THE EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON REPRODUCTIVE CYCLING IN THE ESTUARINE GOBIID FISH, *GILLICHTHYS MIRABILIS*

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ABSTRACT

Investigations were undertaken at several different times during the year to examine the effects of various photoperiods and constant-temperature regimes on reproductive function in the longjaw goby, *Gillichthys mirabilis*, with the intent of evaluating the influence of these factors in regulation of the annual sexual cycle. Testicular regression occurs at any time during the year when fish are exposed to constant temperature of 24°C and above, independent of photoperiod. Similar results were obtained with female fish, but 22°C is the thermal threshold. It is concluded that the gonadal regression observed in the Alviso population of this species during the summer months is a consequence of increasing temperature. At high temperatures, the transformation of spermatogonia to spermatocytes is blocked, and in females vitellogenesis is inhibited. The degree of gonadal regression is temperature-dependent. Gonadal recrudescence is dependent on low temperatures (10°-20°C) and will not occur if fish are exposed to high temperatures (24°C or above) regardless of photoperiod. At low temperatures, short photoperiods accelerate recrudescence. Between January and June spermatogenesis and oogenesis are maintained at temperatures between 10° and 21°C; long photoperiods are more effective in this respect, but not essential. Termination of the reproductive season in this species is not endogenously timed. Regression is not "obligatory" since gonadal involution does not occur at the "normal" time if fish are exposed to temperatures of 20°C or below.

The survival of any species in a seasonally changing environment is dependent on the development of mechanisms that permit it to adjust physiological functions to changes in the environment. Studies of reproductive timing and how the environment influences this timing are of importance in understanding the ecology of any species.

Compared with the wealth of information available on the systematics, ethology, and physiology of fishes, there is little knowledge concerning how external factors regulate their reproduction. Some investigations have been undertaken to elucidate the role of environmental factors in regulating the reproductive cycles of various teleosts. The relationship of environmental factors to the reproductive cycles of gobies has not received experimental consideration. Moreover, experimental work with the environmental control of teleost reproductive cycles has been confined to fewer than 20 species representing only 8 families.

Photoperiod and temperature are presumed to be the most important factors (i.e., the most studied) influencing the neuroendocrine centers that control gonadotropin secretion in teleosts (de Vlaming, 1972a). The experimental conditions employed in a majority of the previous studies, however, are diverse and the results contradictory. In fact, most of the experimental work was too poorly controlled and too brief in duration to allow proper assessment of the role of the environment in synchronizing fish reproduction (de Vlaming, 1972a).

The subject of the present study is the longjaw goby, *Gillichthys mirabilis*. It is distributed

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from central California south to Magdalena Bay, Baja California, and the Gulf of California south to Mulegé on the west coast, and south to Agiabampo Bay on the east coast (Barlow, 1963). The typical habitat of this species is the intertidal region of coastal sloughs. Barlow (1961, 1963) discussed the systematics and some aspects of the ecology of *G. mirabilis*. The population of *Gillichthys* used in these studies occurs in the Alviso salt ponds located at the southern end of San Francisco Bay, Calif. Carpelan (1957) described seasonal changes in the hydrobiology of these ponds.

De Vlaming (1972b) described the reproductive cycle of *G. mirabilis* and suggested that seasonal temperature changes may be involved in regulating sexual cycling in this species. The spawning period is protracted, extending from December to June. Gonadal regression occurs in July; the gonads remain regressed during August and September. Gonadal recrudescence begins in late September, reaching completion by early December.

The aim of the present study was to determine the effects of various light and constant temperature regimes on gonadal function in *G. mirabilis*, with the hope of evaluating the influence of these factors in regulation of the annual sexual cycle. The phenological data on reproductive cycling in this species presented by de Vlaming (1972b) was used as a basis for these studies. Some of the previous studies with teleosts have shown that the effect of the environmental synchronizer(s) varies with the stage of gonadal maturity. Consequently, the effects of photoperiod and constant temperature treatments were examined during different phases of the gonadal cycle.

MATERIALS AND METHODS

Samples of *G. mirabilis* were captured in the Alviso habitat at several different times during the year and thus in different phases of gametogenesis. Since males were more abundant in these samples, a greater number of experiments were conducted with this sex. Several fish from each sample were sacrificed, and the gonads examined at the time of capture; these fish served as a reference for the experiments that followed. In the following discussion the fish sacrificed from the samples from nature will be referred to as initial controls. In many of the experiments, samples of fish from the natural habitat were collected and sacrificed upon termination of the experiment; these fish will be referred to as terminal controls. To facilitate quantification of gonadal response, animals of approximately equal size were utilized in these experiments.

Experimental fish were maintained in 56- (no more than 10 fish per tank) or 132-liter (no more than 18 fish per tank) tanks. Recirculating filtered seawater was used in all of these experiments. The bottom of each tank was covered with fine gravel. The experimental tanks were housed in constant temperature rooms ($\pm$ 1.5°C). Temperatures selected for these experiments are within the range normally experienced by this species during the year.

Various photoperiods were also employed in these experiments. Light was provided by 20-w warm-white fluorescent bulbs suspended above the tanks. Salinity was maintained at a constant 35 ‰, and pH between 8.0 and 9.5 (these pH's are consistent with those experienced by the fish in the Alviso ponds). The fish in these experiments were provided with a varied diet consisting of brine shrimp, chopped fish, boiled egg white, and beef kidney and liver; all fish ate voraciously.

Upon termination of each experiment, the weight and standard length of each fish were recorded. Gonads were weighed and prepared for histological examination in the same manner as previously reported (de Vlaming, 1972b). Gravimetric data are expressed in absolute weights since it was shown (de Vlaming, 1972b) that gonadal weight is independent of body weight (and length) in the size range used. Spermatogenesis and oogenesis were divided into six and five recognizable phases (Tables 1 and 2) to facilitate quantitative evaluation of gametogenetic activity.

Statistical comparisons of gonadal weights between experimental groups were made by using the Mann-Whitney *U* test (Siegel, 1956, p. 184-193). This nonparametric test is suitable for small sample sizes and can be used to determine

TABLE 1.—Criteria used for histologically staging testes of *Gillichthys mirabilis*.

Stage	Histological characteristics of testes
0	"Regressing testis." Seminiferous lobules characterized by large numbers of pyknotic nests of degenerating cells (spermatozoa, spermatids, and spermatocytes); phagocytes observed free within the lobules.
1	"Quiescent testis." Seminiferous lobules small in diameter. Germinal epithelium consists of only spermatogonia. Lumen of the lobules contain only few residual spermatozoa, and the sperm duct is collapsed.
2	"Mitotic phase." Same as Stage 1, with the exception that mitotic figures are observed in the spermatogonia.
3	"Meiotic phase or active spermatogenesis." Testicular lobules larger than in Stages 1 and 2; germinal epithelium consists of spermatogonia, spermatocytes, and spermatids.
4	"Prespawning testis." Seminiferous lobules large and distended with sperm. Germinal epithelium consists of relatively few spermatogonia.
5	"Postspawning testis." Seminiferous lobules small and contain relatively few sperm; sperm duct expanded and containing residual sperm.

