J. comp. Physiol. 94, 309–319 (1974)

The Effects of Melatonin on Lipid Deposition in Cyprinodontid Fishes and on Pituitary Prolactin Activity in *Fundulus similis*

Victor L. de Vlaming, Martin Sage, Connie B. Charlton, and Bob Tiegs

Department of Biology, Marquette University, Milwaukee, Wisconsin, and The University of Texas, Marine Science Institute, Port Aransas, Texas, USA

Received March 25, 1974

Summary. The effect of melatonin treatment on lipid deposition in *Fundulus similis* and *Cyprinodon variegatus* was examined. Melatonin administration stimulates depletion of lipids in *Fundulus* and *Cyprinodon* acclimated to a long photoperiod; the action of this indolamine on lipid metabolism is independent of injection time in *Fundulus*, but not in *Cyprinodon*. Treatment of *Fundulus* with melatonin also causes a decrease in body fat in May when animals are maintained on a short photoperiod. However, an increase in lipids was observed in *Fundulus* acclimated to a short photoperiod in July and treated with melatonin. These data suggest that the effects of melatonin on lipid deposition may depend on season and photoperiod conditions. Pituitary prolactin activity is reduced in *Fundulus* treated with melatonin. The possibility that prolactin mediates the effects of melatonin on lipid metabolism is discussed.

Introduction

In most aquatic environments in the north temperate latitudes food availability varies seasonally and annual cycles of growth, reproduction and fattening are normally observed in fishes. Annual cycles of fattening in teleost fishes frequently show a negative relationship to gonadal cycles (e.g., Nikolsky, 1963; Lasker, 1970; de Vlaming, 1971). Environmental factors such as temperature and photoperiod are used as cues to maintain annual reproductive cycles in fishes (for review see, de Vlaming, 1972), but little is known about environmental control of fattening cycles.

The role of the endocrine system in regulating lipid metabolism in teleosts has received little attention. Meier and his co-workers (Lee and Meier, 1967; Meier, 1969; Joseph and Meier, 1971; Meier *et al.*, 1971) have shown that mammalian prolactin has a marked affect on lipid deposition in *Fundulus grandis* and *F. chrysotus*. More specifically, there is a diurnal variation in the lipogenic response to prolactin; injections given early in the photoperiod cause lipid catabolism whereas injections given later in the photoperiod stimulate fat deposition. This diurnal lipogenic response to mammalian prolactin has been verified in *F. kansae* (Mehrle and Fleming, 1970), *F. similis* and *Cyprinodon variegatus* (de Vlaming and Sage, 1972). Recent data obtained in our laboratories suggest that fish prolactin also affects lipid metabolism, that fattening is controlled by photoperiod and that there is a diurnal rhythm in pituitary prolactin activity in *F. similis* (Sage and de Vlaming, 1973). Evidence is also available which implies that prolactin secretion varies seasonally in *Gasterosteus aculeatus* and is regulated

by photoperiod changes (Lam and Hoar, 1967; Lam and Leatherland, 1969). The translation of photoperiod information into prolactin secretion is not presently understood.

The mammalian pineal is presently viewed as a gland of internal secretion; photosensitivity of the pineal's biochemical rhythms, coupled with the photodependency of effects of pinealectomy has led many investigators to infer that the pineal acts as a neuroendocrine transducer of photoperiod information for endocrine and physiological timing (for review see, Reiter, 1973). A large body of evidence has accumulated which suggests that the active pineal principle(s) in mammals may be one or more of the biogenic amines synthesized by the gland (e.g., Antón-Tay, 1971; Fraschini *et al.*, 1971). Two of these indolamines, melatonin and serotonin, increase plasma prolactin in rats (Kamberi *et al.*, 1971). Furthermore, Donofrio and Reiter (1972) suggested that the pineal gland secretions decrease pituitary prolactin levels in the rat. In contrast, pinealectomy of rats was reported to increase pituitary prolactin and decrease plasma prolactin levels (Relkin, 1972; Relkin *et al.*, 1972).

