

EFFECTS OF PHOTOPERIOD-TEMPERATURE REGIMES AND PINEALECTOMY ON BODY FAT RESERVES IN THE GOLDEN SHINER, *NOTEMIGONUS CRYSOLEUCAS*

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ABSTRACT

Various photoperiod-temperature regimes were examined for their effects on total fat content (excluding gonads) in *Notemigonus crysoleucas*; experiments were conducted during several different phases of the reproductive cycle. In *Notemigonus*, fattening normally occurs in fall and early winter concomitant with the early phases of gonadal development. Body fat stores are progressively depleted during the prespawning and spawning seasons. Warm temperature (25°C) normally favored body fat depletion in *Notemigonus*. Short photoperiod (9L/15D) accentuated the lipid depleting effects of warm temperatures. Low temperatures (12°-15°C) usually promoted lipid deposition. Short photoperiods complimented the lipid anabolic effects of low temperatures. Thus, a given photoperiod can have opposite effects on body fat levels depending on temperature. A long photoperiod, in combination with warm temperatures, is required for final gonadal maturation and results in a reduction of lipid reserves. Short photoperiod-warm temperature regimes have similar effects on fat levels, but bring about gonadal regression. Thus, the effects of photoperiod-temperature regimes on lipid metabolism are apparently not totally dependent on the effects of these environmental factors on reproduction.

The effects of pinealectomy on lipid reserves varied depending on the phase of the natural reproductive cycle when the organ was removed, as well as, with the photoperiod-temperature regime under which the experimental animals were maintained. At 25°C and under a 15.5L/8.5D photoperiod, fat levels were frequently lower in pinealectomized than in sham animals. The opposite was usually true for fish exposed to a 9L/15D-25°C regime. Lipid reserves were normally greater in pinealectomized than in sham operated fish maintained on 15.5L/8.5D-12°C regime. Body fat composition was frequently less in pinealectomized than in sham operated animals exposed to a 9L/15D-12°C regime. Pinealectomy reverses the effects of photoperiod on lipid metabolism at a particular temperature. These results suggest that the pineal body is involved in regulating physiological functions and may serve as a photoreceptor and/or transducer of photoperiod information.

In most temperate-latitude aquatic environments food availability varies seasonally and annual cycles of growth, reproduction, and fattening are normally observed in teleost fishes. Lipid reserves may be used to meet the energy demands of reproduction, and seasonal fattening cycles in teleosts may be related to sexual cycling (Lovern 1934; Lühmann 1953; Love 1957; Idler and Bitners 1960; Woodhead 1960; Nikolsky 1963; Wilkins 1967; Lasker 1970; de Vlaming 1971). Environmental factors such as photoperiod and temperature are used as cues to maintain annual reproductive cycles in fishes (for reviews see de Vlaming 1972, 1974), but little is known about environmental control of fattening.

The pineal body of most fishes has sensory organ characteristics (e.g., Rudeberg 1966, 1969; Omura and Oguri 1969; Owman and Rudeberg 1970;

Bergmann 1971; Oksche et al. 1971). Histological examination of the pineal in various teleosts also reveals secretory gland characteristics (e.g., Takahashi 1969; Chèze and Lahaye 1969; Chèze 1970; Rizkalla 1970; Hafeez 1971). In mammals the pineal appears to function as an endocrine gland and the indolamine, melatonin, may be one of the hormones of this organ (cf. Reiter 1973). Histochemical and biochemical data show that the teleost pineal has an active indolamine metabolism (Quay 1965; Hafeez and Quay 1969, 1970; Fenwick 1970; Owman and Rudeberg 1970). Very little, however, is known about the physiological role of the pineal in teleost fishes.

De Vlaming, Sage, Charlton, and Tiegs (1974) showed that melatonin treatment results in body lipid depletion in *Fundulus similis* and *Cyprinodon variegatus* acclimated to a long photoperiod. In *F. similis* acclimated to a short photoperiod during May, melatonin therapy also resulted in fat depletion, but body fat deposition

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was observed in *F. similis* acclimated to a short photoperiod during July and treated with this indolamine (de Vlaming, Sage, Charlton, and Tiegs 1974). These investigators concluded that the pineal might be somehow involved in regulating body fat reserves in teleosts. The data of de Vlaming, Sage, Charlton, and Tiegs (1974) and others (Fenwick 1970; Urasaki 1972a, b, c) indicate that the influence of the pineal on physiological functions in teleosts may vary depending on season and photoperiod conditions.

