area of northern Michigan. The ponds were sunny with few overhanging trees, and the average depth was .5 meters. The sample contained 20 males and 20 females. Gender was determined by the size of the tympanum behind the eye. The tympanum of males is generally twice the size of the eye, and in females the tympanum and eye are the same size. Also, the males usually have yellow throats as opposed to the white throats of females (Tynning, 1990). The males were kept in separate styrofoam coolers from the females, with

each of 8 coolers containing an inch of pond water and a few rocks. The coolers were topped with a screen to contain the frogs and the dragonflies used as food, and the water was changed once a week. The coolers were kept in an indoor environment with limited sunlight and an average temperature of 60 degrees. Each frog was fed one dragonfly or other available insect every other day. The frogs were given seven days to acclimate to the environment before the experiments were begun.

The frogs were assigned into four equal groups (male melatonin, female melatonin, male control, and female control). Each group contained 10 frogs, and within each sex, each frog was randomly assigned to either the melatonin or control condition. On June 6, before injections were begun, each subject was leg-banded by small, numbered bands according to which of the 4 conditions it belonged. Each frog was also pre-weighed and pre-assessed for initial color values.

Oddly, on June 7, one day after the frogs were banded, the banded foot of almost every frog was red and swollen. After the feet did not respond to antibiotic for a possible infection, all of the bands were removed the next day and the frogs were soaked in an inch of antibiotic solution (penicillin, streptomycin, and water) for 3 hours. Subjects were allowed to recover for two days before beginning injections, in which time most of the feet returned to normal. For two of the frogs, the foot eventually died, but the frogs were kept in the experiment. Since a new way of identifying the frogs was needed, the frogs were separated into 8 coolers, and their subject number was identified by natural markings and size that differentiated the frogs from each other. Toe clipping was not used to avoid any additional stress.

On June 9, injections were given to all four groups. Giving injections to the control groups controlled for the effect of the injections on the stress of the frogs. The frogs were given daily injections (at dusk) subcutaneously. The experimental group received 100 μg of melatonin in .1 ml of a 1% alcohol : saline (amphibian ringer) solution. The melatonin powder was difficult to dissolve in the ringer solution, so it was administered in suspension. The control group received only the .1 ml of 1% alcohol : saline solution. Several tests were then performed as the frogs continued to receive the injections at the same time everyday. After one week, the injections were reduced to every other day.

Experiment I: Pigmentation

After one week, the pigmentation of the frogs of each group was again compared against the color scale. Since the pigmentation of the melatonin group was not significantly lighter than the initial values, the injections were increased to 200 μg of melatonin in solution. The colors were monitored twice more (for a total of four times) throughout the study for any other changes.

Experiment II: Gonadal Atrophy

After the injections were completed on June 17, an extraction and biopsy of the gonads for all subjects was performed. Before dissection, a final overall body weight

was recorded for each frog. After dissection the oviducts of the females and the testes of the males were extracted and weighed. Each set of testes and oviducts was preserved in formalin for later analysis. The ratio of each subject's testes or oviduct weight : body weight was determined. The testes and oviducts were then sectioned by embedding each one in paraffin, freezing it, and using a microtome to prepare 10-20 cross-sections of 50 μ m thick. The cross-sections were frozen on slides until staining. The slides were then prepared by a Hematoxylin/Eosin staining procedure, which consisted of several steps including rinsing of the slide with ethanol and xylene to remove the paraffin, and rinsing the slide after the Hematoxylin and then the Eosin is applied by dipping the slides in solutions. Harris Hematoxylin is a purple organelle stain and Eosin is an orange cytoplasmic stain. The slides were then ready for examination under the microscope. The appearance of the testes and oviducts of the experimental group was compared against the control group for the following characteristics: For the females, thickness of the oviduct wall was recorded. The thickness of 10 cross-sections per subject was measured, and the mean of the thicknesses was compared across subjects. For the males, number of sperm in each of 10 testis cross-sections was recorded by observing how dense the cross-sections were with sperm (tails could be seen through microscope). The densities were rated from + (few or none) to ++++ (very dense). Again, the means were compared across subjects.