Several light and electron microscope studies have shown that the sensory cells present in the teleost pineal are similar to the ciliary type of photosensory cells present in the retina of the eye (for review see, Oksche *et al.*, 1971). Electrophysiological studies (for reviews see, Fenwick, 1970a; Oksche *et al.*, 1971) have shown a light induced potential in the pineal of various teleosts. Histological examination of the pineal in various teleosts reveal secretory gland characteristics (e.g., Takahashi, 1969; Rizkalla, 1970; Hafeez, 1971). Chéze and Lahaye (1969) presented evidence for photic influence on the secretory activity in the pineal of *Gambusia affinis*. In the glandular appearing pineal of *Symphodus melops* there is a diurnal variation in stainable granulation (Chéze, 1969). Furthermore, melatonin has been isolated from the pineal gland of teleosts (Fenwick, 1970b). Despite the probable role of the pineal as a transducer of environmental light information and the occurrence of melatonin in the epiphysis, little data is available linking the pineal to physiological and endocrine function in the lower vertebrates.

This investigation was initiated to examine the effects of melatonin treatment on fattening in *Fundulus similis* and *Cyprinodon variegatus*. In addition, the effect of melatonin treatment on pituitary prolactin activity in *F. similis* was investigated.

Materials and Methods

The *Fundulus* and *Cyprinodon* used in these studies were obtained at various times during the year by seining in Corpus Christi Pass near Port Aransas, Texas. The sexually mature male fish used for experimental purposes were placed in 18 liter plastic aquaria (no more than 10 fish per aquarium) supplied with aerated sea water. All *Fundulus* used in these experiments weighed between 7.2 and 8.9 and body weight of the *Cyprinodon* varied between 3.6 and 5.1 g. These animals were acclimated to different temperature and photoperiod regimes for various periods before beginning treatment with melatonin (see Results). Salinity was maintained at a constant $27^0/_{00}$ in all experiments to reduce the possibility of variation in endogenous prolactin levels. The longest daylength in Port Aransas is approximately 13.5 hours, so we will refer to a 13 hour light, 11 hour dark (13L/11D) regime as a long photoperiod and 10L/14D as a short photoperiod. Fish were fed daily *ad libitum* on a commercial fish food (Tetra-Min).

Whether the effect of melatonin on fattening varied according to injection time was studied in some experiments. In these experiments, fish were divided into four groups which served as early and late injection groups and their respective controls. The experimental groups received daily intraperitoneal injections of melatonin (Sigma Chemical Co.) at 2 hours or 8 hours after the onset of the photoperiod; controls for each group were injected with the vehicle (marine teleost saline) at the same time. In some experiments a group of animals was sacrificed on the day of collection to determine the initial level of body fatness; these groups will be referred to as initial controls. Comparison of experimental groups to the initial controls allows one to access the effects of laboratory maintenance on lipid levels, as well as whether hormone treatments actually cause a change in body fatness or simply maintain the initial condition.

In all experiments animals were killed 24 hours after the last injection by severing the spinal cord. Following removal of the gonads, the animals were weighed and placed in a vacuum oven (65° C) for 48 hours for desication. Dried carcasses were weighed and then extracted in a solution of chloroform, ether and methanol (1:1:1) for 12 days. Following the extraction and another drying period, the carcasses were again weighed. The difference in the two dry weights was taken to be body lipids. In some experiments the testes were weighed to obtain information on gonadal activity.

The effect of melatonin on pituitary prolactin levels was investigated in *Fundulus similis* collected in January and February. Experimental conditions, a 11L/13D photoperiod at ambient temperature, were essentially those of the natural environment. Experiments were conducted in January and February using essentially the same acclimation and injection procedures. Experimental fish received a total of five daily injections of melatonin (5 μg/fish) four hours after the onset of the photoperiod; control animals received saline injections at the same time. Animals were sacrificed at two different times of day, either 2–3 hours or 6–7 hours after the onset of the photoperiod. The fish were killed by decapitation, the pituitary dissected out and acetone dried. Pituitary prolactin was assayed by its effect in dispersing pigment of the xanthophores in *Gillichthys mirabilis*; this assay has been described in detail (Sage and Bern, 1972).