The objectives of the present investigation were to examine the effects of various photoperiod-temperature regimes on body lipid reserves in the cyprinid teleost *Notemigonus crysoleucas* and to determine if pinealectomy altered the response of this fish to the experimental regimes. The possible relationship between reproductive activity and body fat reserves was also examined. That is, the effects of photoperiod-temperature regimes and pinealectomy on reproductive activity were determined and compared to the data on fat metabolism. The effects of pinealectomy on reproductive activity and fattening were examined during different phases of the natural sexual cycle (at various times of the year) to determine if physiological responses vary seasonally.

MATERIALS AND METHODS

Samples of *N. crysoleucas* were collected in ponds around the area of Menomonee Falls, Wis. (lat. 43°10'N) at several different times during the year. The reproductive cycle consists of a spawning season which extends from May through July. There is a postspawning season during August and September in which the gonads regress. From October through February there is a gonadal preparatory period, in which spermatogonia proliferate slowly and spermatocytes appear in the testes. Vitellogenesis is initiated during this period. March and April can be referred to as the prespawning period; during this time, final gonadal maturation occurs (i.e., spermatozoa fill the testes and ovaries are distended with mature oocytes). Several fish from each field sample were sacrificed, the gonads examined and body lipid levels determined at the time of collection; these fish served as a reference for the experiments that followed. In the following discussion the fish sacrificed at the time of collection will be identified as initial controls.

Sham operated and pinealectomized fish were maintained under various photoperiod and constant temperature regimes (see Results) in 114- or 285-liter tanks supplied with aerated and filtered dechlorinated tap water. Temperatures selected for these experiments are within the range normally experienced during the year in nature by this species. Illumination was a combination of incandescent and cool white fluorescent bulbs which gave a light intensity of 200 to 275 lx at the surface of each tank. Fish were fed ad libitum on a commercial fish food (Tetra-Min)²; animals maintained at warm temperatures were fed twice daily whereas fish at low temperatures were fed only once a day. All *Notemigonus* used in these studies weighed between 12 and 17 g.

For pinealectomies, fish were anesthetized in buffered tricaine methane-sulfonate (1:4,000). Each fish was then wrapped in cheesecloth and submerged in water so that only the top of the skull was emergent. The section of skin covering the pineal area was cut and folded back to reveal the parietal bone. Using a diamond-edged wheel saw (diameter = 2.2 cm) attached to a dental drill, three sides of a rectangle (5×4 mm) were cut in the parietal bone. This bone flap was then lifted forward toward the animal's mouth to expose parts of the cerebrum and midbrain. The pineal could then be easily removed using a gentle suction applied through a Pasteur pipette. After removal of the pineal, the parietal bone and the epithelial flaps were individually sealed into place with Eastman's 910 Adhesive. Sham operations consisted of raising the parietal bone flap without removing the pineal. Removal of the pineal can be completed within 2 min in this species.

The effects of pinealectomy on reproductive function were assessed by gravimetric and histological techniques. Fish were sacrificed by severing the spinal cord. Body weight and gonadal weight were recorded immediately after sacrifice. Gravimetric data are expressed in terms of the gonosomatic index (GSI) (gonadal weight/body weight × 100) since gonadal size in this species depends on body weight. After weighing, gonads were fixed in Bouin's solution and embedded in paraplast for histological examination. The data obtained on the effects of pinealectomy on reproductive function in *Notemigonus* are the subject of another report (de Vlaming 1975). GSI

²Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

data are presented here without statistical comparisons or extensive discussion so that comparisons can be made with the results on body lipid reserves. After removal of the gonads, the bodies of the fish were extracted to remove lipids. The procedure used to measure body fat content has been previously described (de Vlaming, Sage, Charlton, and Tiegs 1974; de Vlaming et al. in press). Basically this technique consists of extracting in a methanol:chloroform:ether solution (1:1:1). Body fat content is expressed as a function of dry body weight.

RESULTS

Preparatory Season

The effects of pinealectomy on body lipid reserves were first examined during the gonadal preparatory period (January). The results of this experiment are summarized in Table 1.

Body lipid reserves were significantly depleted in both male and female sham operated fish maintained at 25°C (whether on a long or short photoperiod) compared to the initial January controls.

TABLE 1.—The effects of pinealectomy on body lipid reserves in *Notemigonus* maintained on various photoperiod-temperature regimes during the gonadal preparatory season (30-day treatment).

