Photothermal manipulation of reproduction in Indian major carp: a step forward for off-season breeding and seed production

S. K. Sarkar[1,*], A. Saha[1], S. Dasgupta[1,3], S. Nandi[1], D. K. Verma[1], P. Routray[1], C. Devaraj[1], J. Mohanty[1], N. Sarangi[1], A. E. Eknath[1] and S. Ayyappan[2]

[1]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India
[2]Indian Council of Agricultural Research, Krishi Bhavan, New Delhi 110 114, India
[3]Present address: Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Andheri (W), Mumbai 400 061, India

We report here for the first time significant advancement of sexual maturation and off-season breeding of Indian major carp (IMC) species, rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrihinus mrigala*) through photothermal manipulation during winter at least 4–5 months prior to their normal spawning season (June–August). The spent fishes were subjected to different photothermal regimes such as long photoperiod in combination with above ambient water temperature (LP–AAT), simulated natural photoperiod with ambient temperature (SNP–AT) under controlled condition and natural photoperiod under pond condition (NP–AT). Both the sexes of the IMCs attained suitable gonadal maturity for induced spawning between 100 and 124 days of rearing under LP–AAT condition during winter months. However, the broods under the SNP–AT and NP–AT regimes did not show any sign of maturity of gonads. Matured females of all the species induced to spawn successfully and produced a similar quantity of eggs and spawns as reported in the case of pre-monsoon spawning in the species, except for mrigal. The spawning success of individual species ranged between 66.6% and 100%. The results clearly reveal that the photothermal manipulation does modulate physiological rhythm for altering spawning times and provides scope for off-season production of spawn, fry and fingerlings of IMC almost throughout the year to facilitate aquaculture in a most effective way.

Keywords: Carp, off-season seed, maturity, photothermal manipulation, spawning.

SEASONALITY of reproduction in fish is synchronized with the seasonal changes in climate, day length and food availability. For most animals and many fish species, the seasonally changing pattern of day length coordinates reproductive development. In order to use 'light' for the purpose, the perception of light and changing pattern of photoperiod must be measured and this information must be provided as a suitable 'signal' for integration by the neuro-endocrine cascade, which ultimately initiates and then modulates reproductive development[1,2]. Thus, this pathway of regulation and methods has been used in the present study and elsewhere[3–5] to activate off-season spawning in order to produce year-round spawn for fry production. In order to put this hypothesis into practice, one would design the experimental alteration in photoperiod carefully considering the biological clock running under the ambient light cycle. Besides, the relationship of light and temperature with maturation in fish may have to be taken into consideration as, it is clearly envisaged that temperature plays a crucial role in regulating reproductive cycle in many fishes, particularly in carp[6].

Since the first success achieved in induced spawning in Indian major carp (IMC) through hypophysation in 1957 (ref. 7), more attention has been paid to address the problems associated with the routine brood management practices, such as water quality and nutrition to obtain gonadal maturity suitable for induced spawning[8,9] to meet the commercial requirement of fish seed. In spite of much advancement in the breeding technology[10], including multiple breeding[9], neither gonadal maturation nor spawning has been possible so far during September–March every year in these species, which remains a bottleneck for year-round spawn production.

Though research on manipulation of reproduction and spawning time in Indian fish has been initiated a few decades ago, it is but restricted to only different air-breathing fishes, such as *Heteropneustes fossilis*[11,12], *Channa punctatus*[13,14] and *Clarius batracus*[15,16], and minor carp, *Cirrhinus reba*[17,18], probably due to their easy maintenance under laboratory conditions. Manipulation of reproduction in IMC, particularly in *Catla catla* has been attempted through photoperiod alteration recently[19]. Studies have revealed that photoperiod per se plays an important role in the seasonal maturation of ovary and testes in catla under ambient thermal condition[20,21]. But no ovarian recrudescence was reported during the preparatory phase (February–March), and thus failed to demonstrate advanced gonadal maturity in respect to their normal spawning season[21]. So far, no information is available on manipulation of physiological rhythm of reproduction in any other major carp species in India.

The present study is concerned with the activation of neuro-endocrine rhythm through manipulation of external factors, such as photoperiod and temperature to activate off-season spawning in IMC. Thus, we report here a technique and devise of modulating reproduction rhythm of carp, such as rohu, catla and mrigal to obtain maturity of gonads at least 3–4 months prior to their normal spawning season in Orissa, by exogenous orientation of photoperiod and temperature (patent filed in the name of Indian Council of Agricultural Research, No. Del 1689).