Results

An experiment was initiated in January (natural photoperiod = 10.5 hrs light) with *F. similis* which had been acclimated for nine days to a long (13L/11D) photoperiod at 9° to 12° C. One group of animals received 15 daily injections of 3 μg/fish of melatonin two hours after the onset of the light phase and another group was treated with melatonin at eight hours after the onset of the light phase. Body lipid levels were significantly lower in both groups of melatonin treated fish compared to the respective control groups (Table 1). Body lipids did not differ in the two melatonin groups. Testicular size was also significantly lower in the melatonin-treated groups. In this experiment, as well as in the experiments reported below, treatment with melatonin caused fish to become pale.

Another experiment was initiated in May 1971 (natural photoperiod = 13 hrs light) with *F. similis* which had been acclimated for seven days to a 13L/11D photoperiod at 15.5° to 18° C. Treatment procedures were as mentioned above except that experimental animals received ten daily 5 μg/fish injections of melatonin. Lipid levels in all four groups of fish decreased compared to the initial controls (a sample sacrificed at the time of collection). Lipid depletion was, however, greater in the melatonin treated groups (Table 2). Dry lipid indices in both groups treated with the indolamine were significantly less than in the respective saline injected control groups. The gonosomatic index was significantly lower in both groups receiving melatonin.

Table 1. The effects of melatonin treatment in January on body lipids in male *Fundulus similis* acclimated to a long (13L/11D) photoperiod[a]

Treatment	*n*	Body lipids[b]	Gonosomatic index
		mg/g dry weight ($\bar{X} \pm$ S.E.)	Wt. gonads/body wt. $\times 100$ ($\bar{X} \pm$ S.E.)
Saline injected 2 hrs after onset of photoperiod[c]	10	69.2 ± 2.8	4.93 ± 0.55
Melatonin injected 2 hrs after onset of photoperiod[c] (3 μg/fish/day)	7	53.7 ± 3.1[d]	3.45 ± 0.40[d]
Saline injected 8 hrs after onset of photoperiod[c]	6	71.8 ± 3.9	5.13 ± 0.66
Melatonin injected 8 hrs after onset of photoperiod[c] (3 μg/fish/day)	8	54.4 ± 3.0[d]	3.06 ± 0.46[d]

[a] Temperature varied between 9° and 12° C.
[b] Less gonadal lipids.
[c] Animals given a total of 15 injections.
[d] Significantly less ($P < 0.05$) than saline injected controls.

Table 2. The effect of melatonin treatment in May 1971 on body lipids in male *Fundulus similis* acclimated to a long (13L/11D) photoperiod[a]

Treatment	*n*	Body lipids[b]	Gonosomatic index
		mg/g dry weight ($\bar{X} \pm$ S.E.)	Wt. gonads/body wt. $\times 100$ ($\bar{X} \pm$ S.E.)
Initial controls (sacrificed at time of collection)	9	73.9 ± 3.5	4.53 ± 0.26
Saline injected 2 hrs after onset of photoperiod[c]	8	56.4 ± 5.0	5.94 ± 0.23
Melatonin injected 2 hrs after onset of photoperiod[c] (5 μg/fish/day)	8	43.0 ± 3.3[d]	4.56 ± 0.14[d]
Saline injected 8 hrs after onset of photoperiod[c]	8	57.6 ± 6.1	6.35 ± 0.19
Melatonin injected 8 hrs after onset of photoperiod[c] (5 μg/fish/day)	8	36.7 ± 4.8[d]	4.37 ± 0.27[d]

[a] Temperature varied between 15.5° and 18° C.
[b] Less gonadal lipids.
[c] Animals given a total of 10 injections.
[d] Significantly less ($P < 0.01$) than saline injected controls.