Treatment	n	Sex	Dry lipid index ¹ $\bar{X} \pm SE$	Gonosomatic index ² $\bar{X} \pm SE$
Initial controls (January)	5	M	248 ± 11	1.48 ± 0.09
	9	F	207 ± 10	3.03 ± 0.25
15.5L/8.5D photoperiod:				
25°C:				
Sham operated	6	M	136 ± 7	2.82 ± 0.07
	8	F	164 ± 11	4.67 ± 0.42
Pinealectomized	5	M	119 ± 5*	0.34 ± 0.18
	10	F	139 ± 8*	2.88 ± 0.37
12°C:				
Sham operated	6	M	235 ± 10	1.95 ± 0.16
	8	F	211 ± 7	3.42 ± 0.08
Pinealectomized	6	M	246 ± 9	1.20 ± 0.04
	10	F	237 ± 5*	3.47 ± 0.13
9L/15D photoperiod:				
25°C:				
Sham operated	5	M	118 ± 8	0.94 ± 0.09
	8	F	96 ± 6	1.78 ± 0.22
Pinealectomized	6	M	106 ± 5	0.89 ± 0.16
	9	F	109 ± 11	1.64 ± 0.21
12°C:				
Sham operated	6	M	263 ± 12	1.28 ± 0.11
	10	F	244 ± 9	3.40 ± 0.21
Pinealectomized	5	M	250 ± 15	1.19 ± 0.18
	8	F	221 ± 6*	4.12 ± 0.35

¹Dry lipid index = mg lipid, less gonadal lipids/g dry body wt.

²Gonosomatic index = wt of gonads (g)/g body wt x 100.

*Significantly ($P < 0.05$) different than sham operated controls maintained under same photoperiod-temperature regime.

In both sexes of sham operated fish exposed to the long photoperiod-low temperature regime body lipid stores were maintained at the initial levels. Short photoperiod-low temperature treatment, however, caused a significant increase in fat stores in sham operated females, but not in sham operated males.

In sham operated control fish maintained on a long photoperiod, body lipid levels were significantly higher in the group at 12°C than in the group at 25°C. Fat levels were also significantly greater in sham operated *Notemigonus* maintained at 12°C than at 25°C on a short photoperiod. These data suggest that low temperatures, regardless of daylength, either maintain or favor body lipid deposition in this species.

At 12°C, a short photoperiod was more effective in maintaining or stimulating lipid deposition than a long photoperiod. Specifically, body fatness (in both sexes) was significantly greater in sham operated fish exposed to the short photoperiod-low temperature regime than in animals maintained on the long photoperiod-low temperature regime. Thus, short daylengths seem to compliment the effects of low temperatures on fat deposition. Body lipid levels were significantly lower in sham operated animals maintained on the short photoperiod-warm temperature regime than in fish exposed to the long photoperiod-warm temperature regime. Body fat depletion in *Notemigonus* at warm temperatures is therefore accentuated by short daylengths.

In animals maintained on the 15.5L/8.5D-25°C regime, body lipid levels were significantly lower in the pinealectomized group than in sham operated group. Pinealectomy also retarded fat deposition in female fish exposed to the 9L/15D-12°C regime. In contrast, body fat levels were significantly greater in the female pinealectomized fish than in the sham operated females maintained on the long photoperiod-low temperature regime. Body lipid levels in pinealectomized fish did not differ significantly from lipid levels in control sham operated animals under any of the other experimental conditions. These data suggest that the effects of pinealectomy on lipid metabolism depend on photoperiod and temperature conditions.

The effects of pinealectomy on reproductive function are discussed elsewhere (de Vlaming 1975). One should note, however, that pinealectomy retarded the stimulatory effects of the 15.5L/8.5D-25°C regime on gonadal maturation;

under these same conditions body lipid levels were also significantly lower in pinealectomized than in sham operated fish. Ovarian GSI was significantly higher in the pinealectomized than in sham operated group exposed to the 9L/15D-12°C regime; however, body fat levels were significantly lower in pinealectomized than in sham operated fish under these conditions. These data imply that the effects of pinealectomy on lipid deposition do not necessarily depend on the effects of this organ on reproductive activity.

Prespawning Season

The effects of pinealectomy on body fat reserves were examined again during the prespawning season (Table 2).

Regardless of photoperiod, low temperature treatment of both sexes of sham operated *Notemigonus* resulted in a significant increase in body fatness, compared to the initial March controls. Warm temperature treatment, however, caused a depletion of body lipids in sham operated fish compared to the initial controls; this fat depletion was observed in both photoperiod groups.

Body fatness (in females) was significantly greater in sham operated control fish exposed to the 9L/15D-12°C regime than in animals maintained on the 15.5L/8.5D-12°C regime. These data further confirm the suggestion that short daylengths compliment the effects of low temperatures on fattening. Body lipid levels (both sexes) were significantly lower in sham operated animals maintained on the short photoperiod-warm temperature regime than in sham operated fish exposed to the long photoperiod-warm temperature regime. The lipid depletion which occurs at warm temperatures is thus accentuated by short daylengths.

