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Synchronization of Plasma Sexual Steroid Concentrations and Gonadal Cycles in the Sleeper, *Eleotris acanthopoma*

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Hurng-Yi Wang, Ching-Feng Weng, Ming-Chung Tu and Sin-Che Lee (2001) Synchronization of plasma sexual steroid concentrations and gonadal cycles in the sleeper, *Eleotris acanthopoma*. *Zoological Studies* **40**(1): 14-20. This paper describes the possible influences of environmental factors on the reproductive cycle of *Eleotris acanthopoma* in the Kaoping River estuary. For over 1 yr, gonad gross morphologies of the fish were examined by light microscopy, and plasma 17 beta-estradiol (E2) and testosterone (T) were analyzed by ELISA. In male *E. acanthopoma*, plasma T concentrations show a single seasonal cycle, with a peak in the summer (June to August) following the profile of the gonadosomatic index (GSI). In females, plasma E2 concentrations are significantly elevated during summer in accordance with the GSI. Low temperatures and a short photoperiod in the winter correlate well with the arrest of gonad maturation. Annual patterns of plasma E2 and T levels are similar to those of GSI changes existing in *E. acanthopoma* of either sex. These findings indicate that both temperature and photoperiod dominantly affect the reproductive cycle of *E. acanthopoma*.

Key words: Photoperiod, Temperature, Annual cycle, *Eleotris acanthopoma*, Estradiol (E2) and Testosterone (T).

Reproduction is an important factor ensuring the continuation of a species by recruitment of the next generation. Most fish employ external fertilization, and their embryos and larvae must be adapted for more or less unstable environments including variable salinities, temperatures, light, water currents, food supplies, and predation. Reproductive adaptations would be expected to occur during the breeding season to ensure the maximum survival of offspring. The number of surviving offspring depends to a large extent on the number of eggs laid, and this in turn is governed by fecundity and the number of mature females themselves. In order to understand reproductive biology, particularly the reproductive cycle of fish, studies on the timing and duration of breeding, the process of gonad maturation, the sex ratio, and fecundity must be included. The sex steroid levels in gonads and peripheral blood plasma are useful indicators of steroidogenic secretion during a particular stage of the sexual cycle, and in association with changes in gonad condition, have fostered an understanding of the endocrine control of reproduction in teleosts. Determinations of steroid hormones and gonad condition during the annual reproductive cycles of various teleosts have been reported (Singh and Singh 1987, Matsuyama et al. 1991). Moreover, alterations of steroid hormones have implications in studying the effects of the environment (temperature, photoperiod, pollution, or habitat degradation) on the reproductive biology of fish (Bromage et al. 1982, Okuzawa et al. 1989, Shimizu et al. 1994).

Gobioids comprise a large group of marine, freshwater, or estuarine fishes; *Eleotris acanthopoma* of the Eleotrinae is one of the most abundant species in Taiwan, living mainly in estuaries of the west coast. Abundant nutrients in rivers have attracted this species to widespread habitats. The re-

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cent discovery of 35 freshwater and 81 brackish water fish species in the Kaoping River has proven it to be the most important habitat for river fishes in southern Taiwan (Han et al. 1997). *Eleotris acanthopoma* is one of few typical estuarine species that completes its entire life cycle within the estuaries with a limited migration range. Its year-round occurrence and short life span permit the study of seasonal changes of biological characteristics. We therefore chose *E. acanthopoma* from the Kaoping River to examine the relationships of histology and sex steroids in gonads during the reproductive cycle, and to correlate these changes with environmental factors such as temperature, photoperiod, and currents in the surrounding areas.

MATERIALS AND METHODS

The study site (22°30'N, 120°24'E) is located under a 1100-m long bridge at a distance of 2500 m from the mouth of the Kaoping River. The lowest water level remains at 1-2 m deep, with the water heavily polluted by sewage discharges from petrochemical plants on the river banks. The river bed is mainly muddy sand with patches of gravel. Twenty to 30 specimens of *Eleotris acanthopoma* were collected monthly using electric gill nets from May 1996 to Aug. 1997 and transferred live to the laboratory for the experiment. Fish were sexed based on the morphology of the gonads. However, it was difficult to distinguish the sex due to the immaturity of the gonads during the non-reproductive season. After anesthetization with ice, the fish were sexed and weighed (7.01-30.30 g), and standard length measured (7.5-11.2 cm). Blood samples were taken from the heart using a heparinized microsyringe; they were then centrifuged, and the plasma stored at –40 °C until steroid assay. The ovaries and testes were dissected out and weighed, and the gonadosomatic index (GSI) was calculated following the equation GSI = (GW/BW) × 100, where GW and BW are gonad weight (g) and body weight (g), respectively.