Table 3. The effect of melatonin treatment in May 1972 on body lipids in male *Fundulus similis* acclimated to a short (10L/14D) photoperiod[a]

Treatment	n	Body lipids[b]	Gonosomatic index
		mg/g dry weight ($\bar{X} \pm$S.E.)	Wt. gonads/body wt. $\times 100$ ($\bar{X} \pm$S.E.)
Initial controls (sacrificed at time of collection)	12	39.6 ± 1.5	5.83 ± 0.66
Saline injected 8 hrs atfer onset of photoperiod[c]	8	81.1 ± 2.6	13.37 ± 0.81
Melatonin injected 8 hrs after onset of photoperiod[c] (2 μg/fish/day)	8	60.8 ± 1.3[d]	7.24 ± 0.48[d]

[a] Temperature varied between 17° and 19° C.
[b] Less gonadal lipids.
[c] Animals given a total of 11 injections.
[d] Significantly less ($P < 0.001$) than saline injected controls.

In May 1972 a group of *F. similis* was collected and acclimated for 14 days to a short photoperiod at 17° to 19° C. Body lipids were considerably lower in the initial controls of May 1972 than in May 1971 (Tables 2 and 3). This difference could be due to the warmer temperatures and the more advanced stage of gonadal development in 1972. Furthermore, the May 1971 sample was obtained early during the month, whereas the 1972 sample was obtained late in May. Experimental animals were treated daily with 2 μg of melatonin or saline for 11 days. On this short photoperiod body lipids and testicular size increased in both control and melatonin treated groups compared to the initial controls (Table 3). Fat content and testicular weight in the melatonin-treated animals were significantly less ($P < 0.001$) than in the control fish.

The above experiments were conducted during the prespawning and spawning seasons. An experiment was begun in the postspawning season (July) with both *Fundulus* and *Cyprinodon*. Both species were acclimated for ten days to a long (13L/11D) photoperiod at 21.5° to 24° C prior to treatment with melatonin. The *Cyprinodon* were divided into four groups which received eight daily injections of melatonin (5 μg/fish) or saline at two or eight hours after the onset of the light phase. The *Fundulus* received the same number of injections and dose at eight hours after the onset of the light period (Table 4). In both saline injected *Cyprinodon* groups fat levels increased compared to the initial controls, but decreased in the melatonin treated groups. Compared to the respective control groups the lipid indices in the melatonin groups were significantly lower. The group receiving melatonin injections early in the photoperiod had significantly lower ($P < 0.05$) fat content than the group receiving melatonin late in the light period. Body fatness decreased in both the saline and melatonin injected *Fundulus* (Table 4). Lipid levels were, however, significantly lower ($P < 0.001$) in the melatonin treated fish than in the control group.

Table 4. The effect of melatonin treatment in July on body lipids in male *Fundulus similis* and *Cyprinodon variegatus* acclimated to a long (13L/11D) photoperiod[a]

Treatment	*n*	Body lipids[b] mg/g dry weight ($\bar{X} \pm$ S.E.)
	Cyprinodon	
Initial controls (sacrificed at time of collection)	11	74.3 ± 5.7
Saline injected 2 hrs after onset of photoperiod[c]	6	84.6 ± 2.1
Melatonin injected 2 hrs after onset of photoperiod[c] (5 μg/fish/day)	5	65.3 ± 4.4[d]
Saline injected 8 hrs after onset of photoperiod[c]	6	86.7 ± 3.8
Melatonin injected 8 hrs after onset of photoperiod[c] (5 μg/fish/day)	5	73.3 ± 1.9[d]
	Fundulus	
Initial controls	12	62.4 ± 2.6
Saline injected 8 hrs after onset of photoperiod[c]	8	57.1 ± 4.1
Melatonin injected 8 hrs after onset of photoperiod[c] (5 μg/fish/day)	10	37.7 ± 1.8[d]

[a] Temperature varied between 21.5° and 24° C.
[b] Less gonadal lipids.
[c] Animals given a total of 8 injections.
[d] Significantly less ($P < 0.01$) than saline injected controls.

Another group of *Fundulus* was collected in July and acclimated for 30 days to a short (10L/14D) photoperiod at 22° to 24° C. These animals were divided into four groups, one group treated daily with 5 μg of melatonin early in the light phase for ten days and another group receiving melatonin late in the light period. The other two groups served as controls (Table 5). In contrast to the results obtained in the other experiments, lipid levels in both melatonin treated groups were significantly greater ($P < 0.01$) than in the respective control groups. These data suggest that the effect of melatonin on fattening may depend on photoperiod and season. No differences were observed in lipid levels in the groups treated with melatonin early and late in the light period.