Body fat levels (both sexes) were significantly lower in the pinealectomized group than in the sham operated group maintained on the 15.5L/8.5D-25°C regime. In animals maintained on a long photoperiod-low temperature regime, body lipid levels were significantly greater in the pinealectomized fish than in the shams. Pinealectomized females contained significantly more fat than sham operated females exposed to the short photoperiod-warm temperature regime. In contrast, body lipid levels (both sexes) were significantly lower in pinealectomized than in sham operated fish maintained on the 9L/15D-12°C

TABLE 2.—The effects of pinealectomy on body lipid reserves in *Notemigonus* maintained on various photoperiod-temperature regimes during the prespawning season (21-day treatment).

Treatment	n	Sex	Dry lipid index ¹ X ± SE	Gonosomal index ² X ± SE
Initial controls (March)	5	M	209 ± 10	1.96 ± 0.17
	9	F	184 ± 12	3.42 ± 0.09
15.5L/8.5D photoperiod:				
25°C:				
Sham operated	6	M	140 ± 11	1.35 ± 0.07
	8	F	131 ± 5	2.41 ± 0.15
Pinealectomized	6	M	112 ± 10*	0.80 ± 0.09
	7	F	107 ± 8*	1.58 ± 0.22
12°C:				
Sham operated	6	M	232 ± 8	2.00 ± 0.10
	7	F	244 ± 7	3.41 ± 0.26
Pinealectomized	6	M	251 ± 6*	1.79 ± 0.14
	7	F	264 ± 7*	3.18 ± 0.33
9L/15D photoperiod:				
25°C:				
Sham operated	8	M	129 ± 5	2.02 ± 0.15
	6	F	114 ± 6	1.74 ± 0.11
Pinealectomized	6	M	143 ± 9	1.62 ± 0.04
	7	F	132 ± 8*	2.03 ± 0.13
12°C:				
Sham operated	5	M	243 ± 5	1.31 ± 0.05
	5	F	267 ± 8	3.79 ± 0.28
Pinealectomized	6	M	187 ± 11*	1.20 ± 0.07
	4	F	240 ± 6*	3.43 ± 0.27

¹Dry lipid index = mg lipid, less gonadal lipids/g dry body wt.
²Gonosomal index = wt of gonads (g)/g body wt x 100.
 *Significantly ($P < 0.05$) different than sham operated controls maintained under same photoperiod-temperature regime.

regime. At a low temperature, pinealectomy interferes with the complimentary effects of short photoperiods on lipid deposition. At a warm temperature, however, pinealectomy reverses the accentuating effects of short photoperiods on body lipid depletion.

Interestingly, body lipid levels in pinealectomized females maintained on the 9L/15D-12°C regime did not differ significantly from fat levels in sham operated females exposed to the 15.5L/8.5D-12°C regime. Fat levels were not significantly different in pinealectomized females on the 15.5L/8.5D-12°C regime and sham operated females on the 9L/15D-12°C regime. Similarly, body fat levels in pinealectomized fish (both sexes) exposed to the 9L/15D-25°C regime did not differ significantly from lipid levels in shams maintained on the 15.5L/8.5D-25°C regime. These data indicate that pinealectomy reverses the effects that photoperiod has on lipid levels at a given temperature.

Pinealectomy reverses the stimulatory effects of a long photoperiod-warm temperature regime, causing gonadal regression (de Vlaming 1975); under these conditions body lipid levels in pinealectomized animals are lower than in shams. Short photoperiods in combination with warm

temperatures induce gonadal involution in *Notemigonus*. Pinealectomy under these conditions prevents gonadal regression, stimulating gonadal development and spawning. Body lipid reserves were significantly greater in pinealectomized fish than in shams maintained on the 9L/15D-25°C regime. Under the other photoperiod-temperature regimes, pinealectomy resulted in changes in body fat reserves without appreciably altering reproductive activity. Possibly then, changes in lipid metabolism due to pinealectomy may influence reproductive activity, but apparently the effects of pinealectomy on fat deposition are not totally dependent on changes in sexual activity.

Spawning Season

The effects of pinealectomy on body fat stores were again examined during the early spawning season (late April, Table 3).