Enzyme-linked immunosorbent assay (ELISA)

The plasma levels of 17 beta-estradiol (E2) and testosterone (T) were determined with a commercial ELISA kit (NEOGEN, Lexington, KY, USA) after ethyl ether extraction. The cross reactivities of anti-estradiol rabbit antibody were 100% for 17 beta-estradiol, 1% for testosterone, 0.41% for estriol, 0.1% for estrone, and a trace amount for other steroid derivatives. The cross reactivities of anti-testosterone rabbit antibody were 100% for testosterone, 100% for dihydrotestosterone, 0.86% for androstenedione, 0.1% for estriol, 0.05% for 17 beta-estradiol, and a trace amount for other steroid derivatives. A 20-ul extracted sample was applied for each E2 or T assay. The sensitivities of E2 and T were 10 and 5 pg/ml, respectively. Intra-assay variations were 8.7% (n = 12) for E2, and 9.6% for T. Interassay variations were 17.8% (n = 8) and 20.3%, respectively.

Light microscopy

Gonads were fixed in 4% formalin, embedded in paraffin, sectioned at 10 µm, and stained with Mayer's hematoxylin and eosin for histological examination. The sex of the fish was also determined through histological section examination.

Statistics

All gonadal weight, GSI, E2, and T data are expressed as the mean ± S.E.M. Differences throughout the spawning season were determined by one-way analysis of variance (ANOVA), and means among them were compared with the non-spawning season by t-test. The values of each group as compared with the non-spawning season are shown with $p < 0.05$ as the level for significance.

RESULTS

Fluctuations of water temperature in the Kaoping River during the study period are shown in figure 1A. The water temperature was low between Dec. and the following Feb. (about 24 °C), rising gradually from Apr., and remaining at a high level between June and Aug. (above 30 °C). Changes of photoperiod showed a similar trend to that of water temperature (Fig. 1B). The photoperiod was short between Dec. and the following Feb. (below 12 h), increasing gradually from Apr., and maintaining at a longer period during June and July (above 14 h). Generally, the increase of ovarian weight (wet, g) in *Eleotris acanthopoma* commenced in May, and remained at significantly ($p < 0.05$) high levels from June to Aug. (Fig. 1C). After Aug., ovarian weight decreased. Compared to that in other months, the testis weight (wet, g) reached a steady high level from June to Aug.. However, the extent of the testicular change was much smaller than that of the ovary. Accordingly, changes of gonadal weight in *E. acanthopoma* accompanied alterations of water temperature and photoperiod.

Changes in the gonadosomatic index (GSI = [ovarian weight, g/ body weight, g] × 100) and plasma E2 in female over a 1-yr period are shown in figure 2A. GSI profiles of female were the lowest in Jan., increasing gradually toward a peak in July (reproductive period, June-Aug., GSI = 5%-8%). Plasma E2 levels showed a similar pattern as changes of GSI during the study period, and ranged from 2 to 4 ng/ml in females between June and Aug.. Similarly, the changing trends of male GSI and plasma T levels agree well with those of GSI and plasma E2 levels in females during the same period (Fig. 2B). Changes of GSI (%) in males were much lower than those in females. In addition, the alteration of plasma T levels in males was generally lower (about 1-2 ng in the period). The gonads (testis and ovary) gradually increased in weight from Mar., and were fully grown between June and Aug., resulting in a large proportion of GSI ($p < 0.05$) being concentrated in this period. Considering the plasma steroid analysis and histological examination of gonads, the spawning season should be confined to June-Aug. (Figs. 2-4). In the beginning of May, the ovary of the female contained small oocytes in the early cortical alveolus stage (Fig. 3A, B). The ovary was more active while attaining gonad maturity with most vitellogenic oocytes recorded during the spawning season, but the ovary regressed after Aug. (Fig. 3C, D). The multimodal oocytes viewed from ovarian sections indi-