*For correspondence. (e-mail: sksampadsarkar@yahoo.co.in)

Matured male and female brood fishes of rohu, catla and mrigal were carefully selected from the brood-rearing pond located at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, India. The fishes were induced to breed to confirm that our experiments were initiated with spawned fishes only. In the preliminary experiment, eight spent females and eight males were distributed equally into two groups and kept in separate cemented cisterns under long photoperiod (light : dark hours; 15.5 L : 8.5 D) in combination with above ambient temperature at 27–29°C (LP–AAT, lights on at 0530 h). The rearing condition and feeding schedule were maintained in the pattern as described below. Both the males and females attained suitable maturity in March 2007 when they were subjected to induced breeding. The induced breeding experiments revealed that out of four matured females and four males, three of each spawned successfully with an average spawn production of 1.33×10^5/kg body wt. The success of the induced breeding programme in March, at least 2–3 months prior to their normal spawning season in Orissa, encouraged us to take up another systematic experiment to bring advanced maturity in all the species of IMC through photoperiod and temperature (photothermal) manipulation of reproduction. A total of 10 females and 10 males of individual species, viz. rohu, catla and mrigal, which have already spawned once (spent fish), were reared in separate ponds sex-wise for another 2–3 months. At the end of September, the healthy females and males of rohu and catla, weighing between 600 and 1200 g, were selected from the ponds and randomly distributed in different groups with each group comprising the same number of male and female of individual species (Table 1). Thereafter five such groups were stocked in separate circular cement tanks (dia 2.4 m × height 1.2 m), and two groups were marked by fin clipping and stocked in separate ponds in order to subject them to different photothermal (photoperiod and temperature in combination) regimes. The females and males of mrigal, each three in number, were included into each experimental cistern and pond in the middle of November after conditioning for a week and examining for initial gonado-somatic index (GSI). Each indoor cistern was provided with artificial aeration and water depth was maintained at about 90 cm. The rearing density of fish was maintained at 3 fish/m^3. The fishes were acclimatized to indoor condition under ambient temperature (25–26°C) for a week. They were fed at the rate of 3% of body weight twice daily with a formulated balanced feed containing 31.3% crude protein, 11.7% crude lipid and 3.9 kcal/g gross energy levels. Partial water exchange was done fortnightly keeping ambient temperature variation within a range of ± 1°C. Some selected parameters of tank water such as pH, dissolved oxygen (DO), free CO_2, total alkalinity, dissolved ammonia and conductivity were estimated monthly following standard methods[22].

One female of individual species from each group was sacrificed for examining the GSI. The remaining fishes in the two groups were subjected to either simulated natural photoperiod in combination with ambient temperature ranging between 23.0°C and 26.0°C (SNP–AT; lights on at 0530 h) under indoor condition, or, to natural photoperiod with ambient temperature (NP–AT) under pond condition. The remaining three groups were subjected to long photoperiod (light : dark hours; 15.5 L : 8.5 D) in combination with above ambient temperature at 28.6 ± 1.2°C (LP–AAT, lights on at 0530 h). Natural photoperiod hours were recorded daily, which varied with respect to month (i.e. from October about 11 h 57 min to December of about 10 h 59 min, thereafter to about 11 h 20 min in February). The photoperiod hour recorded each day was provided artificially the next day for SNP–AT treatment. The dawn and dusk periods were maintained with the help of low-watt fluorescence lamp fitted above each cistern. Almost similar water temperature as recorded in pond water, was maintained throughout the experimental period in the SNP–AT cisterns by exchanging of pond water whenever required. Artificial lights were provided in each tank with 23 W fluorescence lamps controlled with electronic timers for regulating the duration of illumination in different tanks. Only tanks under long photoperiod regimes were provided with thermostat-controlled heaters to maintain water temperature consistently during the experiment period. The progress of gonadal development was monitored by assessing secondary sexual characteristics as described by Chaudhuri[23]. GSI was recorded by sacrificing one female from each group at the beginning of the experiment and thereafter in the middle of December and January in case of rohu and catla, whereas the same for mrigal was done at the end of January and February. The suitable state of gonadal

Table 1. Details of species combination and number of fishes that responded to induced breeding experiments under LP–AAT regime

