The female reproductive cycle of the european common carp, *Cyprinus carpio*, at a Transvaal fish farm: Gonadal morphometric development

C.H. Fouché, J.F. Vermaak, J.H.J. van Vuren and H.J. Schoonbee
Research Unit for Fish Biology, Rand Afrikaans University, Johannesburg

The annual reproductive cycle of *Cyprinus carpio* was investigated using morphometric parameters of the body and gonads, including fecundity estimations, gonadosomatic indexes as well as histological evaluation and classification of oocyte developmental stages. It was concluded the *C. carpio* has an extended spawning season under temperate conditions, from early spring (September) to late summer (February). Results further suggest that by manipulating temperature and daylight period during late winter (August) it would be possible to induce spawning during this period.
S. Afr. J. Zool. 1985, 20: 172 – 176

Die voortplantingsiklus van *Cyprinus carpio* is oor 'n periode van 'n jaar ondersoek deur waardes vir die morfometriese parameters van die liggaam en gonades te bepaal. Fekunditeit en gonadosomatiese indekse is bereken terwyl die gonades histologies ondersoek en die stadium van oösietontwikkeling bepaal is. Vanuit die resultate kan afgelei word dat *C. carpio* oor 'n verlengde broeiseisoen onder gematigde omgewingstoestande beskik. Die broeiseisoen strek vanaf die vroeë lente (September) tot laat somer (Februarie). Deur die manipulering van temperatuur en dagliglengte is dit waarskynlik moontlik om broei-aktiwiteit reeds aan die einde van die winter (Augustus) te bewerkstellig.
S.-Afr. Tydskr. Dierk. 1985, 20: 172 – 176

C.H. Fouché,* J.F. Vermaak, J.H.J. van Vuren and H.J. Schoonbee
Research Unit for Fish Biology, Department of Zoology, Rand Afrikaans University, P.O. Box 524, Johannesburg, 2000 Republic of South Africa

*To whom correspondence should be addressed

Received 7 March 1984; accepted 26 March 1985

Investigations by authors such as Henderson (1963); Reisman & Cade (1967), Baggerman (1972); Bromage, Whitehead, Elliot, Breton & Mathy (1982); Hanyu, Asahima & Shimizu (1982) and Richter, Eding, Leuven & van der Wijst (1982) showed that photoperiod and environmental temperature are amongst the major factors affecting the breeding cycle. These and other related physical conditions may vary from one season to the next, affecting gonadal maturation and development in fish. For this reason a clear seasonal pattern, which might enable the fish farmer to predict with accuracy the onset of the spawning season, is not always possible to predict. The breeding cycle of fish is usually determined with the aid of parameters such as gonadosomatic index (GSI), fecundity and gonadal histomorphology (Egami & Hasokawa 1973; De Vlaming 1972, 1974; Sundararaj & Vasal 1976).

In the present investigation, the above-mentioned parameters were employed using randomly selected sexually mature carp collected monthly from fish ponds in order to assess more accurately gonadal development, maturation of the gonads and the onset of the actual spawning season under temperate conditions.

Material and Methods

Carp were collected during consecutive months between September 1981 (spring) and August 1982 (end of winter) with the aid of seine-nets from fish ponds at the Marble Hall Provincial Fisheries Station, Transvaal, Republic of South Africa. Ten sexually mature females were selected after the fish were dissected and the gonads evaluated (Kesteven 1960). The body mass and fork length of each individual specimen were recorded. Gonads were removed and the stage of ovarian development evaluated according to Kesteven (1960) as briefly described in Table 1, and greatest circumference of each ovary was constantly taken from the right ovary according to Babiker & Ibrahim (1978).

The GSI (gonadosomatic index) was calculated by employing the following formula:

$$\frac{\text{Gonad mass (g)}}{\text{Body mass (g)}} \times 100$$

Fecundity estimations were done with the formula described by Babiker & Ibrahim (1978).

$$F = 1/2\ \frac{N_1}{m_1} + \frac{N_2}{m_2} M$$

where m_1 and m_2 = mass of egg subsamples, N_1 and N_2 = number of eggs in subsamples, and M = total mass of the ovary.