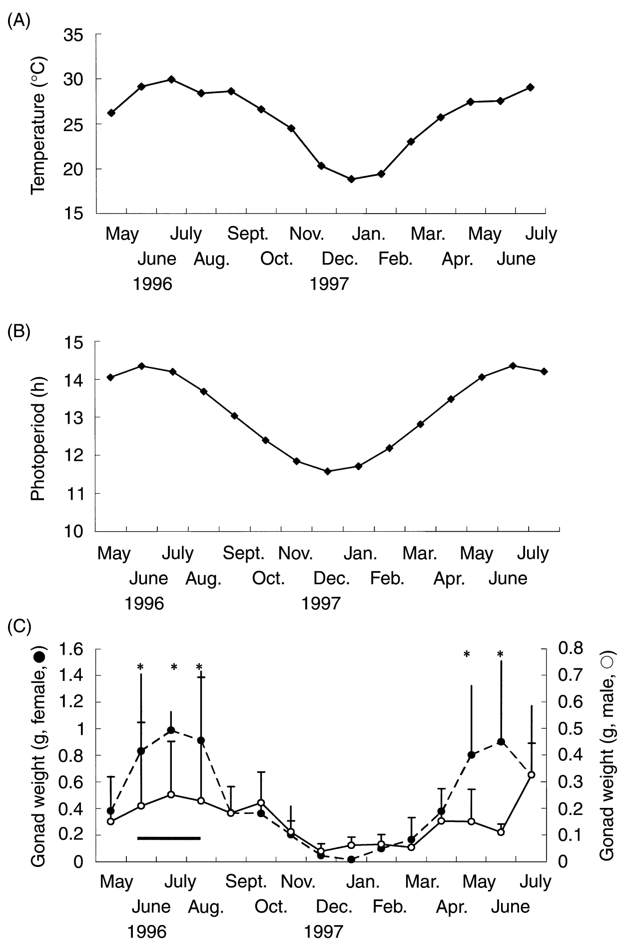

Fig. 1. Pattern of (A) water temperature (°C), (B) photoperiod (h), and (C) gonadal weight (g) in male and female *Eleotris acanthopoma* fish during the study period (from May 1996 to July 1997). The black line represents the spawning season. An asterisk (*) represents a significant difference at $p < 0.05$ in female gonad weight between the spawning period and non-spawning season. The sample size in each month is 5 or 6 fish.