Species	Rohu		Catla		Mrigal	
Sex	Female	Male	Female	Male	Female	Male
Stocking details in each cistern (n = 3)	4	3	3	2	2	1
Experimental period	October–February		October–February		November–February	
Fish matured	9	9	6	6	3	3
Fish spawned	7	9	6	6	2	3
Spawning response (%)	77.7	100	100	100	66.6	100

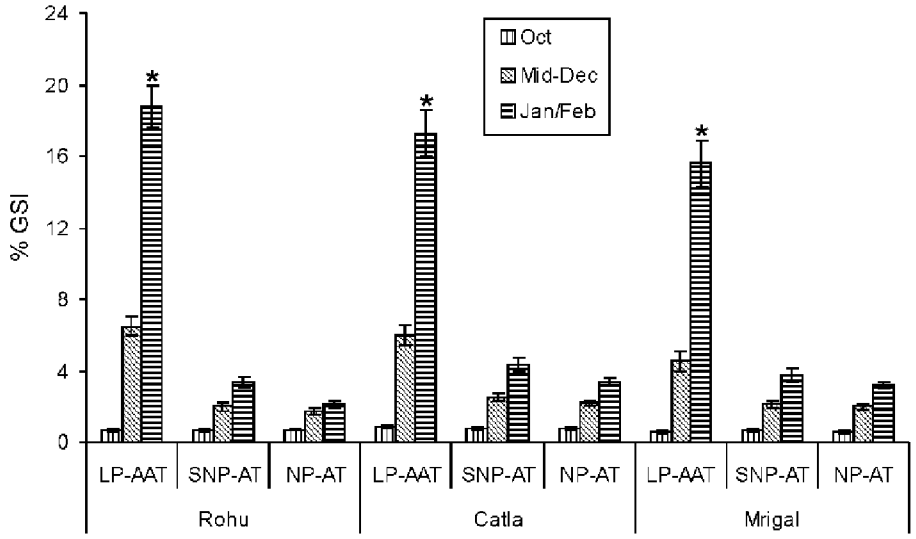

Figure 1. Changes in gonado-somatic index (GSI) value (%) in Indian major carps under different photothermal regimes. GSI values were determined in the respective months for the fishes kept under different photothermal regimes. LP–AAT, long photoperiod in combination with above ambient temperature; SNP–AT, simulated natural photoperiod with ambient temperature, and NP–AT, natural photoperiod with ambient temperature. Asterisk denotes significant differences among the values under different photothermal regimes.

maturity in females and males was assessed as described earlier[24]. The selected females and males were injected with a single dose of Ovaprim (Glaxo India Ltd), 0.4–0.5 ml/kg body wt and 0.2–0.25 ml/kg body wt respectively. The induced spawning experiments were carried out in January and February in rohu, whereas the same were performed in February for catla and mrigal. The spawning responses in terms of certain parameters such as latency period (Lat-P), fertilization rate (FR), hatching rate (HR) and egg and spawn production (EP and SP; per kg body wt) were determined for individual species as described by Dasgupta *et al.*[24].

The effects of photoperiod and temperature on GSI and breeding response parameters were statistically analysed using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) to determine significant differences among different treatments with the help of SPSS software.

Certain physicochemical parameters of tank and pond water such as pH, DO, free CO_2, total alkalinity, dissolved ammonia and conductivity ranged from 7.1–7.3, 2.1–5.0 mg/ml, 12–16 mg/ml, 100–130 mg/ml, 0.08–0.18 mg/ml and 0.298–0.323 mohm respectively, during the experimental period. Most of the water parameters remained within the optimum level required for carp brood rearing.

The mean value of GSI in rohu ($n = 3$) increased from October ($0.7 \pm 0.09\%$) to mid-January, when it reached its highest value of $18.8 \pm 1.22\%$ under LP–AAT treatment, whereas the maximum mean GSI values recorded were $0.69 \pm 0.07\%$ and $0.7 \pm 0.08\%$ under SNP–AT and NP–AT treatments respectively (Figure 1). The catla and mrigal females ($n = 3$) attained a maximum mean GSI value of similar range in February from the initial respective mean values of $0.8 \pm 0.03\%$ and $0.7 \pm 0.03\%$ in October and November respectively, under the LP–AAT regime (Figure 1). Under SNP–AT condition, the highest mean GSI values of catla and mrigal ($n = 3$) remained low as recorded in rohu, whereas under NP–AT condition they ranged from 2.8% to 3.4% in catla and mrigal (Figure 1). Males attained gonadal maturity as assessed by spermiating condition at the end of December in rohu ($n = 3$) and at the end of January in catla and mrigal ($n = 3$). No maturity in males was observed in any species subjected to either SNP–AT or NP–AT treatment.