In sham operated fish maintained at 25°C (both photoperiod groups), the lipid index was significantly lower than that of the initial controls. Body lipid reserves were also significantly depleted in sham operated female fish exposed to the 9L/15D-25°C regime, but not in sham operated

females maintained on the 15.5L/8.5D-25°C regime compared to the initial controls. Low temperature treatment during the spawning season caused a significant increase in body lipid content of sham operated female fish regardless of photoperiod. The short photoperiod-low temperature regime stimulated fat deposition in sham operated males; however, body lipid levels did not differ significantly in the initial controls and sham operated males maintained on the long photoperiod-low temperature regime.

At 25°C, body lipid stores were slightly higher in sham operated female fish maintained on a long photoperiod than in females exposed to a short photoperiod; the reverse was true for males. At 15°C, sham operated fish maintained on a short photoperiod were significantly fatter than sham operated fish on the long photoperiod regime.

Lipid reserves were significantly lower in female pinealectomized than in sham operated female fish exposed to the 15.5L/8.5D-25°C regime. In fish (both sexes) maintained on the long photoperiod-low temperature regime, body lipid stores were significantly greater in pinealectomized than in sham operated control fish. Body fat content was significantly greater in both sexes of pinealectomized fish than in shams exposed to the 9L/15D-25°C regime. In contrast, lipid content in pinealectomized fish was significantly lower than in sham operated animals on the 9L/15D-15°C regime.

Body lipid reserves were not significantly different in pinealectomized female fish maintained on the 9L/15D-15°C regime compared to the females exposed to the 15.5L/8.5D-15°C regime. Fat composition of pinealectomized females exposed to the long photoperiod-low temperature regime did not differ significantly from fat composition in sham operated females which experienced the short photoperiod-low temperature regime. No significant difference was observed in body fatness in pinealectomized females on the 9L/15D-25°C regime and sham operated females on the 15.5L/8.5D-25°C regime. Similarly, body lipid stores were approximately the same in pinealectomized females maintained on the long photoperiod-warm temperature regime and in sham operated females exposed to the short photoperiod-warm temperature regime.

Many of the fish maintained on the 15.5L/8.5D-25°C regime spawned; under these conditions pinealectomy resulted in the initiation of gonadal regression. Compared to sham operated animals

TABLE 3.—The effects of pinealectomy on body lipid reserves in *Notemigonus* maintained on various photoperiod-temperature regimes during the early spawning season (21-day treatment).

Treatment	n	Sex	Dry lipid index ¹ $\bar{X} \pm SE$	Gonosomatic index ² $\bar{X} \pm SE$
Initial controls (Late April)	6	M	91 ± 2	3.10 ± 0.22
	6	F	88 ± 3	6.53 ± 0.97
15.5L/8.5D photoperiod: 25°C:				
Sham operated	7	M	56 ± 4	2.79 ± 0.25
	7	F	87 ± 6	5.13 ± 1.04
Pinealectomized	5	M	45 ± 5	2.10 ± 0.09
	8	F	62 ± 4*	4.08 ± 0.73
15°C:				
Sham operated	5	M	91 ± 5	2.16 ± 0.09
	6	F	114 ± 6	5.88 ± 0.89
Pinealectomized	5	M	132 ± 8*	2.38 ± 0.14
	6	F	129 ± 6*	7.81 ± 1.22
9L/15D photoperiod: 25°C:				
Sham operated	5	M	68 ± 5	1.16 ± 0.15
	9	F	74 ± 6	2.87 ± 0.52
Pinealectomized	6	M	110 ± 6*	2.67 ± 0.16
	5	F	101 ± 7*	3.50 ± 0.88
15°C:				
Sham operated	6	M	155 ± 10	3.12 ± 0.17
	5	F	138 ± 8	6.43 ± 0.66
Pinealectomized	5	M	124 ± 9*	3.01 ± 0.15
	7	F	117 ± 5*	5.89 ± 0.57

¹Dry lipid index = mg lipid, less gonadal lipids/g dry body wt.

²Gonosomatic index = wt of gonads (g)/g body wt x 100.

*Significantly ($P < 0.05$) different than sham operated controls maintained under same photoperiod-temperature regime.

exposed to the long photoperiod, body lipid levels in pinealectomized fish were significantly lower. Gonadal regression was also initiated in sham operated fish maintained on the 9L/15D-25°C regime; spawning was observed in pinealectomized animals on this regime. Body lipid levels were significantly greater in pinealectomized fish than in sham operated fish maintained on the short photoperiod-warm temperature regime. In animals maintained at a low temperature (both photoperiods), GSIs did not differ significantly in the pinealectomized and sham operated groups. Sham operated fish, however, were significantly fatter than the pinealectomized animals experiencing the 9L/15D-15°C regime. Furthermore, under the 15.5L/8.5D-15°C regime, pinealectomized fish contained significantly more fat than the sham operated controls.