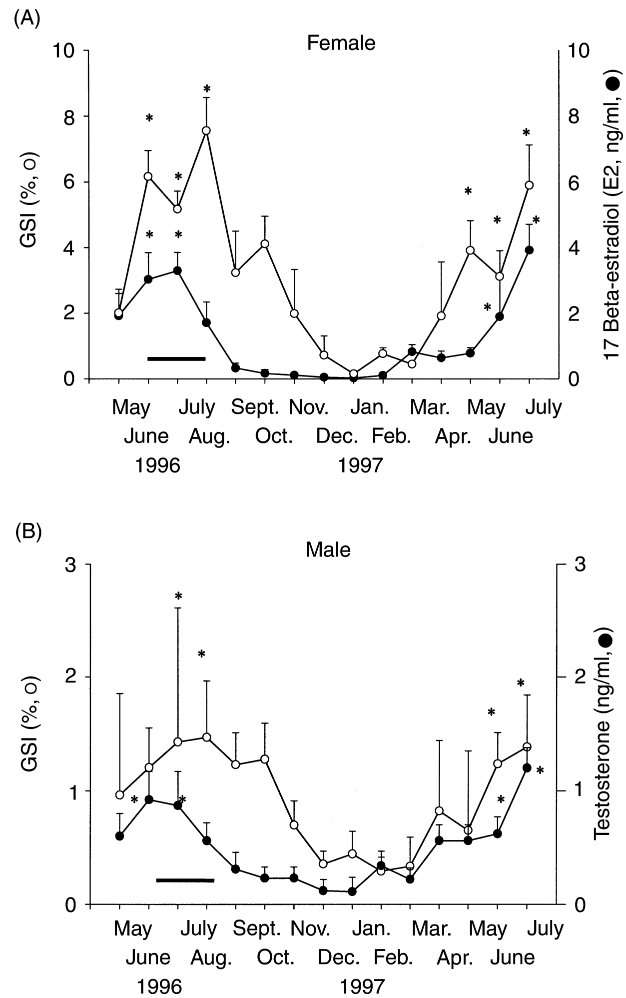

Fig. 2. Reproductive cycle of (A) E2 (ng/ml) and GSI (%) in female, and (B) T (ng/ml) and GSI (%) in male *Eleotris acanthopoma* fish during the study period (from May 1996 to July 1997). The black line represents the spawning season. An asterisk (*) represents a significant difference at $p < 0.05$ between the spawning period and non-spawning season. The sample size in each month is 5 or 6 fish.

cated that the female carries out multiple ovulations during the spawning season, which is considered to be an asynchronous type of spawning (Fig. 3D). In the male, the testis consisted of undeveloped germinal cells (spermatogonia and spermatocytes) at the beginning of May (Fig. 4A) after which the spermatogenic tubules grew gradually (Fig. 4B), reaching a maximum during the spawning season (June to Aug.) when more spermatozoa had matured (Fig. 4C). Like the ovary, testis regressed after Aug. when vacuoles formed after spermiation (Fig. 4D).

DISCUSSION

The annual cycle of steroids indicated that high plasma levels of E2 in females ($p < 0.05$) and testosterone in males ($p < 0.05$) correspond well with high gonadal activity (high GSI index and active vitellogenesis or spermatogenesis) in the study period. The synchronous spawning season of June-Aug. in both sexes of *Eleotris acanthopoma* was determined from seasonal profiles of GSI and plasma steroids, and successive observations of gonad histology. The presence of oocytes in different size groups suggests that the female *E. acanthopoma* may spawn several times in parallel with high levels of E2 during the spawning season. High levels of E2 during spawning also occur in other species which spawn several times during the season (Kadmon et al. 1985, Matsuyama et al. 1990). In teleost fishes, androgens (T and 11-ketotestosterone, 11-KT) in the blood are effective in stimulating male characters (Borg 1994). Some fishes display peaks of androgen levels during the spawning period (Schulz 1984, Dye et al. 1986, Fitzpatrick et al. 1986, Ouchi et al. 1988, Scott and Sumpter 1989, Mayer et al. 1990, Cerda et al. 1997) as were noted in the present report. In other studied fishes, however, both T and 11-KT in males peak during prespawning, rather than during spawning periods (Stuart-Kregor et al. 1981, Scott et al. 1984, Methven and Crim 1991,