Melatonin treatment resulted in an approximately tenfold reduction in prolactin activity in the pituitary glands of *Fundulus similis* (Table 6). This effect was observed in pituitary glands obtained 2–3 hours and 6–7 hours after the onset of the photoperiod. Essentially identical results were obtained in the experiments initiated in January and February.

Table 5. The effects of melatonin treatment in July on body lipids in male *Fundulus similis* acclimated to a short (10L/14D) photoperiod[a]

Treatment	n	Body lipids[b]
		mg/g dry weight ($\bar{X} \pm$S.E.)
Saline injected 2 hrs after onset of photoperiod[c]	8	58.3 ± 3.6
Melatonin injected 2 hrs after onset of photoperiod[c] (5 μg/fish/day)	8	78.3 ± 3.4[d]
Saline injected 8 hrs after onset of photoperiod[c]	8	54.0 ± 4.1
Melatonin injected 8 hrs after onset of photoperiod[c] (5 μg/fish/day)	6	73.5 ± 3.5[d]

[a] Temperature varied between 22° and 24° C.
[b] Less gonadal lipids.
[c] Animals given a total of 10 injections.
[d] Significantly greater ($P<0.01$) than saline injected controls.

Table 6. The effect of melatonin treatment in January on pituitary prolactin activity in *Fundulus similis* acclimated to a 11L/13D photoperiod

Treatment	n	Pituitary prolactin activity
		Log units[a] ($\bar{X} \pm$S.E.)
Saline injected controls (killed 2–3 hrs after onset of photoperiod)	6	1.42 ± 0.08
Saline injected controls (killed 6–7 hrs after onset of photoperiod)	6	1.17 ± 0.20
Combined controls	12	1.29 ± 0.11
Melatonin injected (killed 2–3 hrs after onset of photoperiod)	5	0.60 ± 0.27[b]
Melatonin injected (killed 6–7 hrs after onset of photoperiod)	6	0.50 ± 0.00[b]
Combined melatonin injected	11	0.55 ± 0.11[b]

[a] See Sage and Bern (1972).
[b] Significantly less ($P<0.01$) than respective controls.

Discussion

The data presented here show that melatonin has a marked effect on lipid deposition in cyprinodontid fish. In *F. similis* and *Cyprinodon variegatus* melatonin treatment reduced body lipids if animals were exposed to a long photoperiod. Lipid deposition is also reduced if *Fundulus*, maintained on a short photo-

period, are administered melatonin in May, but body fats increase if animals are held on a short photoperiod in July and treated with melatonin. These results suggest that the effects of melatonin on fattening in this species may vary with season and photoperiod acclimation. The variation in response to melatonin in May and July could have been a consequence of temperature and/or dose differences in the two experiments. Other recent investigations, however, have shown that the effects of pinealectomy or melatonin treatment vary with season and photoperiod acclimation (Fenwick, 1970c, d; Urasaki, 1972a, b; de Vlaming *et al.*, 1974). It is of interest to note that preliminary results obtained in one of our laboratories (VLV) indicate that melatonin can influence lipid deposition in *Ictalurus natalis* and two species of hylid frogs. Results presented in Tables 1–3 show that melatonin therapy can also affect gonadal development. The effects of melatonin on reproductive function in *F. similis* are discussed in more detail elsewhere (de Vlaming *et al.*, 1974).