Experiment III: Amplexic Clasping

The frogs in each group were also pretested before the injections for release calls. They were each clasped manually by placing the thumb and forefinger on either side of the pectoral area. The number of calls in a one-minute test was observed. One week after the injections were begun, each frog was clasped again for number of release calls per minute. The frogs were tested twice more throughout the study for number of release calls.

Body weight was recorded twice more during the experiment, one of which was recorded immediately before extracting the testes and oviducts. These were recorded to measure possible stress on the frogs and to notice any competition for food in each cooler.

Through the 4-week duration of the experiment, several frogs escaped from the coolers and two coolers were knocked over. Only some of the frogs were recovered. Injections continued to be given to the remaining ones.

RESULTS

The data for each subject is presented in Appendix A. Raw values are given as well as means for each group. A "-" indicates that the frog was lost at some point during the experiment, and that no data exists for those dates. The number of subjects available for final analysis, therefore, decreased in relation to the number available at the beginning. A more comprehensive view of the means is available

in Table 1, as well as the standard deviations in parentheses for each of the means. It is important to notice not only the differences between the 4 groups, but also the differences between the "Overall Mel" and the "Overall Control" groups. Several of the means were analyzed for significant differences by using simple independent groups t-tests.

Pigmentation: T-tests were performed between the mel and control groups of the same sex for each date. T-tests were also performed between the overall groups. Several groups did not show any significant differences. Looking at Table 1, initially the groups did not differ significantly in their color, which provides an accurate baseline measure for all. It seems that the melatonin did not have any effect during the first three days of injections when the color was recorded on 6-12-92, since there are no significant differences. When the amount of melatonin was increased, however, and the color was again recorded on 6-21-92, Table 1 shows that 2 values were significant. First, the male melatonin group had a significantly lower color value for 6-21-92 than did the male control, with $p < .01$. This shows that for the males, melatonin had a marked effect on skin-lightening. This was not seen for the females. Nevertheless, Table 1 also shows that considering the overall mel and control groups on 6-21-92, there is a strong significant difference ($p < .001$). So, for the entire population of mel, the melatonin had a significant skin-lightening effect, although this is more evident in the males. One oddity is that when the color was again measured on 7-8-92, no significant differences appear. Looking at Figure 1, we can see that there still may be some visible difference between groups on 7-8-92, but since the errors for each mean are so high, the differences are not significant for $p < .05$.

Gonadal Atrophy: This can be seen by studying the gonad/body weight ratio means shown in Table 1. It is not useful to study solely the testes or oviduct weights, since body weight will positively correlate with testes or oviduct weight. The ratio controls for differences in body weight means across the groups. The males did not show a difference in ratios between mel and control groups. The melatonin, therefore, did not have any effect on decreasing the mass of the testes, nor did it decrease the sperm count in the testes, also shown in Table 1. For the females a significant difference does exist between mel and control. The mass of the oviducts was less for the melatonin groups ($p < .05$). The thickness of the oviduct wall, however, was not significantly different between the two groups.

Body Weight: The mean body weight of each group decreased as shown by Figure 2 between 6-5-92 and 7-10-92. This decrease was only significant for the male control group, where 18.69g is significantly less than the initial mean of 23.72 ($p < .05$). Only this group is significant due to the small standard deviations of the means, unlike the other groups, where the variability was much higher. The purpose of recording body weight was to notice primarily a general trend in the body weights; therefore, the graph provides useful information.

Clasping: Zero release calls were observed for every frog at all recording times. When frogs were clasped they made no sound at all. These zeroes were not included in the tables, but the discussion of this occurrence will nonetheless be covered.

DISCUSSION

There are many elements of this study to consider when discussing the results. The most important factor to note is that the frogs underwent an extreme amount of stress while in captivity. The stress could be seen in several ways. As each parameter is discussed, the effect of stress on the results will also be discussed.