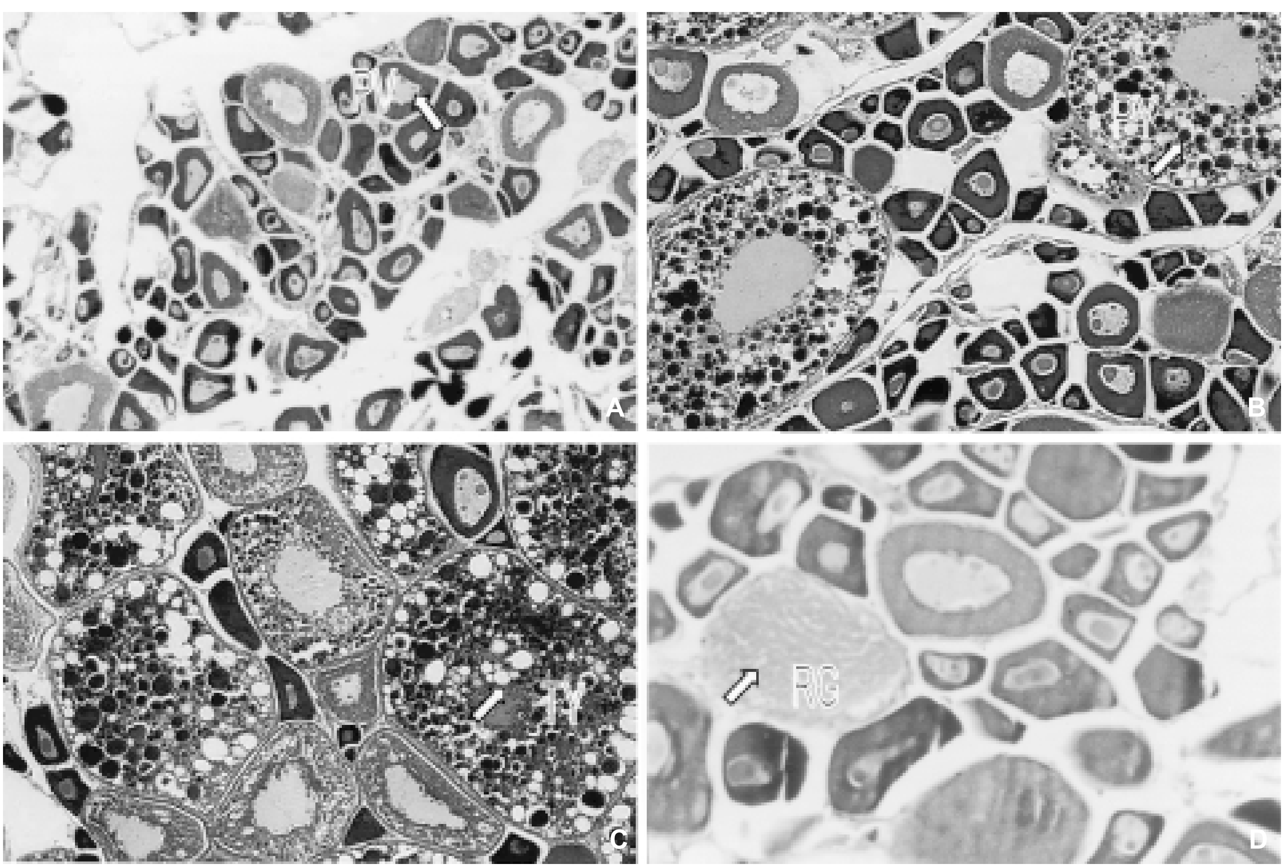

Fig. 3. Typical pattern of ovarian histological examination results in female *Eleotris acanthopoma* during the study period (from May 1996 to July 1997). (A) Primordial oocytes in the immature stage (PV, April), (B) onset of vitellogenesis (PY, May), (C) process of vitellogenesis (TY), and (D) completion of vitellogenesis (June to August) (×400 magnification). PV, provitellogenic oocyte; PY, primary yolk stage; RG, regression; TY, tertiary yolk stage.

Mayer et al. 1992). In the present study, we measured plasma T levels by using an ELISA kit which utilizes antibodies with low cross reactivities with 11-KT. Therefore, we cannot exclude the existence of 11-KT in male *Eleotris acanthopoma*, but further experimentation is necessary to clarify this point.

One study of an endemic cyprinid (Wang et al. 1995), *Zacco pachycephalus*, in the Kaoping River showed a bimodal seasonal cyclic pattern in summer which was otherwise not found in *E. acanthopoma*. The different reproductive patterns observed may be due to different strategies used by the fish or be the result of different study methods. The reproductive cycle of *Z. pachycephalus* is determined indirectly by the occurrence of juveniles, which may be influenced greatly by the circumstances of the study periods. However, since the environments of freshwater and brackish water differ distinctly, fish living in such diversified environments may evolve different reproductive strategies. It will be interesting to study the reproductive period of gobies, such as *Rhinogobius*, living only in the freshwater environment of the Kaoping River. Because both *Rhinogobius* and *Eleotris* belong to the same family, a similar reproductive cycle pattern may be expected within limits of evolutionary constraints. Therefore, environmental factors may also play important roles in shaping patterns of the reproductive cycle. On the contrary, understanding the annual cycle may provide a glimpse into environmental changes, including habitat degradation, contamination, or pollution.