TABLE 2.—Criteria used for histologically staging ovaries of *Gillichthys mirabilis*.

Stage	Histological characteristics of ovaries
I	"Regressing ovary." Atretic follicles predominate in the ovary. Only nonyolky oocytes and oogonia present.
II	"Quiescent phase or phase of oogonial proliferation." Ovary characterized by nonyolky oocytes with a basophilic cytoplasm, and a diameter of less than 75 μ. Granulosa not fully organized around the developing oocytes.
III	"Phase of active vitellogenesis." Ovary characterized by developing yolky oocytes whose diameter is between 75 μ and 640 μ. Granulosa fully organized around the oocytes.
IV	"Prespawning condition." Ovary characterized by oocytes whose diameter is in excess of 640 μ. Yolk vesicles abundant.
V	"Postspawning condition." The ovary is wine-red in color; the tunica albuginea thick, highly vascularized, and folded. Postovulatory follicles predominate in the ovary. The stroma of the ovary appears disorganized, yet highly vascularized.

whether two independent groups have been drawn from the same population.

RESULTS

EFFECTS OF CONSTANT TEMPERATURE AND PHOTOPERIOD ON FISH WITH REGRESSING GONADS

To examine the influence of low and high temperature treatments at different photoperiods on gonadal recrudescence, fish with regressing testes (Stage 0) and ovaries (Stage I) were collected in July 1967. Fish were exposed to 13°C, at a short (8L/16D) or long (15L/9D) photoperiod, or to 27°C at a short photoperiod (8L/16D).

The effects of these treatments on testicular and ovarian weights are summarized in Figure 1. The testes (Stage 0) and ovaries (Stage I) of all of the initial controls were regressing. After 57 days, testicular and ovarian weights increased significantly ($P < 0.01$) at both photoperiods at 13°C, and were also significantly greater ($P < 0.01$) than those of fish from nature sacrificed at the same time. Ovarian weights of females exposed to 8L/16D at 13°C were significantly greater ($P < 0.01$) than those of fish exposed to 15L/9D at the same temperature.

Testes of all fish at 13°C were in the meiotic phase of spermatogenesis (Stage 3) whereas those of all fish in the September sample from nature were only in Stage 2 (mitotic proliferation phase). The ovaries of all fish at 13°C were in phases of vitellogenesis (Stage III); the oocytes of fish at 8L/16D were, however, in a

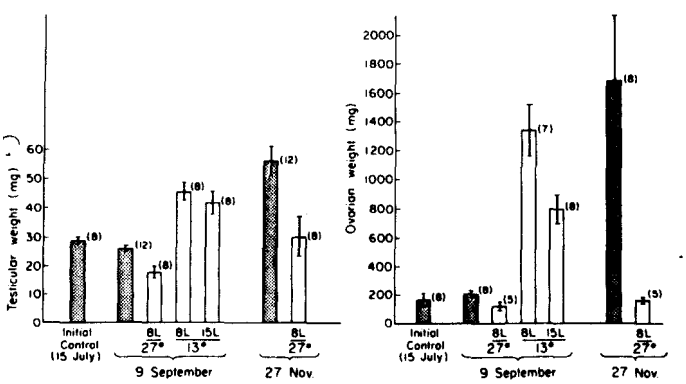

FIGURE 1.—Effect of 13°C at a long (15L/9D) photoperiod and 13° and 27°C at a short (8L/16D) photoperiod on testicular and ovarian weight in *Gillichthys mirabilis*. Mean gonadal weight is illustrated by histograms; the mean is bracketed by one standard error. Shaded histograms represent gonadal weight of samples collected in nature; open histograms, experimental groups. Light (hours per 24 hr), temperature, and dates on which fish were sacrificed are recorded below the histograms. Sample sizes are indicated atop each histogram.

later stage of vitellogenesis. The ovaries of all fish in the September nature sample were in the quiescent phase (Stage II).

After 57 days at 27°C, testicular weights were significantly lower ($P < 0.05$) than those of the initial July controls. The testes of all fish were in the quiescent phase (Stage 1), even after 134 days. In contrast, the testes of all fish in the November sample from nature were in Stage 3.

Ovarian weights of fish exposed to 27°C (both the September and November samples) did not differ significantly from those of the initial July controls, but they were significantly lower ($P < 0.01$) than ovarian weights of both 13°C groups. The ovaries of the 27°C treated fish revealed only resting oocytes (Stage II). However, the ovaries of fish in the November sample from nature were in Stage III, IV, or V.

Thus, low temperatures promote gonadal recrudescence in *Gillichthys*, independent of photoperiod. Short photoperiods may accelerate the rate of gonadal recrudescence at low temperatures. A short photoperiod, however, failed to initiate gonadal recrudescence at high temperature. High temperatures act by blocking vitellogenesis and the transformation of spermatogonia to spermatocytes.

The influence of constant temperature and photoperiod on testicular recrudence was examined again in July 1968, using 13° or 20°C, with a short (8L/16D) and a long (15L/9D) photoperiod. A fifth group was exposed to 24°C at a long photoperiod (15L/9D). Each group was sampled after 45, 70, and 120 days (Figures 2 and 3).

In the initial July controls testes were regressing (Stage 0). At 24°C testicular weights remained low throughout the experiment; in September and November testicular weights at this temperature were significantly lower ($P < 0.05$) than those of the initial July controls. Moreover, at 24°C testes remained in the regression or quiescent phase (Stage 0 or 1) throughout the experiment (Figure 3).

At 20°C testicular weights remained essentially the same as in the initial July controls

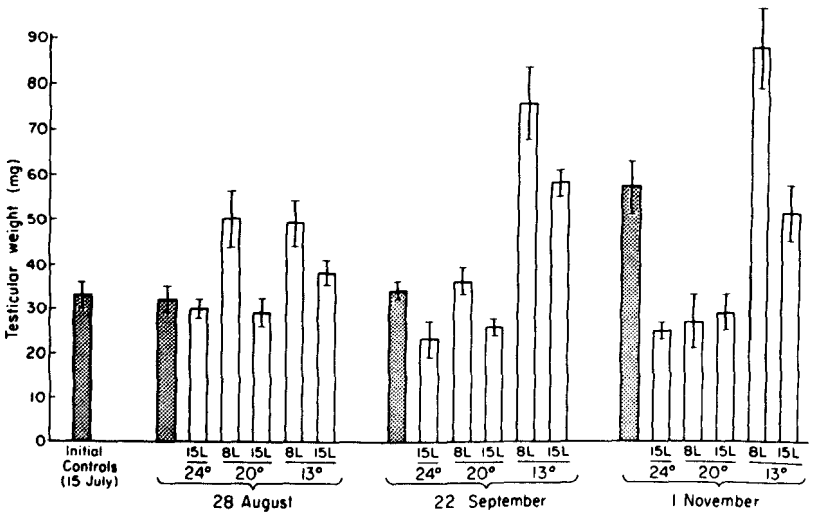

FIGURE 2.—Effect of 24°, 20°, and 13°C treatments at short (8L/16D) and long (15L/9D) photoperiods on testicular weight in *Gillichthys mirabilis*. Mean testicular weight is represented by histograms; the mean is bracketed by one standard error. Shaded histograms represent testicular weight of samples collected in nature; open histograms, experimental groups. Light (hours given per 24 hr), temperature, and dates on which fish were sacrificed are recorded below the histograms. For sample sizes, see Figure 3.