All the matured fishes from the LP–AAT group were subjected to induced breeding programme using Ovaprim as an inducing agent. Seven out of nine females of rohu had spawned totally in January and February, whereas all six catla females had spawned fully in February (Table 1). In the case of mrigal, two out of three females responded fully to induced spawning in February (Table 1).

Table 2 shows that rohu females could be successfully induced bred under the LP–AAT regime for 117.3 ± 6.96 days, whereas the catla females attained suitable gonadal maturity for induced spawning within 115 ± 5 days of exposure under the same regime. Though the mrigal successfully induced bred on 100 days of exposure to the same regime, there was low egg production compared to the other two species, followed by poor fertilization and hatching success resulting in low spawn production.

In Indian subcontinent, catla (*C. catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) are the most important carps of commercial value. Originally they

Table 2. Breeding response parameters of Indian major carp induced bred with Ovaprim during off-season

Species	Rohu	Catla	Mrigal
Month	January and February	February	February
Sample size	$n = 7$	$n = 6$	$n = 2$
Body weight (kg)	0.89 ± 0.07	1.1 ± 0.07	0.52 ± 0.02*
Temperature (°C)	25.6 ± 0.8	26.5 ± 1.5	27.0
Average exposure period (PP; days)	117.3 ± 6.96	115 ± 5	100
Latency period (h)	8.39 ± 0.22	7.8 ± 0.7	8.4 ± 0.1
Egg production (10^5/kg)	1.41 ± 0.27	0.9 ± 0.08	0.37 ± 0.02*
Fertilization success (%)	84.2 ± 4.31	81.1 ± 3.1	62.0 ± 8.0*
Hatching success (%)	73.3 ± 8.8	86.1 ± 2.8	52.0 ± 6.0*
Spawn production (10^5/kg)	0.85 ± 0.22	0.6 ± 0.1	0.12 ± 0.07*

Values are mean ± SEM. Asterisk denotes significant differences ($P < 0.05$) among the values of individual parameters.

were riverine fishes, domesticated under pond condition for artificial propagation. These carps prefer to graze on different pond niches for their feeding. As a result they are usually exposed to environmental light differently. However, they spawn during the monsoon season under natural condition[25]. Consequently, these fishes show gonadal recrudescence as early as March in Assam (lat. ca. 25°N), June in Orissa (lat. ca. 20°N) and July in other parts of India depending on their geographical location[25]. Since the IMCs show gonadal recrudescence from March to June under natural condition, at a time when both photoperiod and temperature are increasing, it is surmised that these two factors may be involved in initiating gonadal recrudescence. The present study has demonstrated that experimental alterations in day length and temperature, for example, long photoperiod and above ambient temperature earlier in the year than would be expected under the ambient light cycle considered as the internal clock running 'late' and followed by a corrective phase advance in the internal physiological rhythm, have subsequently accelerated the rate of maturation, and advancing the spawning time in these species even at the preparatory phase of the reproductive cycle. However, alterations in photoperiod alone could not initiate any changes in the rate of maturation during the preparatory phase[21]. Furthermore, natural photoperiod as well as simulated natural photoperiod failed to initiate gonadal recrudescence in any of the carps under treatment, as evident by changes in the GSI during the experimental period. Thus, from the present study it is clear that both long photoperiod and above ambient temperature than that of ambient cycle are required to obtain full maturation suitable for off-season induced spawning in IMC during winter months. Similar influences of photoperiod and temperature on gonadal activity were demonstrated in other cyprinid species, such as *Cyprinus carpio*[6], *Notemigonus crysoleuces*[3] and *Carassius auratus*[26]. However, the influence of photoperiod may vary among the species and even among different strains of the same species[27].