Changes of water temperature and photoperiod correlate with changes of GSI and steroids in the annual reproductive cycle of fishes (Lam 1983). In previous studies, high temperatures and long daylength were positively correlated with changes of GSI and plasma steroids in summer spawners. On the other hand, high temperatures and long daylength were

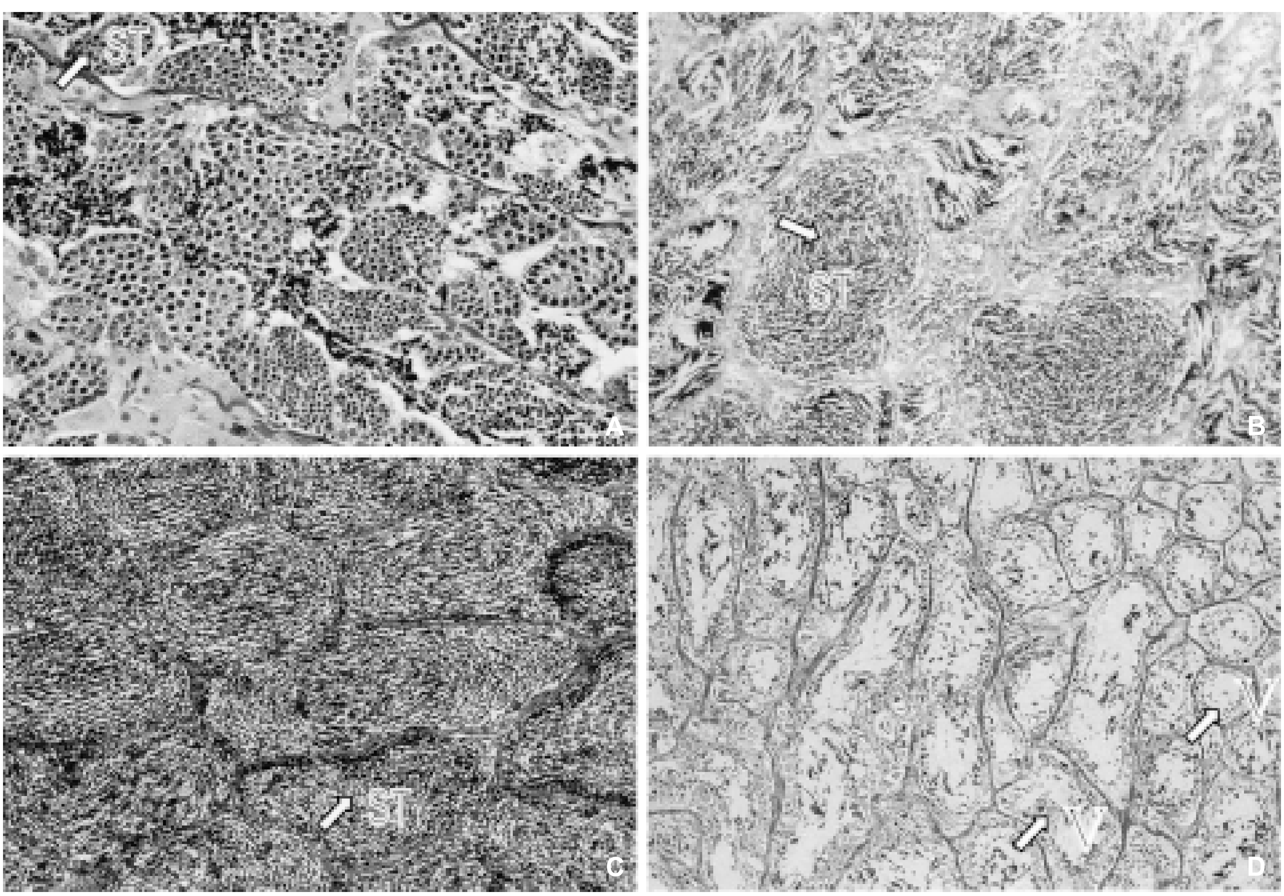

Fig. 4. Typical pattern of testicular histological examination results in male *Eleotris acanthopoma* during the study period (from May 1996 to July 1997). (A) Solid mass of cells in spermatogenic tubules (ST) in the immature stage (April), (B) spermatogenesis in spermatogenic tubules (May) containing spermatozoa and spermatogonia, (C) gametogenesis (June to August), spermatogenic tubules containing spermatozoa, and spermatids in addition to spermatocytes and spermatogonia, and (D) vasculosa (V) after spermiation (June to Auguet) (x400 magnification). Arrows indicate ST in (A), (B), and (C), and V in (D), respectively.