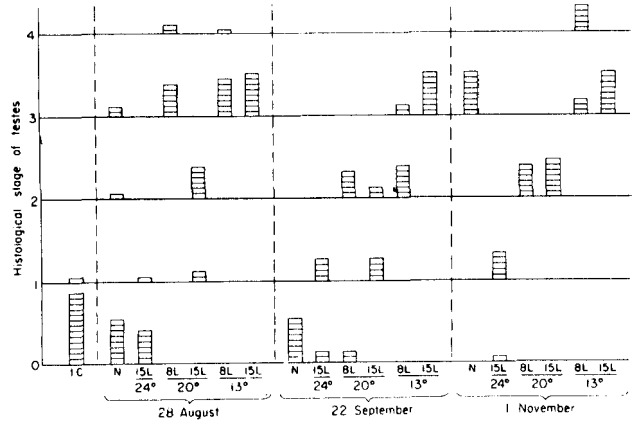

FIGURE 3.—Effect of 24°, 20°, and 13°C treatments at short (8L/16D) and long (15L/9D) photoperiods on testicular histology in *Gillichthys mirabilis*. I.C. refers to initial controls (15 July) and N, to samples from natural population. Each box represents the testicular condition of one fish.

with one exception. Testicular weights of the 20°C group at 8L/16D sacrificed in August were significantly greater ($P < 0.05$) than those of the July controls. With the exception of this same group, active spermatogenesis was not initiated in fish exposed to 20°C. Mitotic proliferation of spermatogonia was, however, stimulated by this treatment. In contrast, active spermatogenesis was initiated by August at 13°C, regardless of photoperiod. Active spermatogenesis was not initiated in the natural population until after 22 September. Testicular weights of both groups at 13°C sacrificed in September were significantly greater ($P < 0.01$) than those of the initial July controls and those of the September sample from nature. Some photoperiod effect was evident at 13°C since testicular weights at 8L/16D were significantly higher ($P < 0.05$) than those of the 15L/9D group by September. By November, the testes of a majority of the fish in the 13°C-8L/16D group were in the prespawning condition (Stage 4) whereas the testes of all fish in the 13°C-15L/9D group were in Stage 3; testicular weights of these two groups were also significantly different ($P < 0.01$).

These data indicate that 24°C inhibits testicular recrudescence by blocking the transformation of spermatogonia to spermatocytes and also retards mitoses in the spermatogonia. Low temperatures promote testicular recrudescence; the rate of recrudescence at a low laboratory temperature was faster than in the natural population. At low temperatures, short photoperiods accelerate testicular recrudescence. With the exception of the one sample at 20°C-8L/16D sacrificed in August, 20°C stimulates little or no testicular recrudescence, only mitotic proliferation of spermatogonia.

EFFECTS OF CONSTANT TEMPERATURES AND PHOTOPERIOD ON FISH IN STAGES OF ACTIVE GAMETOGENESIS (MAY)

Responses of fish in phases of active gametogenesis (in May) were examined by exposing two groups of fish for 67 days to 13°C, at a short (8L/16D) and a long (15L/9D) photoperiod, and 27°C at a long photoperiod (15L/9D). The effects of these treatments on gonadal weights are summarized in Figure 4.

At the beginning of treatment, testes and ovaries were in Stages 3 and III, respectively. In the July sample from nature, sacrificed with the experimentals, ovarian (Stage I) and testicular (Stage 0) regression was occurring. Testes and ovaries of fish at 27°C regressed as in nature; in both sexes gonad weights were significantly lower ($P < 0.01$) than initial levels.

In both groups at 13°C spermatogenetic activity remained at the initial levels and the testes did not show the regression seen in nature or at 27°C. Ovarian weights in the 13°C group at a long photoperiod were significantly lower ($P < 0.05$) than those of the initial May sample

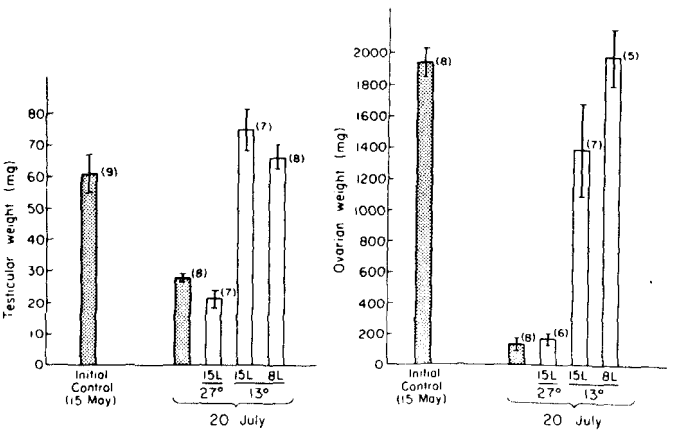

FIGURE 4.—Effect of 27° and 13°C treatments at short (8L/16D) and long (15L/9D) photoperiods on testicular and ovarian weight in *Gillichthys mirabilis*. Mean gonadal weight is represented by histograms; the mean is bracketed by one standard error. Shaded histograms represent gonadal weight of samples collected in nature; open histograms, experimental groups. Light (hours given per 24 hr), temperature, and dates on which fish were sacrificed are recorded below the histograms. Sample sizes are indicated atop each histogram.

and those of the 13°C group at a short photoperiod. Ovaries in both 13°C groups were, however, in active vitellogenesis (Stage III). These results could indicate a photoperiod influence in vitellogenesis. But one must observe caution in interpreting these data since this difference in ovarian condition may simply be a problem of beginning experiments with fish in various stages of oogenesis. Data presented by de Vlaming (1972b) revealed the nonsynchrony of gametogenesis in this species (i.e., fish in different stages of gonadal development were common in monthly samples between November and June).

High temperatures apparently cause testicular and ovarian regression in spring, at least when the photoperiod is long, whereas low temperatures prevent gonadal regression and are required for spermatogenesis and vitellogenesis.