DISCUSSION

Both temperature and photoperiod have a distinct effect on body lipid reserves in *N. crysoleucas*. During the prespawning and spawning seasons, low temperature treatment (12°-15°C) favors increases in body fat stores in both sexes of *Notemigonus*, regardless of photoperiod conditions. Low temperature treatment of *Notemigonus* during the gonadal preparatory season did not result in lipid deposition, but did maintain body fat composition at a level equivalent to that in the initial controls (sacrificed at the onset of the experiment). Animals collected during the preparatory period (January) were very fat. In fact, body fat stores in this species reach a peak in late December, January, and early February. The failure of laboratory low temperature treatment to stimulate lipid deposition during the preparatory season could be due to the presence of sufficient fat reserves in the initial controls. Regardless of season or photoperiod, high temperature (25°C) acclimation favors body lipid depletion in males. During the preparatory and prespawning seasons, body fat depletion is also observed in females exposed to warm temperatures. Compared to initial controls (animals sacrificed at the beginning of the experiment), lipid levels in *Notemigonus* maintained at warm temperatures during the early spawning season were not appreciably altered. The failure of warm temperature treatment to deplete lipid reserves in females during the early spawning season may be

due to the relatively low levels of fat in the initial controls. Fish maintained at warm temperatures were fed twice daily whereas fish exposed to low temperatures were fed only once a day; warm temperature animals consumed four to five times more food than the low temperature fish. Since the fish in all experiments were fed ad libitum, the differences in body lipid levels should not be due entirely to higher metabolic rates at elevated temperatures. Lipid synthesis and deposition is also promoted at low temperatures in several other teleost species (Blazka 1958; Brown 1960; Dean and Goodnight 1964; Knipprath and Mead 1968; de Vlaming and Pardo 1975). The means by which temperature acts to control lipid metabolism is not fully understood, but Kinne (1960) reported that the efficiency of food conversion in *Cyprinodon macularis* is maximal at lower temperatures. Furthermore, enzyme systems (Hochachka 1969) and hormones (de Vlaming and Pardo 1975; Pardo and de Vlaming in press) involved in lipid metabolism in fishes appear to be temperature sensitive.

At low temperatures, short photoperiods are more effective than long photoperiods at stimulating lipid deposition in *Notemigonus*. In all of the experiments reported here, body lipid reserves were higher in female fish exposed to the short photoperiod-low temperature regimes than in females maintained on long photoperiod-low temperature regimes. With the exception of the experiment conducted during the prespawning season, similar results were obtained with males. Short photoperiods also compliment the lipid depleting effects of warm temperatures. In two of the three experiments summarized here, body fat reserves were significantly lower in fish (both sexes) exposed to the short photoperiod-warm temperature regime than in animals maintained on the long photoperiod-warm temperature regime. Thus, in *Notemigonus*, the effects of photoperiod on lipid metabolism are temperature dependent. Specifically, in combination with a low temperature, short photoperiods favor body fat deposition, but at a high temperature, short photoperiods accelerate depletion of lipid reserves. The fact that short photoperiods have opposing effects on lipid metabolism depending on temperature is, at the present time, an enigma. Apparently, however, changing environmental temperatures can differentially sensitize *Notemigonus* to daylength. Roberts (1964) showed that photoperiod changes can alter metabolic pat-

terns in sunfish. In *F. similis*, short photoperiods promote fattening whereas long photoperiods result in lipid depletion (de Vlaming, Sage, Charlton, and Tiegs; de Vlaming et al. in press). Other than these studies, little is known concerning the potential role of daylength in controlling fattening cycles in teleosts.

The results presented here on temperature and photoperiod effects on fat storage are consistent with environmental data. Lipid levels are lowest in *Notemigonus* collected during July, August and early September; environmental temperatures are high during this time and daylength is decreasing. From mid-September through December daylength and temperature continue to decrease. Fat stores increase progressively during this time. Beginning in mid or late February, lipid reserves are progressively depleted until late June or July. During this time, daylength and temperature are increasing.