Pigmentation: Past studies have shown that injections of melatonin dramatically lighten skin within 48 hours. Since this did not happen for the frogs after 3 days, it was assumed that the dosage was not high enough and so doubled to 200 µg. This explains the lack of change in values or the slope of the graph for the first week. The colors in all of the frogs faded to grays, however, during this first week. The color was not lightening, but instead, fading. The green hue remained the same, but this fading could be due to stress or lack of sunlight. When the higher dosage of melatonin was in effect, the male mel frogs experienced a significant lightening of color. This also happened for females, but the changes were not significant. A larger sample size or a color scale with more divisions (hues) may resolve this. The question for this section is, why did the colors of almost all of the frogs re-darken by the last recording date? Male control fell slightly, but the rest rose sharply, with the female control even above its initial value. Perhaps the mel frogs became accustomed to the melatonin and the melanin granules were able to readjust, but the reason why this happened is really unknown.

Body weight: The body weights of the frogs were assessed, for one, to tell the frogs apart in their physical descriptions. The assessments were primarily used to monitor competition for food and overall weight loss or gain as the experiment progressed. As can be seen in Appendix A, the weights of a few frogs continued to increase, demonstrating the frogs that were winning in the food competition. The rest of the frogs suffered because their weight constantly dropped. The means for each group dropped because, as discussed earlier, the food supply for the frogs was poor. The male control group dropped significantly with the least amount of variability, but with more subjects in each group, this significance could have possibly been shown for all. It is possible, however, that the melatonin had an effect of keeping the body weight up.

Gonadal atrophy: The testes or oviduct/body weight ratio for the male groups was almost identical between mel and control. It appears that the melatonin had no effect on the weight of the testes. They did not atrophy from the melatonin as was hypothesized. The sperm count between the two groups was likewise no different. For the females, however, the weight of the oviducts by ratio were significantly less