Photoperiod manipulation induces changes in spawning time in several species, including salmonids[4], striped bass[28], scianids[5] and Atlantic cod[29]. In the present study, photothermal manipulation advances the spawning time at least 4–5 months prior to normal spawning time in the natural environment. The degree of advancement was similar in the catla and rohu species considering the exposure time of the fishes to the same photothermal regime. The fishes reared for 115 days or above under LP–AAT regime produced similar quantity of eggs as reported in the case of pre-monsoon spawning in IMC, which is comparatively less than monsoon spawning[24,30]. Thus, the acceleration of spawning time was accompanied by a reduction of egg production clearly noticed in rohu; such reduction in fecundity in the photoperiod-accelerated group is in accordance to the results in pink salmon[31]. It is evident from the breeding response parameters in mrigal that the female did not attain suitable maturity as observed in rohu or catla under the specific LP–AAT regime. Catla being a surface feeder has more contact with the environmental light, compared to rohu and mrigal, which are column and bottom feeders respectively. However, under similar brood fish management practices individual species attain gonadal maturity at different times; generally the mrigal and catla attain maturity prior to rohu under pond condition in Orissa (pers. commun.), which may be attributed to the different exposure levels of similar light intensity under pond condition. A detail study is warranted to evaluate the specific requirement of the individual species, particularly of mrigal, as they are bottom-dwellers and generally breed earlier in the season.

In summary, the photothermal manipulation accelerates sexual maturation in such a way that IMCs could be induced bred during winter at least 4–5 months prior to their normal spawning season. It can also be hypothesized that photoperiod provides signal may be generating important modification of the neuro-endocrine mechanism involved in the initiation of gonadal recrudescence and control of gonadal growth only when an appropriate temperature is being provided to the fish during the rearing period. Thus, photothermal manipulation provides a simple method for altering spawning times and in turn

off-season production of spawn, fry and fingerlings of IMC for commercial culture almost throughout the year to facilitate aquaculture in a most effective way.