EFFECTS OF CONSTANT TEMPERATURES AND PHOTOPERIOD ON FISH IN STAGES OF ACTIVE SPERMATOGENESIS (JANUARY)

In the previous experiment the influence of temperature and photoperiod was examined during the spawning season in spring. Whether fish respond similarly in winter (near the onset of the spawning season) is also of interest, so in January 1968 fish were exposed to 27°, 20°, and 13°C, at a short (8L/16D) or a long (15L/9D) photoperiod for 30-39 days. The influence of these treatments on testicular weight and histology is presented in Figures 5 and 6, respectively.

Testes of the initial January controls were in Stages 3, 4, or 5. As observed in May, testes regressed rapidly at 27°C; testes weights were significantly less ($P < 0.01$) than those of the January controls and February sample from nature. Histological examination confirmed that regression had occurred (testes in Stage 1). No photoperiod effect was seen at 27°C.

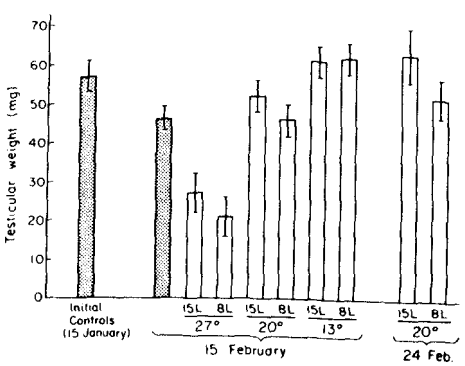

FIGURE 5.—Effect of 27°, 20°, and 13°C treatments at short (8L/16D) and long (15L/9D) photoperiods on testicular weight in *Gillichthys mirabilis*. Mean testicular weight is represented by histograms; the mean is bracketed by one standard error. Shaded histograms represent testicular weight of samples collected in nature; open histograms, experimental groups. Light (hours given per 24 hr), temperature, and dates on which fish were sacrificed are recorded below the histograms. For example sizes, see Figure 6.

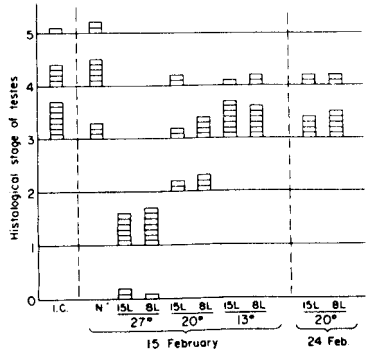

FIGURE 6.—Effect of 27°, 20°, and 13°C treatments at short (8L/16D) and long (15L/9D) photoperiods on testicular histology in *Gillichthys mirabilis*. I.C. refers to initial controls (15 January) and N, to sample from natural population. Each box represents the testicular condition of one fish.

Testicular weights in the 20°C groups remained at approximately the initial level. Both groups at 20°C had significantly heavier ($P < 0.01$) testes than those of the 27°C groups. The testes of all fish at 20°C advanced to Stages 3 or 4 by 24 February, and there was no clear photoperiod effect. Testicular weights of the 13°C groups did not differ significantly from the initial January controls but were significantly greater than those of the 20°C group at a short photoperiod ($P < 0.05$) and those of the 27°C groups ($P < 0.01$). Spermatogenic condition of the testes in the 13°C groups was essentially the same as in the January controls and February sample from nature (Figure 6).

The results of this experiment indicate that spermatogenesis is maintained at 13°C and 20°C (independent of photoperiod) but that this process may occur at a slower rate at 20°C than at 13°C. This difference in effect of these two temperatures must be accepted with some reservation because the variability of gonadal development in the initial controls could introduce a degree of bias into the results. Regardless of photoperiod, testicular regression occurred at 27°C.

EFFECTS OF HIGH TEMPERATURES ON GONADAL FUNCTION IN DIFFERENT SEASONS

Experiments using high temperatures were initiated at different times during the year to determine whether there is a seasonal variation in gonadal susceptibility to such treatment. The conditions employed and results obtained in these experiments are summarized in Table 3.

Experiments I and II indicate that 24°C is a sufficiently high temperature to initiate testicular regression within a relatively short period. With either a long (15L/9D) or a short (8L/16D) photoperiod (Experiment III), 25°C stimulates the completion of testicular regression within 21 days; testes of these fish were in the quiescent phase (Stage 1). A temperature

TABLE 3.—Effect of various high temperature and photoperiod treatments on testicular weight in *Gillichthys mirabilis*.

Experiment numbers	Date	Length of treatment (Days)	Temperature (°C)	Photoperiod (L/D)	Testicular weight (mg) ($\bar{x} \pm SE$)		
					Initial controls	After treatment	(n)
I	May 69	21	24	12/12	79.1 ± 5.2	58.1 ± 3.1**	(7)
II	Feb. 70	15	24	14/10	79.1 ± 9.5	34.4 ± 2.5**	(9)
III	Feb. 68	21	25	15/9	43.7 ± 3.4	25.0 ± 1.6*	(7)
	Feb. 68	21	25	8/16	43.7 ± 3.4	23.2 ± 4.8*	(7)
IV	July 70	30	27	10/14	22.3 ± 7.8	18.1 ± 1.4	(8)
V	Nov. 69	14	27	12/12	64.8 ± 8.8	30.0 ± 2.7**	(8)
VI	Apr. 70	8	27	12/12	97.8 ± 7.4	46.7 ± 3.9**	(10)
VII	July 68	41	24	12/12	33.1 ± 3.2	28.9 ± 2.2	(7)
	July 68	41	28	12/12	33.1 ± 3.2	18.1 ± 2.2*	(8)
VIII	June 68	21	27	12/12	52.2 ± 3.9	26.2 ± 2.7**	(7)
	June 68	21	30	12/12	52.2 ± 3.9	14.8 ± 3.1**	(8)
IX	Apr. 68	8	28	12/12	62.2 ± 5.4	40.1 ± 4.2**	(7)
	Apr. 68	14	28	12/12	62.2 ± 5.4	24.3 ± 1.8**	(9)
	Apr. 68	8	32	12/12	62.2 ± 5.4	15.6 ± 2.8**	(8)

*Significantly less ($P < 0.05$) than initial controls.
**Significantly less ($P < 0.01$) than initial controls.

of 25°C does not, however, block mitotic proliferation of spermatogonia.

Experiment IV (Table 3) indicates that 27°C blocks the initiation of testicular recrudescence. Testicular regression is initiated within 14 days at 27°C (Experiment V); the testes of all fish were in the regression phase (Stage 0). Moreover, Experiment VI suggests that testicular regression is initiated within 8 days at 27°C; the testes of all fish were regressing (Stage 0).