Influence of melatonin

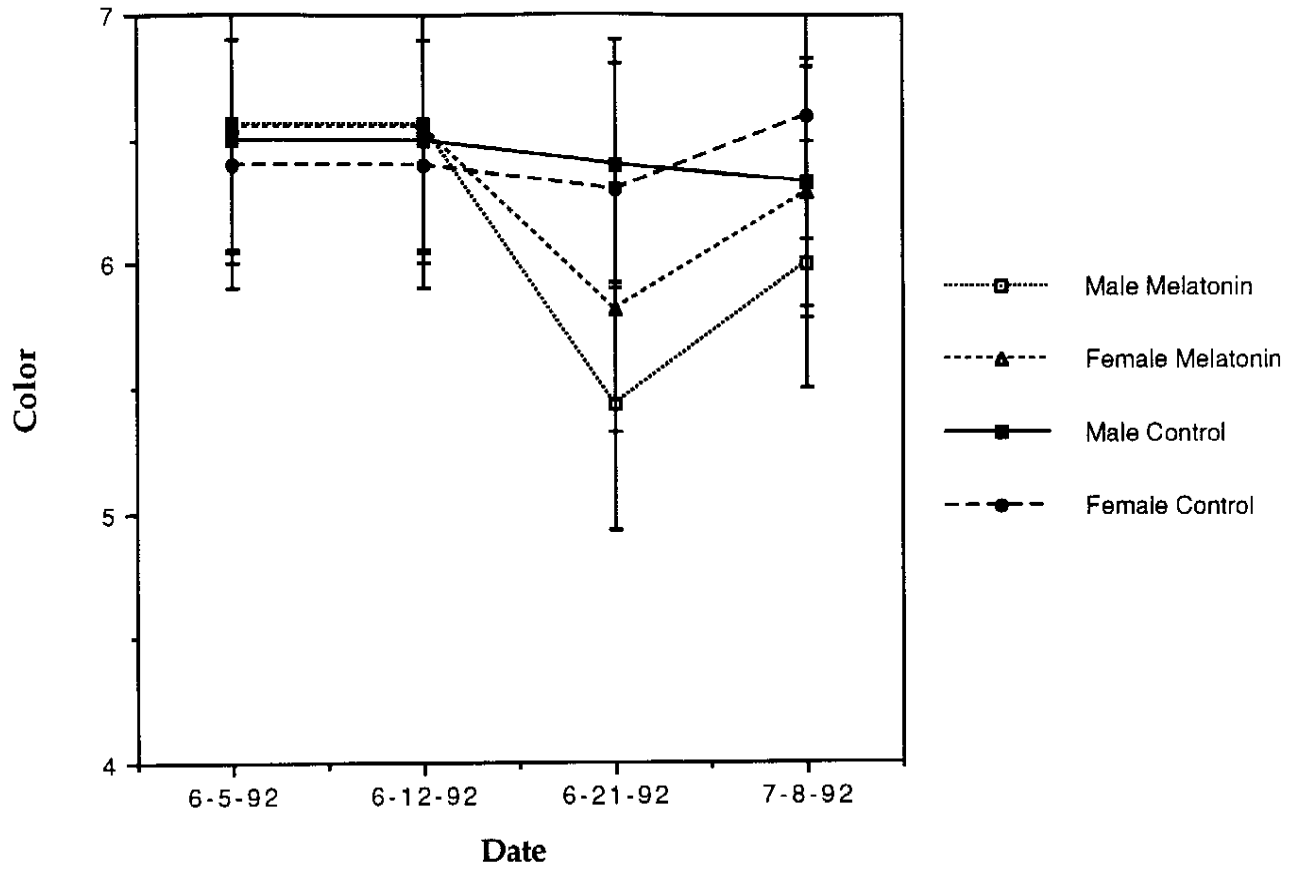


Figure 1.
Frog Color for each group by Date of measurement

Influence of melatonin

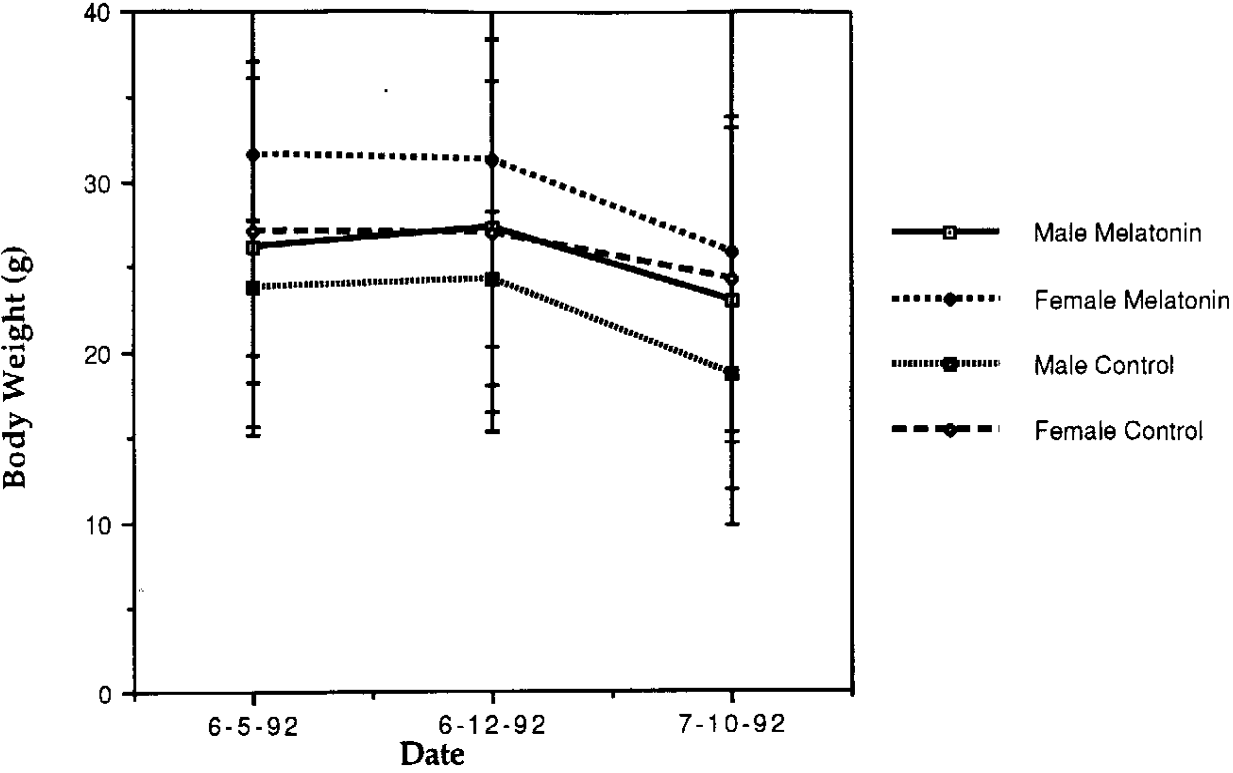


Figure 2.
Body Weight for each group by Date of Measurement

for the melatonin group ($p < .05$), showing atrophy of these reproductive structures. The thickness of the oviduct wall was slightly less for the mel group, but not significantly. Perhaps atrophy in the oviducts does not occur by reducing thickness of the wall, but rather length of the tubes or the full diameter of them. It would still be helpful to do an analysis on the ovaries themselves, but the oviducts are nonetheless structures affected by hormones and inhibitors like melatonin of the reproductive system.

Why did the melatonin cause atrophy in the oviducts but not the testes? Since melatonin is known as an inhibitor, it must inhibit a certain step in the reproductive pathway that leads to the production of androgens. Since these pathways are somewhat different for males and females, the melatonin may only inhibit a step in the female cycle. It is also possible that the female may have fewer receptors for melatonin than the male, showing less of a response, or considering the fact that females weigh proportionately more, a given dosage of melatonin would affect them less. But then, how do we explain male seasonal breeding as well as female? Perhaps the melatonin is still an inhibitor in the male, but in a step that could not be detected by gonadal atrophy. Also, behavior may be modified by melatonin by a method still unknown. Studies concentrating on melatonin, the brain, and the pineal gland directly could investigate this. Pinealectomy, another way of manipulating melatonin levels, would be the next step in studying these parameters for amphibians.

Clasping: As mentioned in the results, no frog gave a release call at any time by simulated amplexic clasping. Frogs were observed to release call occasionally when held for injections, but in the amplexus position, they failed to call ever. Since release calling is a common phenomenon for *Rana clamitans* and is easy to elicit