A progressive decrease in body fat reserves was observed in *Notemigonus* collected during the preparatory, prespawning and spawning seasons respectively. These data indicate that there may be a relationship between lipid stores and reproduction. Other investigators presented evidence of body fat depletion associated with increasing gonadal activity (Lovern 1934; Lühmann 1953; Idler and Bitners 1960; Woodhead 1960; Wilkins 1967; Lasker 1970). A long photoperiod, in combination with warm temperatures, is required for final gonadal maturation and spawning in *Notemigonus* (de Vlaming 1975). These conditions result in depletion of fat stores in this species. The fat depleted from body storage sites could possibly be utilized for the energy demands of reproduction; in females, some of the body lipids may also be converted to yolk precursors and transported to the developing oocytes. So gonadal activation by long photoperiod-warm temperatures regimes may result in mobilization of body lipid reserves. Possibly, however, gonadal maturation may depend on the prior activation of lipid mobilization enzyme systems by long photoperiod-warm temperature regimes. The former hypothesis gains some support from observations of several investigators (e.g., Kobayashi 1953; Egami 1955; Oguro 1956) which indicate that sex steroids stimulate lipid synthesis in fishes. In vitro studies with *Notemigonus* liver preparations imply that estradiol-17 β stimulates synthesis and transport of lipid by this tissue (Shing and de Vlaming unpubl. data). My intention is not to

imply that only sex steroids are involved in regulation of lipid metabolism. Indeed, other hormones such as insulin (de Vlaming and Pardo 1975) and prolactin (see below) have distinct effects on fat metabolism in *Notemigonus*.

Low temperatures, regardless of photoperiod, maintain vitellogenesis and spermatocyte proliferation in *Notemigonus*, but will not stimulate final ovarian or testicular maturation (de Vlaming 1975). Low temperatures also maintain or increase body lipid reserves in this species. These observations lend further support to the suggestion that fat stores are in some way related to reproductive activity.

Short photoperiod-warm temperature regimes cause gonadal regression in *Notemigonus* (de Vlaming 1975). Body fat depletion also occurs under these conditions. Obviously this fat depletion is not associated with increased gametogenic activity. Body lipid reserves also decreased in fish maintained on long photoperiod-warm temperature regimes. Therefore, depletion of body fats may be primarily associated with increased energy requirements at high temperatures. In *Notemigonus*, however, there is some indication that sex steroid secretion is stimulated or remains high in fish maintained on a short photoperiod-warm temperature regime. Specifically, fish exposed to these conditions either develop or maintain nuptial coloration. If such is the case, sex steroids may be involved in mobilization and/or utilization of lipid reserves.

In a majority of the experiments reported here, pinealectomy had a pronounced effect on body fat reserves in *Notemigonus*. The effects of pinealectomy on fat metabolism depend on the photoperiod-temperature regime to which the experimental animals are exposed. In all three experiments, body lipid levels were significantly lower in pinealectomized than in sham operated females exposed to the long photoperiod-warm temperature regime; similar results were obtained with males in one experiment. Body lipid content was significantly greater in pinealectomized than in sham operated females in two of the three experiments where fish were exposed to a short photoperiod-warm temperature regime; similar results were obtained in only one experiment with males. These data indicate that, in fish maintained at warm temperatures, the effects of pinealectomy depend on photoperiod conditions. During the prespawning and spawning seasons, pinealectomy reversed the effects of photoperiod on fish

exposed to a warm temperature. For example, lipid levels were not significantly different in pinealectomized fish exposed to the short photoperiod-warm temperature regime and sham operated animals maintained on the long photoperiod-warm temperature regime; nor was fat content significantly different in sham operated animals exposed to the short photoperiod-warm temperature condition and pinealectomized fish maintained on the long photoperiod-warm temperature regime (Tables 2, 3).

In all three of the experiments summarized here, body fat reserves were significantly greater in pinealectomized than in sham operated females maintained on the long photoperiod-low temperature regime; similar results were recorded in two of the experiments with males. Body fat composition was significantly lower in pinealectomized than in sham operated females exposed to a short photoperiod-low temperature regime; similar differences were noted with males in two of the experiments. These data further confirm the suggestion that the effects of pinealectomy on lipid metabolism in *Notemigonus* depend on photoperiod. In two of the experiments, fat content did not differ significantly in pinealectomized fish maintained on the long photoperiod-low temperature regime and sham operated animals exposed to the short photoperiod-low temperature regime; nor were significant differences noted in fat levels when the reverse comparison was made (Tables 2, 3).

The data obtained at both high and low temperatures indicate that the pineal in *Notemigonus* may have some role in receiving and/or integrating light information. Such a suggestion seems likely since the pineal may either facilitate or retard lipid deposition in this species. Several morphological and electrophysiological studies suggest that the pineal in some teleosts functions as a photoreceptor (cf. de Vlaming 1974). Light microscope studies on the pineal of *Notemigonus* also indicate a sensory function (Vodicnik and de Vlaming unpubl. data). If the pineal in *Notemigonus* is a photoreceptor involved by some means in measuring daylength, then removal of this organ from fish maintained under different photoregimes might be expected to have variable effects on lipid metabolism. Urasaki (1972a, b) has also shown that the effects of pinealectomy on reproductive function in *Oryzias latipes* vary with photoperiod conditions.