When initial controls are undergoing testicular regression, 28°C stimulates a more rapid completion of regression than does 24°C (Experiment VII). This is evident since the testes of a majority of the fish at 28°C were in the quiescent phase (Stage 1) whereas those of the 24°C group were in Stage 0. A 30°C temperature (Experiment VIII) causes a more complete testicular regression than does a 24°C temperature (i.e., testicular weights of the 30°C group were significantly lower ($P < 0.01$) than those of the 27°C group). Both of these treatments caused the completion of testicular regression within 21 days; the testes of a majority of fish in both groups were in the quiescent phase (Stage 1). Similarly, 32°C stimulates a more rapid and more complete testicular regression than 28°C (Experiment IX); testicular weights after 8 days were significantly lower ($P < 0.01$) in the 32°C group than those of the 28°C group. Testes of all fish at both temperatures were regressing (Stage 0). After 14 days at 28°C, testicular weights were still significantly higher ($P < 0.05$) than testicular weights after 8 days at 32°C (Experiment IX).

With the exception of the experiments in which the initial controls were fish with regressing gonads, all high temperature treatments summarized in Table 3 caused a significant decrease in testicular weights. These data indicate that the rate of testicular regression and the degree of testicular regression are temperature dependent. These data also indicate that under laboratory conditions testicular involution is initiated relatively soon after exposure to high temperatures and that regression can be completed rapidly in this species.

Experiments similar to those discussed above were conducted with female *Gillichthys* (Table 4). Beginning with fish in active vitellogenesis (Stage III), 25°C initiates ovarian regression (Stage I) within 21 days, independent of photoperiod (Experiment 1). Ovarian weights in the two photoperiod groups were not significantly different. Beginning with fish having regressing ovaries (Experiment 2), 27°C caused the completion of ovarian regression. Experiment 3 suggests that ovarian regression is complete within 21 days at 30°C; the ovaries of a majority of the initial controls were in phases of active vitellogenesis (Stage III), whereas following treatment, the ovaries of all fish were in the quiescent phase (Stage II).

Experiment 4 (Table 4) indicates that 32°C stimulates a more rapid rate of ovarian regression than does 28°C; ovarian weights of the 32° and 28°C groups were significantly different ($P < 0.01$) after 8 days of treatment. Ovarian regression was occurring in all fish. After 14 days at 28°C, further ovarian regression occurred, but involution had still not been completed.

Therefore, temperatures between 25° and 32°C initiate ovarian regression within a relatively short time; all high temperature treatments caused a significant decrease in ovarian weights. Apparently the completion of ovarian regression is temperature dependent.

EFFECTS OF 21°-22°C AND PHOTOPERIOD TREATMENT ON GONADAL FUNCTION

The previous experiments suggest that gonadal regression occurs at 24°C but not at 20°C. To define more precisely the thermal threshold for gonadal involution, the effects of 21°-22°C treatments were examined in April, May, September, and November. The conditions employed and results obtained in these experiments are summarized in Table 5.

Beginning in April with fish in active spermatogenesis or in the prespawning condition (Stage 3 or 4), there was no change in testes after 17-day treatment at 22°C (15L/9D). However, after 30-day treatment at 22°C and a short photoperiod (10L/14D), there was a significant decrease ($P < 0.01$) in both ovarian and testicular weights (Experiment 11—Table 5). Testes

TABLE 4.—Effect of various high temperature and photoperiod treatments on ovarian weight in *Gillichthys mirabilis*.

Experiment number	Length of treatment (Days)	Temperature (°C)	Photoperiod	Weight of ovaries (mg) ($\bar{x} \pm SE$)		
				Initial controls	After treatment	(n)
1	21	25	15L/9D	1,744 ± 422	381 ± 81**	(7)
	21	25	8L/16D	1,744 ± 422	480 ± 108**	(8)
2	30	27	10L/14D	484 ± 98	188 ± 4*	(8)
3	21	30	12L/12D	1,638 ± 219	190 ± 76**	(8)
4	14	28	12L/12D	3,042 ± 305	654 ± 181**	(6)
	8	28	12L/12D	3,042 ± 305	1,790 ± 423**	(6)
	8	32	12L/12D	3,042 ± 305	871 ± 312**	(8)

*Significantly less ($P < 0.05$) than initial controls.
**Significantly less ($P < 0.01$) than initial controls.

of the May controls were in active spermatogenesis (Stage 3) or in the prespawning conditions (Stage 4); ovaries of the May controls were in active vitellogenesis (Stage III). The testes of six of the eight fish at the short photoperiod, however, were regressing (Stage 0); ovaries in this group were regressing (Stage I) or in the quiescent phase (Stage II).

In contrast to the effects of short photoperiod at 22°C, a long photoperiod (in May) did not initiate testicular regression. Although testicular weights in this group were significantly less ($P < 0.05$) than those of the initial May controls, the testes of all fish were in active spermatogenesis (Stage 3). Ovarian weights of fish at a long photoperiod were also significantly lower ($P < 0.01$) than those of the initial May controls; ovarian regression (Stage I) was occurring in all fish.

Beginning in September, 26-day exposure to a short photoperiod (10L/14D) at 21°C stimulated a significant increase ($P < 0.05$) in ovarian and testicular weights when compared to gonadal weights in the initial controls (Experiment 12—Table 5). In contrast, neither ovarian nor testicular weights in the long photoperiod group were significantly altered. The testes of the September controls were in Stage 2, and the ovaries of this group were in early stages of vitellogenesis (Stage III). After short photoperiod treatment, the gonads of all fish were in the meiotic phase of spermatogenesis (Stage 3) or vitellogenesis (Stage III); the long photoperiod, however, did not stimulate spermatogenesis (testes in this group were in Stage 1) and caused ovarian regression (Stage I).

Beginning in November (Experiment 13), testicular weights were maintained at the initial level for 21 days at 22°C and a short photoperiod (10L/14D); testes of the initial controls and the experimental fish were in Stage 3 or 4. A long photoperiod at 22°C, however, caused the initiation of testicular regression (Stage 0); testicular weights in this group were significantly

TABLE 5.—Effect of 21° and 22°C treatments on testicular and ovarian weight in *Gillichthys mirabilis*.

Experiment number	Beginning date	Length of treatment (Days)	Temperature (°C)	Photoperiod		Gonadal weight (mg) ($\bar{x} \pm SE$)		
						Initial controls	After treatment	(n)
10	April	17	22	15L/9D	males:	97.8 ± 7.4	99.2 ± 8.8	(10)
11	May	30	22	10L/14D	males:	88.4 ± 7.1	43.3 ± 2.3**	(8)
					females:	1,263 ± 186	681 ± 242**	(7)
11	May	30	22	15L/9D	males:	88.4 ± 7.1	70.6 ± 6.1*	(8)
					females:	1,283 ± 186	631 ± 216**	(6)
12	September	26	21	10L/14D	males:	42.8 ± 7.4	61.5 ± 7.8*	(8)
					females:	233 ± 39	364 ± 44*	(6)
12	September	26	21	15L/9D	males:	42.8 ± 7.4	36.0 ± 4.2	(7)
					females:	233 ± 39	166 ± 21	(6)
13	November	21	22	15L/9D	males:	68.5 ± 9.7	46.9 ± 6.3**	(10)
13	November	21	22	10L/14D	males:	68.5 ± 9.7	79.5 ± 5.1	(8)

*Significantly different ($P < 0.05$) from initial controls.
**Significantly different ($P < 0.01$) from initial controls.

lower ($P < 0.01$) than those of the initial November controls.