1. Chaudhuri, H., Environmental regulation of gonadal maturation and spawning in fishes. In *Frontiers in Environmental and Metabolic Endocrinology* (ed. Maitra, S. K.), University of Burdwan, 1997, pp. 91–100.
2. Baggerman, B., Photoperiodic and endogenous control of the annual reproductive cycle in teleost fishes. In *Environmental Physiology of Fishes* (ed. Ali, M. A.), Plenum, New York, 1980, pp. 533–567.
3. De Vlaming, V. L., Effects of photoperiod and temperature on gonadal activity in the cyprinid teleost, *Notemigonus crysoleucas*. *Biol. Bull.*, 1975, **148**, 402–415.
4. Bromage, N. R., Randall, C., Dutson, J., Thrush, M. and Jones, J., Environmental control of reproduction in salmonids. In *Recent Advances in Aquaculture, IV* (eds Muir, J. F. and Roberts, R. J.), Blackwell, Oxford, 1993, pp. 55–65.
5. Thomas, P. and Arnold, C. R., Environmental and hormonal control of reproduction in Sciaenid fish. In *Recent Advances in Aquaculture, IV* (eds Muir, J. F. and Roberts, R. J.), Blackwell, Oxford, 1993, pp. 31–42.
6. Davies, P. R., Hanyu, I., Furukawa, K. and Nomura, M., Effect of temperature and photoperiod on sexual maturation and spawning of the common carp. III. Induction of spawning by manipulating photoperiod and temperature. *Aquaculture*, 1986, **52**, 137–144.
7. Chaudhuri, H. and Alikunhi, K. H., Observation on the spawning in Indian carps by hormone injection. *Curr. Sci.*, 1957, **26**, 381–382.
8. Nandi, S., Routray, P., Gupta, S. D., Rath, S. C., Dasgupta, S., Meher, P. K. and Mukhopadhyay, P. K., Reproduction performance of carp *Catla catla* (Ham.) reared on a formulated diet with PUFA supplement. *J. Appl. Icthyol.*, 2007, **23**, 684–691.
9. Bhattacharya, S., Dasgupta, S., Datta, M. and Basu, D., Biotechnology input in fish breeding. *Indian J. Biotechnol.*, 2002, **1**, 29–38.
10. Gupta, S. D. and Rath, S. C., Carp breeding and seed production. In *Handbook of Fisheries and Aquaculture* (ed. Ayyappan, S.), DIPA, Indian Council of Agricultural Research, 2006, pp. 248–264.
11. Sundararaj, B. I. and Sehgal, A., Effects of long or an increasing photoperiod on the initiation of ovarian recrudescence during the preparatory period in the catfish, *Heteropneustes fossilis* (Bloch). *Biol. Reprod.*, 1970, **2**, 413–423.
12. Sundararaj, B. I. and Vasal, S., Photoperiod and temperature control in the regulation of reproduction in the female catfish, *Heteropneustes fossilis*. *J. Fish Res. Board Can.*, 1976, **33**, 959–973.
13. Garg, S. K. and Jain, S. K., Effect of photoperiod and temperature on ovarian activity in the Indian murrel, *Channa* (*Ophicephalus*) *punctatus* (Bloch). *Can. J. Zool.*, 1985, **63**, 834–842.
14. Srivastava, S. J. and Singh, R., Effects of constant photoperiod–temperature regimes on the testicular activity of *Channa punctatus* (Bloch). *Asian Fish. Sci.*, 1992, **5**, 231–240.
15. Singh, M. S. and Joy, K. P., Precocious recrudescence of seminal vesicle and testis in catfish, *Clarias batrachus* (Linn.), subjected to a long photoperiod regime. *Indian J. Exp. Biol.*, 1998, **36**, 1264–1268.
16. Acharia, K., Lal, B., Singh, T. P. and Pati, A. K., Circadian phase dependent thermal stimulation of ovarian recrudescence in Indian catfish, *Clarias batrachus*. *Biol. Rhythm Res.*, 2000, **31**, 125–135.
17. Verghese, P. U., Preliminary experiments on the modification of the reproductive cycle of an Indian carp *Cirrhina reba* (Ham.) by control of light and temperature. *Proc. Indo-Pac. Fish Council*, 1970, **13**, 171–184.
18. Verghese, P. U., Internal rhythm of sexual cycle in a carp *Cirrhina reba* (Ham.) under artificial conditions of darkness. *J. Inland Fish. Soc. India*, 1975, **7**, 182–188.
19. Maitra, S. K. and Chattoraj, A., Role of photoperiod and melatonin in the regulation of ovarian functions in Indian carp *Catla catla*: basic information for future application. *Fish Physiol. Biochem.*, 2007, **33**, 367–382.
20. Bhattacharya, S., Dey, R. and Maitra, S. K., Photoperiodic regulation of annual testicular events in the Indian major carp *Catla catla*. *Acta Zool.*, 2005, **86**, 71–79.
21. Dey, R., Bhattacharya, S. and Maitra, S. K., Importance of photoperiods in the regulation of ovarian activities in Indian major carp *Catla catla* in an annual cycle. *J. Biol. Rhythms*, 2005, **20**, 145–158.
22. APHA, Standard methods for examination of water and wastewater, American Public Health Association, Washington DC, 1998, 20th edn.
23. Chaudhuri, H., Notes on external characters distinguishing sex of breeders of the common Indian carps. *Sci. Cult.*, 1959, **25**, 258–259.
24. Dasgupta, S., Sarkar, S. K., Sarangi, N. and Bhattacharya, S., Variation in spawning responses, egg and larvae production from induced rohu (*Labeo rohita*) during premonsoon and monsoon seasons: Relationship with hormonal changes and oocyte responsiveness during final maturation. *Aquaculture*, 2009, **209**, 320–326.
25. Jhingran, V. G., *Fish and Fisheries of India*, Hindustan Publication, New Delhi, 1975.
26. Kawamura, T. and Hanyu, I., On acceleration of the ovulation in the goldfish. *Jpn. J. Ichthyol.*, 1950, **1**, 157–165.
27. Davies, P. R. and Hanyu, I., Effect of temperature and photoperiod on sexual maturation and spawning of the common carp I. Under condition of high temperature. *Aquaculture*, 1986, **51**, 277–288.
28. Smith, T. I. J. and Jenkins, W. E., Controlled spawning of F1 hybrid striped bass (*Morone saxatiles*) and rearing F2 progeny. *J. World Aquacult. Soc.*, 1989, **15**, 147–161.
29. Hansen, T., Karlser, O., Taranger, G. C., Hamre, G.-I., Holm, J. C. and Kjesbu, O. S., Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture*, 2001, **203**, 51–67.
30. Gupta, S. D., Rath, S. C., Dasgupta, S. and Tripathi, S. D., A first report on quadruple spawning in *Catla catla* (Ham.). *Vet. Arh.*, 1995, **65**, 143–148.
31. Beacham, T. D., Murray, C. B. and Barner, L. W., Influence of photoperiod on the timing of reproductive maturation in pink salmon (*Oncorhynchus gorbuscha*) and its application to genetic transfers between odd- and even-year spawning population. *Can. J. Zool.*, 1999, **72**, 826–833.

ACKNOWLEDGEMENTS. We thank Dr S. N. Mohanty, Head, Fish Nutrition and Physiology Division and Dr J. K. Jena, Head, Aquaculture Production and Environment Division, CIFA, Bhubaneswar for providing facilities to conduct this study.

Received 5 June 2009; revised accepted 18 August 2010