negatively correlated with changes of GSI and plasma steriods in autumn spawners. In summer spawners, for instance, water temperature may play a key role in both initiating and terminating the spawning season of the Japanese sardine, *Sardinops melanostictus* (Matsuyama et al. 1991). One report showed that the GSI of blueline tilefish, *Caulolatilus microps*, exhibited peaks in May and Sept.. Termination of gonadogenesis and the concomitant gonad regression coincided with a rapidly decreasing photoperiod in Oct. (Ross and Merriner 1982). The initiating factor of the breeding season in rose bitterling, *Rhodeus ocellatus ocellatus*, is rising temperature, whereas the terminating one is decreasing daylength at high temperatures (Shimizu et al. 1994). Okuzawa et al. (1989) reported that a cryprinid fish, *Gnathopogon caerulescens*, shows a photoperiodic response during the spawning season, and is temperature dependent. On the other hand, in a study of an autumn spawner, *Acheliognathus rhombea*, both lower temperature and shorter daylength had great effects on increases of GSI and plasma steroid levels (Shimizu et al. 1994). These results indicate that the critical photoperiod for maturation falls between 13 and 14 h of light per day (Asahino and Hanyu 1983) for summer spawners and 12 h per day (Shimizu et al. 1994) for autumn spawners. And, these might be latitude dependent.

It seems that both photoperiod and temperature play major roles in regulating gonadal development of *Eleotris acanthopoma*. In our study, daylength reached 13 h in Apr. and increased to 14 h from May to June with initially increasing GSI and steroid levels (Fig. 1B). Higher temperatures (26 °C) and longer day length (14 h) in summer corresponded well with the spawning season (June-Aug.) of this species. The photoperiod also fit well with termination of the reproductive period. Daylength fell below 13 h in Sept. with the end of reproduction, when the water temperature was still higher than 25 °C. Alternatively, photoperiod plays a more important role than water temperature in termination of the reproductive cycle; however, this needs to be proven in further experiments. We can conclude that the spawning period of *E. acanthopoma* is initiated by lengthening days and increasing water temperatures in early summer, and is terminated by shortened daylength at the end of the reproductive period.

The distributions of *Eleotris* in Taiwan are geographically differentiated: *E. fusca* are only found in the east coast of Taiwan whereas *E. acanthopoma* and *E. melanosoma* live mainly in the west region of the Island and totally absent in the east coast (Wang unpublished). Since the dispersal of *Eleotris* mainly rely on pelagic larval stage (Miller 1998), the water current right after reproductive period will have decisive impact on their distribution and differentiation. Water currents around Taiwan change seasonally. In the winter, the Kuroshio current splits near the southernmost tip of Taiwan, with one of its branches skirting the southern end of Taiwan and then entering the Taiwan Strait. While running near northeastern Taiwan, another branch of the Kuroshio current runs along the east coast and encounters the current from the South China Sea (Chern and Wang 1989). From late spring to early fall, the Kuroshio is replaced by the South China Sea current which flows all the way through the Taiwan Strait when the seawater monsoon prevails whereas Kuroshio passes the east coast of Taiwan (Chern and Wang 1989). As our annual cycle data revealed, the reproductive peak of *Eleotris* is from late June to Aug.. Meanwhile, the water currents of east and west coast will not blend with other and will prevent larvae dispersal from east to west or vice versa. Consequently, the allopatric distribution of different species of *Eleotris* around Taiwan would be contributed by the coordinate of reproductive period and water currents.

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塘鱧血漿性腺類固醇濃度與生殖週期之同步性

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本文描述環境因子對高屏溪河口塘鱧的生殖週期之可能影響。超過一年期以光學顯微鏡研究魚體生殖腺之形態，並以酵素免疫分析血清雌二醇和睪固酮。在雄性塘鱧血清睪固酮濃度顯示了單一週期的變化，且其高峰在夏天（六月到八月）伴隨著生殖腺體重指數。在雌性，血清雌二醇濃度與生殖腺體重指數在夏天顯著變高。冬天的低溫與短光週期誘發生殖腺的成熟。雌性與雄性塘鱧之血清雌二醇和睪固酮的年變化和生殖腺體重指數相似。這些發現指出低溫與短日照顯然影響塘鱧的生殖週期。

關鍵詞：光週期，溫度，年週期，塘鱧，性腺類固醇。

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