In spring spermatogenesis occurs at 22°C only if the photoperiod is long and in autumn only if the photoperiod is short. This temperature causes ovarian regression regardless of photoperiod, suggesting that females may be more sensitive to temperature than males. Similarly, 21°C will promote the initiation of testicular and ovarian recrudescence only if photoperiod is short. Apparently then, the effects of photoperiod at these temperatures are seasonally variable.

EFFECTS OF PHOTOPERIOD AT 20°C ON FISH WITH REGRESSING OR QUIESCENT GONADS

Experiments reported above showed that gonadal recrudescence will not occur at 20°C if treatment is initiated in July but that gametogenesis is maintained at this temperature at other times during the year. Thus, the influence of 36-day 20°C treatment at various photoperiods on gonadal recrudescence was examined in August 1968 (Figure 7). The gonads of the initial August controls were in Stages 0 and 1. Neither testicular nor ovarian weights in any of the experimental groups varied significantly from gonadal weights in the initial August controls, and there were no significant differences in gonadal weights among the experimental groups. Differences in gonadal histology were, nonetheless, evident. Testes of all fish collected from nature in August and September were in the regression phase (Stage 0). In all photoperiod groups testicular regression was complete, and spermatogonial proliferation (Stage 2) or spermatogenesis (Stage 3) was occurring. The testes of 4 of 10 fish at a 15L/9D photoperiod, however, were in the quiescent phase (Stage 1). A majority of ovaries from fish collected from nature in August and September were in the quiescent phase (Stage II). In each experimental group, the ovaries of some fish were in the early phases of vitellogenesis (Stage III); with the exception of one fish, vitellogenesis was initiated in all females at 15L/9D.

These results indicate that gonadal recrudescence is initiated at 20°C if treatment is begun

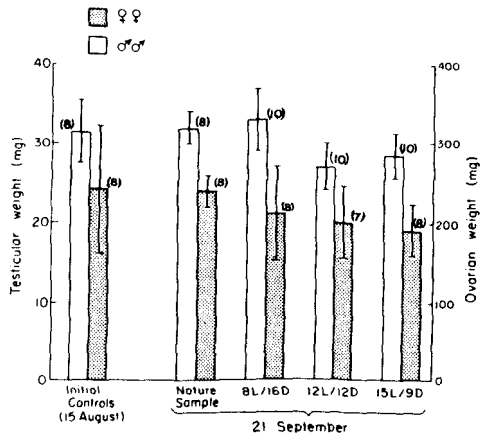

FIGURE 7.—Effect of 36-day 20°C treatment at various photoperiods on ovarian and testicular weight in *Gillichthys mirabilis*. Shaded histograms illustrate mean ovarian weights; open histograms illustrate mean testicular weights; the means are bracketed by one standard error. Photoperiod treatments are given below the histograms. Sample sizes are indicated atop each histogram.

in August. Recrudescence was initiated after 36 days in all photoperiod groups, but a long photoperiod (15L/9D) was most effective in females and least effective in males.

EFFECTS OF 16°C TREATMENT ON GONADAL FUNCTION

Gonadal recrudescence does not normally occur if *Gillichthys* is exposed to 20°C in July. To examine whether recrudescence could be initiated in July at a slightly lower temperature, the effect of 16°C treatment was determined. Before examining the effects of this temperature treatment on recrudescence, an experiment was conducted in January to be certain that gametogenesis could be maintained at this temperature. The ovaries of the initial January controls (Table 6) were in Stage III, IV, or V; the testes of fish in this sample were in Stage 4 or 5. Neither testicular nor ovarian weights were significantly altered by this 90-day treatment, nor was there an evident change in gonadal activity as judged by histological examination.

The testes and ovaries of the initial controls of the July experiment (Table 6) were regres-

sing (Stages 0 and I). Following 80-day treatment at 16°C, ovarian and testicular weights were significantly greater ($P < 0.01$) than those of the initial controls. Testes of the experimental fish were in Stage 3 or 5. Ovaries of fish in the 16°C group were in Stage II or III.

The results of these experiments indicate that spermatogenesis and vitellogenesis are maintained at 16°C. In fish with regressing gonads (collected in July), 16°C treatment initiates spermatogenesis and oogenesis.

EFFECTS OF LONG PHOTOPERIOD TREATMENT (15°C) ON FISH WITH REGRESSING TESTES

In July 1969 an experiment was initiated to determine whether a long photoperiod could block recrudescence at an "intermediate" temperature. All of the initial controls were undergoing testicular regression (Stage 0); the mean (± SE) testicular weight (mg) in this sample was 32.3 ± 2.2. Fish were exposed to 15°C for 30 days at a 15L/9D photoperiod. At the termination of the experiment a sample of fish was taken from nature; the mean testicular weight (± SE) in this group was 20.7 ± 3.2, and the testes of these fish were regressing (Stage 0). Testicular weights ($\bar{x}$ ± SE = 58.3 ± 7.3) of the 15°C group were significantly greater ($P < 0.01$) than those of the initial July controls and the September sample from nature. The testes of nine fish in the 15°C group were in Stage 3, and those of three were in Stage 4. These data suggest that 15°C treatment initiates testicular recrudescence, even when the photoperiod is long.

TABLE 6.—Effect of 16°C treatment (12L/12D) on testicular and ovarian weight in *Gillichthys mirabilis*.

Beginning date	Length treatment (Days)	Sex	Gonadal weight (mg) ($\bar{x}$ ± SE)		
			Initial controls	After treatment	(n)
January	90	Males	71.6 ± 4.9	66.7 ± 4.0	(10)
		Females	1,321 ± 295	1,136 ± 238	(11)
July	80	Males	32.3 ± 2.2	54.5 ± 4.1**	(10)
		Females	256 ± 23	438 ± 34**	(10)

**Significantly greater ($P < 0.01$) than initial controls.

EFFECTS OF 12° AND 20°C TREATMENT ON FISH IN STAGES OF ACTIVE GAMETOGENESIS

Data presented above suggest that gametogenesis may be maintained more effectively at 13°C than at 20°C. Beginning in March 1968, fish were exposed to 20° and 12°C for 21 days to examine this possibility (Table 7). The ovaries of the initial March controls were in phases of active vitellogenesis (Stage III) or the prespawning or postspawning condition (Stage IV or V); the testes of this group were in Stage 3.