in most studies by manual clasping, these results can only be attributed to stress. The physical health of the frogs was poor while in captivity, and their activity in general was greatly reduced. By the third week, they were observed to even be moving around the coolers minimally or not at all in contrast to the jumping they did during the first few days. Some of the larger ones even developed sores on their snouts as a result of constantly trying to jump out by pushing against the screens. When a lack of activity is present, it only makes sense to say that reproductive activity and behavior is also very inhibited. It is true that the ones who do feel inhibited should release call, but they weren't even active enough to do that. Their behavior could almost be described as "physical apathy."

Why would the captivity cause stress? First, each cooler contained 5 frogs; this limited space severely limited the mobility of the frogs and caused them to become inactive. Also, the temperature often got very cold (30 F) and the frogs were unable to burrow in any kind of soil or sediment for warmth. Periods of cold like this could certainly add stress to the frog. The injections themselves put very little stress on the frogs, as they lasted for only 5 seconds and had no side effects or

infections. The leg bands initially caused a great amount of stress, since the frogs could be seen constantly fighting to remove them. Once they were removed and the legs healed, it is doubtful that this continued to be a stressor. One of the largest stressors was the food supply. The frogs ideally ate dragonflies, but during these feeding sessions there was much competition for food and some frogs always ended up eating others' shares. Also, week-long periods of cold and rain prevented the frogs from receiving dragonflies (which are not active enough in this weather to be caught) as well as most other insects in the area. Some frogs did not receive adequate food supply for these two reasons. The frogs were so far removed from their natural environment of food and habitat that their own stress and poor physical health undoubtedly had some effect on the results.