The mechanism by which melatonin influences fat deposition is not completely understood. Our data do imply that melatonin treatment reduces pituitary prolactin activity in *F. similis*. Evidence is also available which shows that both fish and mammalian prolactin have a pronounced effect on fat metabolism in *Fundulus* (de Vlaming and Sage, 1972). The diurnal rhythm in pituitary prolactin activity observed in *F. similis* (Sage and de Vlaming, 1973) suggests photoperiod control of synthesis or release of this hormone; such an effect could be mediated via the pineal. Results presented here and other unpublished data indicate that long photoperiods cause fat depletion in *F. similis*. If this species is treated with prolactin early after the onset of a long photoperiod loss of body fat is accentuated, whereas prolactin injections late in the light phase retard lipid depletion (de Vlaming and Sage, 1972). We presented data (Sage and de Vlaming, 1973) which suggest that in *F. similis* prolactin is stored in the pituitary during the dark phase and release occurs soon after the onset of the light phase in a long photoperiod. Exogenous prolactin given early during the light period apparently potentiates the lipid catabolic effects of endogenous prolactin released early during the light phase. When *Fundulus* is injected with melatonin soon after the light period begins fat depletion is accentuated. If melatonin stimulates release of prolactin soon after the onset of the light phase a decrease in body fat would be expected. The decrease in pituitary prolactin activity following melatonin therapy does imply that this indolamine promotes release. Furthermore, the effects of melatonin on lipid metabolism in *Fundulus* may be mediated, at least in part, by prolactin. Data are available which link melatonin with prolactin release in mammals (Kamberi *et al.*, 1971; Donofrio and Reiter, 1972).

Circulating levels of prolactin in *F. similis* appear to be low during the latter portion of a long photoperiod light phase (Sage and de Vlaming, 1973); if these animals are treated with exogenous prolactin during this time, the effects of a long photoperiod on lipid depletion are reversed (de Vlaming and Sage, 1972). If melatonin stimulates release of prolactin during the latter portion of the light period one might expect fat deposition. However, our data show that *Fundulus* lose body fat when given melatonin injections eight hours after the onset of a

long photoperiod. The lipid depletion resulting from afternoon melatonin therapy could be due to a phase shift in the rhythm of pituitary prolactin secretion.

Short photoperiods cause fat deposition in *F. similis*. Our results (de Vlaming *et al.*, unpublished data) imply that prolactin release is low during the light phase of a short photoperiod. Furthermore, prolactin injections given either early or late during the light period reverse the effects of a short photoperiod, causing a loss of body fats. Melatonin treatment of *Fundulus* collected in May and acclimated to a short photoperiod also resulted in fat depletion. Possibly this effect was mediated by prolactin. In view of results obtained with other teleost species (Sage, 1972, 1973) and the effect of melatonin and prolactin on body fat, the decrease in pituitary prolactin activity observed in *F. similis* following melatonin therapy probably reflects an increase in prolactin release. Existing data suggest that in teleost fishes prolactin cells are under inhibitory hypothalamic control via Type "B" neurosecretory fibers (for review see, Zambrano *et al.*, 1974). Whether melatonin acts via this pathway or directly on the pituitary prolactin cells is not presently known.

The effects of prolactin on lipid deposition in *Cyprinodon variegatus* also vary with injection time (de Vlaming and Sage, 1972). Results presented here show that melatonin causes a loss of body fat in this species when the fish are maintained on a long photoperiod. Injections given early during the light period, however, result in a more pronounced loss of fat than when melatonin is administered during the latter portion of the light phase. In *Fundulus*, melatonin injections given either two or eight hours after the onset of the light period were equally effective in causing fat depletion. The differences in responsiveness to melatonin in the two species may be due to dissimilar patterns of pituitary prolactin release or discrepant responses to photoperiod. Long photoperiods do, in fact, induce fat deposition in *Cyprinodon*.

We do not wish to imply that the effects of melatonin on fattening are mediated solely by prolactin. Indeed, other hormones (e.g., cortisol and gonadotropins) have been implicated in the regulation of lipid metabolism in teleosts. Melatonin may also influence the release of other pituitary hormones which could affect lipid metabolism; in fact, this indolamine apparently has an effect on release of gonadotropin in *Fundulus* (de Vlaming *et al.*, 1974). It is also possible, although in our view unlikely, that melatonin acts directly on stored lipid. In fact, melatonin had no effect on *in vitro* liver lipid metabolism in the golden shiner, *Notemigonus chrysoleucas*, whereas cortisol, prolactin, growth hormone and insulin had profound effects (de Vlaming and Wheeler, unpublished data).