TABLE 7.—Effect of 21-day 12° and 20°C treatments on ovarian and testicular weights in *Gillichthys mirabilis*.

Experimental group	Gonadal weight (mg) ($\bar{x}$ ± SE)			
	Males	(n)	Females	(n)
Initial controls (March)	59.3 ± 6.1	(11)	1,038 ± 198	(11)
12°C treatment	74.7 ± 6.3*	(10)	1,428 ± 106	(8)
20°C treatment	58.8 ± 5.7	(7)	1,375 ± 123	(8)
Terminal controls (April)	62.2 ± 5.4	(8)	3,043 ± 297	(18)

*Significantly greater ($P < 0.05$) than initial controls.

Ovarian weights in the two experimental groups did not differ significantly from those of the initial March controls, but they were significantly less ($P < 0.01$) than ovarian weights of the sample taken from nature at the time of sacrifice (April). Ovaries of the 20° and 12°C groups were in Stage III or IV; the ovaries of a majority of the April sample from nature were in Stage IV.

Testicular weights in the 20°C group were not significantly altered by treatment, but those of the 12°C group were significantly greater ($P < 0.05$) than the testicular weights of the initial March controls. The testes of fish in the 12°C group were in Stage 3 or 4; however, the testes of four of seven fish at 20°C were in Stage 2 and the remainder in Stage 3.

These data indicate that at a 12L/12D photoperiod, vitellogenesis and spermatogenesis will occur at both 12° and 20°C but that the lower temperature is more effective. Neither of these treatments, however, was as effective in promoting vitellogenesis as the factors acting on the natural population.

INHIBITION OF TESTICULAR REGRESSION BY LOW TEMPERATURE

An experiment was initiated in June 1968 to resolve whether low temperature treatment in combination with a short photoperiod could prevent gonadal regression at the "normal" time (early July). The testes of the beginning controls were in the prespawning or postspawning conditions (Stages 4 or 5). Fish were exposed to 10° or 20°C at a 10L/14D photoperiod for 21 days. The testes of fish collected from nature at the time of sacrifice (July) of the experimentals were regressing (Stage 0). Testicular weights in the 10°C group ($\bar{x} \pm SE = 60.7 \pm 4.1$) and the 20°C group ($61.0 \pm 6.2$) did not vary significantly from those (52.2 ± 3.9) in the initial June controls, but those of both groups were significantly greater ($P < 0.01$) than testicular weights of the July sample from nature (32.1 ± 3.1). The testes of 10 fish exposed to 10°C and 6 fish to 20°C were in active spermatogenesis (Stage 3), and 4 in each group were in the prespawning condition (Stage 4). Thus, testicular regression does not occur at the normal time when fish are exposed to temperatures between 10° and 20°C.

COMPARATIVE EFFECTS OF 10° AND 18°C TREATMENT ON GONADAL RECRUDESCENCE

To determine whether there is a differential effect of 10° and 18°C on gonadal recrudescence, a 21-day experiment was initiated in August 1970 with fish having regressing or quiescent gonads (Stages 0 and 1). The effects of these treatments on gonadal weight are presented in Table 8.

TABLE 8.—Effect of 21-day 10° and 18°C treatments (13L/11D) on ovarian and testicular weights in *Gillichthys mirabilis*.

Experimental group	Gonadal weight (mg) ($\bar{x} \pm SE$)			
	Males	(n)	Females	(n)
Initial controls (August)	32.5 ± 2.1	(10)	280 ± 18	(8)
10°C treatment	42.6 ± 4.4*	(8)	339 ± 48	(8)
18°C treatment	41.7 ± 7.3*	(8)	386 ± 33*	(8)

*Significantly greater ($P < 0.05$) than initial controls.

A significant increase ($P < 0.05$) in testicular weights occurred at both 10° and 18°C; testicular weights in the two groups were not significantly different. Spermatogenesis was initiated in all fish in both experimental groups. Ovarian weights in fish exposed to 18°C were significantly greater ($P < 0.05$) than those of the initial August controls; however, ovarian weights in the 10°C group were not significantly different than those of the initial controls or those of the 18°C group. Nonetheless, the ovaries of all fish in both experimental groups were in early phases of vitellogenesis (Stage III).

These data indicate that there is little or no difference in the rate of initiation of gonadal recrudescence at 10° and 18°C. The possibility exists, however, that following the initiation of recrudescence, the rate of testicular and ovarian growth could be different at the two temperatures.

DISCUSSION

In previous work (Barlow and de Vlaming, 1972; de Vlaming, 1972b), the gonadal cycle of *Gillichthys* was observed to be closely correlated with seasonal changes in several environmental variables. Gonadal regression occurs as daylength begins to shorten and temperature reaches a seasonal maximum. The initiation of gonadal recrudescence coincides with the decline in temperature and the continued decrease in daylength. A majority of the spawning in this species occurs when daylength and temperature are increasing. The data presented here suggest that temperature may be important with regard to reproductive cycling, photoperiod acting only to modify the responses to temperature.

GONADAL REGRESSION

In these laboratory experiments, constant temperatures of 24°C and above cause testicular and ovarian regression at any time of the year regardless of photoperiod (i.e., photoperiod does not seem to have an influence on gonadal regression). As temperature is increased above 24°C, shorter treatment periods are required to attain the ovarian and testicular quiescent phases.

High temperatures bring about ovarian regression by inhibiting vitellogenesis and causing atresia of all yolky oocytes. In males, high temperatures apparently increase the rate of meiotic divisions and the process of spermiogenesis; this is apparent because the testes of fish sacrificed soon after the initiation of high temperature treatment are characterized by large numbers of secondary spermatocytes and spermatids. Fish sacrificed after longer treatments at high temperatures, however, are characterized by testes with only primary spermatogonia, suggesting that high temperatures inhibit the transformation of spermatogonia into primary spermatocytes. High temperatures also cause the "flaking off" of cysts of spermatocytes from the germinal epithelium into the lumen of the testicular lobules. Moreover, pyknotic degeneration of spermatocytes, spermatids, and spermatozoa occurs at high temperatures, followed by phagocytosis of cellular debris. Mitotic proliferation of spermatogonia is inhibited above 25°C, but treatment at 27°C for 15 days does not inactivate the sperm remaining in the testes. Weisel (1948) showed that the spermatozoa of *Gillichthys* remain alive in vitro for 2 weeks at 2°-4°C, but at 24°-26°C they are immobile in 33 hr.