One other limitation to the results is that several frogs escaped during various points in the experiment. At one point, two of the coolers were completely knocked over. Many frogs were recovered, but since identification of the frogs was through natural markings only, a few of the frogs may have been misplaced and therefore, greatly confounded the results. Despite this, the significant results can still be stated with a great deal of certainty, but if all of the frogs had remained in their proper places for the duration of the experiment, many more significant results could have been found.

The data presented was somewhat consistent with previous studies on the influences of melatonin on pigmentation and gonadal atrophy. Reiter, et al. (1976) have also tested the effect of melatonin on the gonads, this time with the testes of male hamsters. His results, similar to Wurtman's, show an atrophy of the testes with increased melatonin levels via injections. He states that melatonin is indeed the pineal antigonadotropic factor capable of stunting the development of the reproductive organs in hamsters. However, he reveals an additional important point. One additional criterion must be met before melatonin is capable of acting in a manner inhibitory to the pituitary-gonadal axis: only when the pineal gland is intact and sympathetically innervated can melatonin injections inhibit the gonads. It is possible that melatonin itself is not antigonadotropic but rather acts through the pineal to retard the growth of the sexual apparatus. It appears that the pineal gland is the target organ for exogenously administered melatonin, and that the gonads are a secondary target. So perhaps melatonin injections alone will not produce the expected results. Since the vast majority of these studies center on mammals, we will be able to extend the knowledge that was gained in this study to some of the residents of aquatic environments such as green frogs. It is highly likely that these results could be repeated in other lower vertebrates such as fish and reptiles. The results show that the increased levels of melatonin present in the system inhibit female reproduction physiologically. Further studies are encouraged to study this species or a related species on a behavioral level. What is still not understood is how this occurs on a biochemical level. As Reiter said, does melatonin have no

effect on its own? How does melatonin cause melanin to aggregate? With regard to gonadal atrophy, melatonin must have some effect on other reproductive hormones such as LH or FSH. Where does melatonin inhibit this biochemical pathway? Also, reflecting on previous studies, is there another physiological effect of melatonin that prevents the female from being responsive to clasping? Obviously, much room exists for future studies. The more studies performed on factors that affect seasonal reproduction in lower vertebrates, the more we will understand how our own circadian rhythms have evolved.

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Appendix A. Values of all parameters assessed for each frog.

FROG	COLOR ¹	COLOR	COLOR	COLOR	BODY WEIGHT(g) (6-5-92)	BODY WEIGHT(g) (6-12-92)	BODY WEIGHT(g) (7-10-92)	GONAD WEIGHT(g) (7-10-92)	GONAD/ BODY WT. RATIO	SPERM COUNT ²
MALE MELATONIN										
1	7	7	5	7	47.38	49.30	46.49	.22	.47%	++++
2	6	6	6	-	18.95	20.17	-	-	-	-
3	6	6	6	6	23.26	23.94	24.68	.08	.32%	+++
4	7	7	-	-	44.80	47.79	-	-	-	-
5	7	7	5	6	19.19	22.29	19.53	.09	.46%	+++
6	6	6	6	5	18.07	19.94	13.88	.05	.36%	+++
7	7	7	-	-	17.90	18.53	-	-	-	-
8	7	7	5	6	25.44	23.63	20.05	.09	.45%	+++
9	6	6	5	6	20.25	20.33	12.93	.04	.31%	++
X	6.56	6.56	5.43	6.0	26.14	27.32	22.93	.095	.40%	+++

¹ Color value determined by a green-based color scale with arbitrary values ranging from 1 (very light green) to 7 (very dark green).

² Number of sperm in testis cross-section evaluated on an arbitrary scale of +(few or none) to ++++(very dense).