The data presented here contribute to the view that the pineal is involved in regulating endocrine and physiological functions in teleost fish. The pineal and melatonin have been implicated in regulation of reproductive function, thyroid function, growth and chromatophores (for review see, Fenwick, 1970a). Combined with the evidence of a photosensory and secretory function, these data indicate a probable neuroendocrine role of the fish pineal, possibly based on the hormone melatonin.

This work was supported in part by NSF grant GB 22995 (to MS) and a grant from the Marquette University Committee on Research (to VLV).

References

Antón-Tay, F.: Pineal-brain relationships. In: The pineal gland (G. E. W. Wolstenholm, G. Knight, eds.), p. 213–220. Edinburg and London: Churchill Livingstone 1971

Chéze, G.: Innervation épiphysaire chez "*Symphodus melops*" (Poisson-Labridae). Bull. Soc. Zool. Fr. **96**, 53–59 (1959)

Chéze, G., Lahaye, J.: Étude morphologique de la region épiphysaire de *Gambusia affinis holbrooki*. Incidences histologiques de certain facteurs externes sur le toit diencéphalique. Ann. Endocr. (Paris) **30**, 45–53 (1969)

Donofrio, R. J., Reiter, R. J.: Depressed pituitary prolactin levels in blinded anosmic female rats: role of the pineal gland. J. Reprod. Fertil. **31**, 159–162 (1972)

Fenwick, J. C.: The pineal organ. In: Fish physiology (W. S. Hoar, D. J. Randall, eds.), vol. IV, p. 91–108. New York: Academic Press 1970a

Fenwick, J. C.: Demonstration and effect of melatonin in fish. Gen. comp. Endocr. **14**, 86–97 (1970b)

Fenwick, J. C.: The pineal organ: photoperiod and reproductive cycles in the goldfish, *Carassius auratus* L. J. Endocr. **46**, 101–111 (1970c)

Fenwick, J. C.: Effects of pinealectomy and bilateral enucleation on the conditioned response to light of the goldfish *Carassius auratus* L. Canad. J. Zool. **48**, 175–183 (1970d)

Fraschini, F., Collu, R., Martini, L.: Mechanisms of inhibitory action of pineal principles on gonadotropin secretion. In: The pineal gland (G. E. W. Wolstenholm, G. Knight, eds.), p. 259–273. Edinburg and London: Churchill Livingstone (1971)

Hafeez, M. A.: Light microscope studies on the pineal organ in teleosts fishes with special regard to its function. J. Morph. **134**, 281–314 (1971)

Joseph, M. M., Meier, A. H.: Daily variations in the fattening response to prolactin in *Fundulus grandis* held on different photoperiods. J. exp. Zool. **178**, 59–62 (1971)

Kamberi, I. A., Mical, R. S., Porter, J. C.: Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology **88**, 1288–1293 (1971)

Lam, T. J., Hoar, W. S.: Seasonal effects of prolactin on freshwater osmoregulation of the marine form (*trachurus*) of the stickleback *Gasterosteus aculeatus*. Canad. J. Zool. **45**, 509–516 (1967)

Lam, T. J., Leatherland, J. F.: Effect of prolactin on freshwater survival of the marine form (*trachurus*) of the threespine stickleback, *Gasterosteus aculeatus* in early winter. Gen. comp. Endocr. **12**, 385–387 (1969)

Lasker, R.: Utilization of zooplankton energy by a Pacific sardine population in the California current. In: Marine food chains (D. H. Steele, ed.), p. 265–284. Berkeley: U.C. Press 1970

Lee, R. W., Meier, A. H.: Diurnal variations of the fattening response to prolactin in the golden topminnow, *Fundulus chrysotus*. J. exp. Zool. **166**, 307–316 (1967)

Mehrle, P. M., Fleming, W. R.: The effect of early and midday prolactin injection on the lipid content of *Fundulus kansae* held on a constant photoperiod. Comp. Biochem. Physiol. **36**, 597–603 (1970)