In *Gillichthys* the termination of the reproductive season is apparently not endogenously timed. Regression is not "obligatory" since low temperature treatments (regardless of photoperiod) prevented gonadal involution at the "normal" time (July). These studies imply that the reproductive cycle of this species is primarily under exogenous regulation. A similar situation has been reported in the cyprinodontid, *Epiplatys bifasciatus* which occurs in the Zio River and Lagoon of Lomé of the Republic of Togo, Africa (Loiselle, 1969). *Gillichthys* is thus apparently a potentially continuous breeder but has a reproductive cycle imposed upon it by the increased temperatures of summer. Although little information is available on the causes of termination of reproductive cycles, differences are evident. For example, Baggerman (1957) suggested that since none of the experimental conditions she tested could maintain continuous breeding in *Gasterosteus aculeatus*, termination of the cycle is endogenously controlled. The rate of postspermatogonial regression is also accelerated by warm temperatures, and low temperatures retard the rate of sexual regression in *Fundulus heteroclitus* (Lofts, Pickford, and Atz, 1968).

GONADAL RECRUDESCENCE

In *Gillichthys* gonadal recrudescence does not occur at constant temperatures of 24°C or above, regardless of photoperiod. Long-term experiments indicate that gonadal recrudescence is not initiated in males or females after nearly 4 months at high temperatures (comparable to summer temperatures). High temperatures also retard the early phases or intermediate phases of gametogenesis in *Fundulus heteroclitus* (Burger, 1939), *Phoxinus laevis* (Bullough, 1939), female *Apeltes quadracus* (Merriman and Schedl, 1941), *Enneacanthus obesus* (Harrington, 1956), female *Fundulus confluentus* (Harrington, 1959), *Couesius plumbeus* (Ahsan, 1966), and *Cymatogaster aggregata* (Wiebe, 1968).

Experiments, begun in winter and spring with the longjaw goby in phases of active gametogenesis, indicate that gonadal activity is maintained at 20°C over a wide range of photoperiods; long photoperiods may be more effective in this regard, but more experiments are needed to prove conclusively the influence of photoperiod. Beginning in July with fish having regressing testes, mitotic proliferation of spermatogonia was stimulated, especially with a short photoperiod, but complete recrudescence did not occur at 20°C. However, beginning in August with fish having regressing testes, recrudescence did occur at 20°C; a short photoperiod was more effective in this respect. Beginning in September, testicular and ovarian recrudescence is initiated at 21°C, but only with a short photoperiod. The rate of spermatogenesis was, however, extremely low at these temperatures. Thus, a shift in gonadal responsiveness to 20°C apparently occurs between July and August. The experiment beginning in July was continued for 3 months without the initiation of spermatogenesis whereas the experiment beginning in August was terminated after a much shorter time. The

adaptive significance of the "refractoriness" to 20°C in July may be to prevent "premature" initiation of spermatogenesis should temperatures decrease somewhat during the regression phase.

Although the data presented here are by no means conclusive, they suggest that photoperiod has a variable effect at 20°C. Long photoperiods may be more effective in maintaining spermatogenesis whereas short photoperiods seem to promote a faster rate of recrudescence at 20°C. Moreover, active spermatogenesis is maintained at 22°C only with a long photoperiod. Differences are evident between the sexes since vitellogenesis does not occur at this temperature regardless of photoperiod. Since a majority of spawning in *Gillichthys* occurs when daylength is increasing and recrudescence occurs when daylength is decreasing, the variation in the effects of photoperiod seen here seem reasonable.

That 20°C is not as effective as lower temperatures in maintaining gametogenesis or promoting gonadal recrudescence is consistent with the phenological and climatic data presented by de Vlaming (1972b). Average daily temperatures are below 20°C from early October to mid-June; it is during this period that most of the gonadal activity occurs in this species.

Complete gonadal recrudescence in both male and female *Gillichthys* occurred at 12° and 13°C, suggesting that the decreasing temperatures in autumn are primarily responsible for regulating this process. Constant low temperature treatment promoted a faster rate of recrudescence than occurred in the natural population. Diurnal increases in temperature in the natural habitat during autumn may account for the slower rate of gonadal recrudescence. Short photoperiods augmented the rate of recrudescence at low temperatures in both males and females. Thus, the decreasing photoperiod in autumn probably facilitates, but is not essential for, the effects of decreasing environmental temperatures in promoting gonadal recrudescence.

With a 12L/12D photoperiod, gonadal recrudescence was initiated within 21 days at 10° and 18°C. This suggests that gonadal response in this species is relatively fast; rapid mobilization of energy for gonadal recrudescence may be characteristic of species that spawn more than once in a season. Whether recrudescence was more rapid at 10° or 18°C was not investigated, nor are there sufficient data to indicate whether an optimal temperature exists for the completion of gonadal recrudescence. However, the data presented here show that gonadal recrudescence will occur over a wide temperature range (10°-20°C). This gonadal responsiveness to a wide range of temperatures may reflect an adaptation to the labile thermal environment of this species.

Active gametogenesis was maintained at low temperatures regardless of photoperiod. After treatment at a low temperature in May (Figure 4), more male fish were in the prespawning condition at the longer photoperiod. In the same experiment, however, a short photoperiod was more effective in maintaining vitellogenesis (Figure 4). Perhaps these results reflect a true photoperiod influence, but because of the variable gonadal conditions of the beginning controls no conclusive statements can be advanced. In fact, experiments begun in January with fish revealing less variable gonadal conditions indicated that long and short photoperiod treatments maintained active gametogenesis equally well at low temperature. Photoperiod influences, however, could vary between January and May.

The question arises as to why all fish treated at low temperatures did not progress to the prespawning condition. One possible explanation is that many of the experiments discussed here were relatively short term. In many of the long-term experiments some of the fish could be stripped of ripe eggs and sperm. It is likely, however, that physical factors other than temperature and photoperiod, and also social factors, influence final gonadal maturation and spawning in this species. Indeed, Weisel (1947) has indicated that *Gillichthys* is territorial and has an elaborate courtship behavior. In addition, de Vlaming (1971b) has shown that salinity changes and changes in the availability of food can alter the rate of gametogenesis in *Gillichthys*. Thus, the failure of many of the experimental fish to completely attain the prespawning condition was probably due to the absence of certain conditions in the laboratory sit-

uation. Unfortunately, no data are available on the effects of exogenous factors on the frequency of spawning, fecundity, egg size, or survivorship of the fry in this species, nor are data available on the influence of environmental factors on the spawning act. For a complete understanding of the role of the environment in the physiology and ecology of reproduction in *Gillichthys*, examination of these parameters is needed.

The data presented here indicate that temperature may be the proximate factor regulating the *Gillichthys* reproductive cycle. Zambrano (1971) found that the secretory activity of the pituitary gonadotrophic cells is altered by temperatures, and this provides further support for this suggestion. However, since fish in their natural habitat experience large diurnal temperature fluctuations, the experiments reported here are not conclusive. In addition, *Gillichthys* is capable of regulating its body temperature by behavioral means (de Vlaming, 1971a). These experiments do set the physiological limits with regard to the influence of temperature on reproduction. Understanding the ecological meaning of temperature in reproductive cycling requires experimentation with diurnally fluctuating temperature; experiments of this nature are reported elsewhere (de Vlaming, 1972c).

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