FROG	COLOR	COLOR	COLOR	COLOR	COLOR	BODY WEIGHT(g)	BODY WEIGHT(g)	BODY WEIGHT(g)	OVIDUCT WEIGHT(g)	OVIDUCT/ BODY WT. (X)	OVIDUCT WALL (µg)	STD DEV.
	(6-5-92)	(6-12-92)	(6-21-92)	(7-8-92)		(6-5-92)	(6-12-92)	(7-10-92)	(7-10-92)		(X)	(µg)
1	6	6	6	6	6	74.50	72.75	68.86	.75	1.09%	.5	.2
2	6	6	6	7	7	31.08	31.22	24.98	.04	.16%	.275	.059
3	7	7	5	6	6	23.08	22.78	16.52	.05	.30%	.2	0
4	7	7	6	6	6	23.03	22.65	16.55	.15	.91%	.319	.037
5	6	6	6	6	6	28.54	28.81	22.57	.11	.49%	.336	.019
6	6	6	5	-	-	54.82	52.66	-	-	-	-	-
7	7	7	5	-	-	21.41	20.36	-	-	-	-	-
8	7	7	6	-	-	17.74	18.30	-	-	-	-	-
9	7	7	6	-	-	24.34	23.42	-	-	-	-	-
10	6	6	6	6	6	19.68	20.85	14.52	.06	.41%	.174	.024
11	7	7	7	7	7	30.00	30.17	16.91	.06	.35%	.19	.032
X	6.55	6.55	5.82	6.29	6.29	31.66	31.27	25.84	.17	.53%	.285	

FROG	COLOR	COLOR	COLOR	COLOR	COLOR	BODY WEIGHT(g) (6-5-92)	BODY WEIGHT(g) (6-12-92)	BODY WEIGHT(g) (7-10-92)	GONAD WEIGHT(g) (7-10-92)	GONAD/ BODY WT. RATIO	SPERM COUNT
(6-5-92)	(6-12-92)	(6-21-92)	(7-8-92)	(6-5-92)	(6-12-92)	(6-5-92)	(6-12-92)	(7-10-92)	(7-10-92)		
MALE CONTROL											
1	6	6	7	24.30	25.24	21.32	.12	.56%	++++		
2	6	7	7	22.70	23.36	26.50	.11	.42%	+++		
3	7	6	6	24.68	25.73	17.65	.06	.34%	+++		
4	6	6	6	22.89	24.01	15.53	.04	.26%	++++		
5	7	6	-	30.48	32.82	-	-	-	-		
6	7	6	6	24.21	24.19	-	-	-	-		
7	6	7	6	27.40	26.84	24.74	.13	.53%	+++		
8	6	6	6	19.38	18.45	12.68	.05	.39%	+++		
9	7	7	6	21.93	23.63	20.45	.06	.29%	+++		
10	7	7	7	19.24	18.72	10.61	.05	.47%	+++		
X	6.5	6.5	6.4	6.33	23.72	24.30	.078	.41%	+++1/4		

	(6-5-92)	(6-12-92)	(6-21-92)	(7-8-92)	BODY WEIGHT(g) (6-5-92)	BODY WEIGHT(g) (6-12-92)	BODY WEIGHT(g) (7-10-92)	OVIDUCT WEIGHT(g) (7-10-92)	RATIO	OVIDUCT/ OVIDUCT BODY WT. WALL (µm) (X)	STD. DEV. (µm)
FEMALE CONTROL											
1	6	6	6	6	46.00	47.05	34.56	.33	.95%	.480	.054
2	7	7	6	7	27.60	26.96	23.02	.20	.87%	.458	.049
3	7	7	7	7	29.53	28.78	24.82	.24	.97%	.419	.141
4	6	6	6	-	17.53	17.48	-	-	-	-	-
5	6	6	6	6	34.65	35.00	47.69	.57	1.20%	.495	.055
6	6	6	6	7	27.27	26.75	22.10	.19	.86%	.505	.044
7	6	6	6	6	20.08	19.64	13.75	.11	.80%	.27	.027
8	6	6	6	6	20.52	20.24	13.28	.06	.45%	.122	.021
9	7	7	7	7	17.47	16.59	11.68	.11	.94%	.3	.2
10	7	7	7	7	30.23	31.98	27.26	.22	.81%	.46	.089
X	6.4	6.4	6.3	6.6	27.10	27.05	24.24	.23	.87%	.390	