Meier, A. H.: Diurnal variations of metabolic responses to prolactin in lower vertebrates. Gen. comp. Endocr., Suppl. **2**, 55–62 (1969)

Meier, A. H., Trobec, T. N., Joseph, M. M., John, T. M.: Temporal synergism of prolactin and adrenal steroids in the regulation of fat stores. Proc. Soc. exp. Biol. (N.Y.) **137**, 408–415 (1971)

Nikolsky, G. V.: The ecology of fishes, pp. 209–217. New York: Academic Press 1963

Oksche, A., Ueck, M., Rüdeberg, C.: Comparative ultrastructural studies of sensory and secretory elements in pineal organs. In: Subcellular organization and function in endocrine tissues (H. Heller, K. Lederis, eds.), p. 7–25. Mem. Soc. Endocr. No. **19** (1971). Cambridge: University Press

Relkin, R.: Rat pituitary and plasma prolactin levels after pinealectomy. J. Endocr. **53**, 179–180 (1972)

Relkin, R., Adachi, M., Kahan, S. A.: Effects of pinealectomy and constant light and darkness on prolactin levels in the pituitary and plasma and on pituitary ultrastructure of the rat. J. Endocr. **54**, 263–268 (1972)

Rizkalla, W.: The morphology of the pineal organ in the teleost, *Clarias lazers* C.V. Acta biol. Acad. Sci. hung. **21**, 25–35 (1970)

Sage, M.: Prolactin content of pituitaries from euryhaline teleosts transferred from sea water to fresh water. Proc. IV Int. Congr. Endocrinol. Wash., p. 139. Amsterdam: Excerpta Medica (1972)

Sage, M.: The relationship between the pituitary content of prolactin and blood sodium levels in the mullet (*Mugil cephalus*) transferred from sea water to fresh water. Contr. Mar. Sci. (in press, 1974)

Sage, M., Bern, H. A.: Assay of prolactin in vertebrate pituitaries by its dispersion of xanthophore pigment in the teleost *Gillichthys mirabilis*. J. exp. Zool. **180**, 169–174 (1972)

Sage, M., de Vlaming, V. L.: A diurnal rhythm in hormone effectiveness and in pituitary content of prolactin in *Fundulus similis*. Tex. Rpt. Biol. Med. **31**, 101–102 (1973)

Takahashi, H.: Light and electron microscope studies on the pineal organ of the goldfish, *Carassius auratus* L. Bull. Fac. Fish. Hokkaido Univ. **20**, 143–157 (1969)

Urasaki, H.: Role of the pineal gland in gonadal development in the fish *Oryzias latipes*. Annot. Zool. Jap. **45**, 152–158 (1972a)

Urasaki, H.: Effects of restricted photoperiod and melatonin administration on gonadal weight in the Japanese killifish. J. Endocr. **55**, 619–620 (1972b)

de Vlaming, V. L.: The effects of food availability and salinity on reproductive function in the estuarine goby, *Gillichthys mirabilis*. Biol. Bull. **141**, 458–471 (1971)

de Vlaming, V. L.: Environmental control of teleost reproductive cycles: A brief review. J. Fish. Biol. **4**, 131–140 (1972)

de Vlaming, V. L., Sage, M.: Diurnal variation in fattening response to prolactin treatment in two Cyprinodontid fishes, *Cyprinodon variegatus* and *Fundulus similis*. Contr. Mar. Sci. **16**, 59–63 (1972)

de Vlaming, V. L., Sage, M., Charlton, C. B.: The effects of melatonin treatment on gonosomatic index in the teleost, *Fundulus similis*, and the tree-frog, *Hyla cinerea*. Gen. comp. Endocr. **22**, 433–438 (1974)

Zambrano, D., Clarke, W. C., Hawkins, E. F., Sage, M., Bern, H.: Influence of 6-hydroxydopamine on hypothalamic control of prolactin and ACTH secretion in the teleost fish, *Tilapia mossambica*. Neuroendocrinology (in press, 1974)

Dr. Victor L. de Vlaming
Biology Department
Marquette University
Milwaukee, Wisconsin 53233, USA