The effects of the pineal on lipid metabolism in

Notemigonus, however, do not depend entirely on light information. For example, in fish maintained on a long photoperiod during the prespawning and spawning seasons, pinealectomy accentuated lipid deposition at low temperatures and lipid depletion at a high temperature. Temperature may not, however, act on the pineal directly. High temperatures cause lipid catabolism and low temperatures favor lipid deposition. Temperature may act directly on lipid metabolism enzyme systems or indirectly to stimulate hormone secretion from various endocrine glands. In *Notemigonus*, light information serves only to modify the effects of temperature on lipid metabolism. Thus, the pineal could function at all temperatures as a light receptor and/or integrator. Light information may be differentially interpreted (at different temperatures or at different times of the year) at some other level such as the hypothalamus and/or pituitary.

Whether the pineal in *Notemigonus* exerts its effects on lipid metabolism via neural or hormonal pathways is not presently known. Most morphological studies on the teleost pineal have stressed the dual sensory and secretory appearance of this organ (cf. de Vlaming 1974). A dual sensory-secretory function is also indicated by light microscope studies on the pineal of *Notemigonus*. Histochemical and biochemical data show that the teleost pineal has an active indolamine metabolism (Quay 1965; Hafeez and Quay 1969; Fenwick 1970; Owman and R deberg 1970). Melatonin has inhibitory effects on reproductive function in various teleosts (Fenwick 1970; Urasaki 1972c; de Vlaming, Sage, and Charlton 1974). Melatonin treatment decreases lipid reserves in *F. similis* maintained on a long photoperiod-low temperature regime (de Vlaming, Sage, Charlton, and Tiegs 1974); if melatonin acts as the mediator of pineal action in *Notemigonus*, one might then expect pinealectomy to increase fat levels in fish exposed to a long photoperiod-low temperature regime. Pinealectomy did indeed have these results under this regime. During July, melatonin therapy of *F. similis* exposed to a short photoperiod-low temperature regime stimulated lipid deposition (de Vlaming, Sage, Charlton, and Tiegs 1974). If the mediator of pineal activity in *Notemigonus* is melatonin, one might predict that pinealectomy would decrease body lipid stores in animals exposed to a short photoperiod-low temperature regime. Such results were observed in the experiments reported here. Interestingly,

lighting conditions were reported to alter the secretory activity in the glandular appearing pineals of *Gambusia affinis* and *Symphodus melops* (Chèze and Lahaye 1969; Chèze 1970). Possibly then, the pineal in some teleost species may function as a neuroendocrine transducer of photoperiod information. If the pineal is a neuroendocrine organ, melatonin could conceivably be one of the hormones produced by this organ. Although the pineal of *Notemigonus* does seem to be involved in photoreception, available evidence does not allow one to conclusively state that this organ is neuroendocrine in nature or that pineal-produced melatonin functions as a chemical messenger.

The pineal may modify fat stores in *Notemigonus* by influencing hypothalamic and/or pituitary function. Indeed, de Vlaming and Vodcnik (in press) showed that pinealectomy alters hypothalamic gonadotropin releasing activity and pituitary gonadotropin levels. Several investigators reported that prolactin has a pronounced affect on lipid metabolism in teleost fishes (Lee and Meier 1967; Meier 1969; Mehrle and Fleming 1970; Joseph and Meier 1971; Meier et al. 1971; de Vlaming and Sage 1972; Sage and de Vlaming 1973; de Vlaming et al. in press; Pardo and de Vlaming in press). Furthermore, melatonin treatment significantly reduces pituitary prolactin activity in *F. similis* (de Vlaming, Sage, Charlton, and Tiegs 1974). These authors suggested that the effects of melatonin on lipid metabolism in this species may be due in part to the effects of this indolamine on pituitary prolactin release. Whether pinealectomy alters pituitary prolactin secretion is not presently known. Investigations are presently in progress to examine this possibility. Prolactin does stimulate lipid depletion from in vitro liver preparations of *Notemigonus* incubated at high temperatures and promotes fat synthesis in liver preparations incubated at low temperatures (Pardo and de Vlaming in press).

The effects of pinealectomy on lipid reserves in *Notemigonus* may result from changes in reproductive activity. This suggestion seems rather unlikely since pinealectomy frequently resulted in significant changes in body fat levels without appreciably altering gonadal activity.

The data presented here favor the view that the pineal is a photoreceptor or integrates light information and plays an important role in regulating physiological processes in teleost fishes.

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