Table 1 Stages of ovarian development (Kesteven 1960)

Maturity stage	External characteristics of ovary.
I Virgin	Small thread-like transparent organ situated underneath the bladder; no oocytes visible.
II Maturing virgin	Small pear-shaped pink organ; oocytes not yet visible.
III Developing (early)	Ovary is opaque and reddish with blood capillaries; occupies about half of body cavity; eggs visible to the eye as whitish granular.
IV Developing (late)	Ovary reddish and opaque eggs clearly discernible; ovary occupies about two-thirds of body cavity.
V Gravid	Ovary fills ventral body cavity; eggs completely round and only appear translucent a few days prior to spawning.
VI Spawning	Roe runs with slight pressure; most eggs translucent with few opaque eggs left in ovary.
VII Spawning/spent	Ovary not yet fully empty; occasional residual oocytes may be visible in empty flaccid parts of the ovary.

winter (June – July) during which months the frequency of occurrence of this stage amongst the fish analysed, never exceeded 10%. Stage IV ovaries occurred during all the months of the investigation with a definite peak during the months April to September (autumn – early spring). The above-mentioned four ovarian stages were the most prominent during the summer and autumn and the relative occurrence thereof is presented in Figure 1.

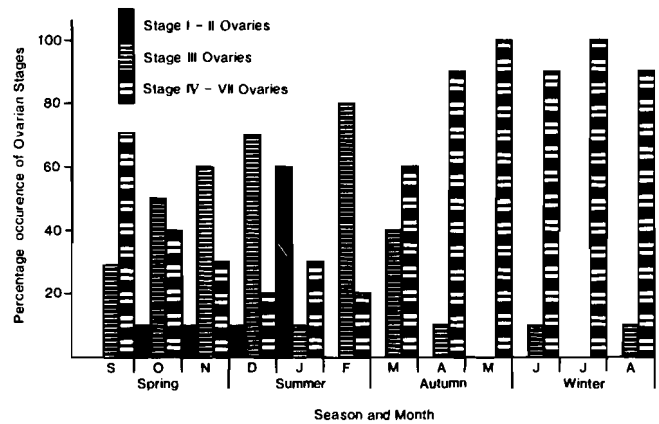

Figure 1 Seasonal occurrence of developmental stages of ovaries of *C. carpio* in fish ponds at Marble Hall, Transvaal.

The stage of oocyte development was histologically determined by making use of a mid-portion section of each ovary. Standard methods for sample fixation and dehydration (Humason 1979) were employed and the samples imbedded in plastic by the use of the Sorval technique (Du Pont Company). Sections of 3 – 5 µm were cut with the aid of a Reichert ultramicrotome and were transferred to microscope slides according to the procedure described by Frangioni & Borgioli (1979). Sections were stained using azocarmine-azan or haematoxylin methods (Humason 1979) and mounted with entelan (Merck). The oocyte stages were studied under an Olympus Vanox light microscope (×40 – 1000).

Oocyte numbers were determined with a light microscope (×40). Four areas between the ovary wall and lumen were selected for this purpose. Mean values were calculated for each oocyte stage observed in the four areas of the ovary per individual. The mean values of each oocyte stage were then determined for all the individuals investigated per month. Standard errors were negligibly small and were therefore not tabulated. Oocytes were classified according to Malhotra, Jyoti & Gupta (1978) who distinguished between previtellogenic (Stages I – III) and vitellogenic oocytes (Stages IV – VI) based on the size of the oocyte.

Results

The results obtained for the body mass (g) and fork length (cm) and the GSI (%) of the fish collected monthly are presented in Table 2. The age of the fish investigated varied from 1,6 – 4,0 years.

Stages I and II ovaries were predominant in January (midsummer) with a frequency of occurrence of 60%, and absent during autumn and winter, reappearing in spring and early summer (December) with a frequency of occurrence of approximately 10%. With the exception of January, the frequency of occurrence of Stage III ovaries amongst fish analysed, clearly showed a tendency to build up from spring (September) reaching the highest value in February, declining towards

The frequency of occurrence and the distribution of oocyte stages during the four seasons are given in Figure 2. From this it is evident that the lowest number of Stage I oocytes was found during mid-winter (June – July) with a gradual increase in spring reaching a peak in December and January after which a gradual decline occurred from late summer (February) towards winter. In the case of Stage II oocytes the increase in numbers from mid-winter towards summer is the most dramatic for all stages with highest values obtained between December and February (summer) when values exceeding 10 counts (per microscope field) were obtained. Stage III oocytes reached maximum numbers during December (5 per microscope field). The number of Stage IV oocytes reached a minimum value of below 0,5 counts per microscope field in September and October, increased to 2 counts per microscope field in December which was followed by a decrease in numbers during January. However, an increase in numbers started during February to reach a maximum value during March. In the case of Stage V oocytes, the lowest counts were obtained from December to March after which an increase occurred with a peak in June. There was a corresponding increase in the number of Stage V oocytes and Stage IV ovaries during the period March to September. The majority of oocytes present in Stage IV ovaries were Stage V oocytes and the increase in the presence of Stage IV ovaries from February to May showed clear resemblance to the rise of Stage IV oocytes. The number of Stage IV oocytes already reached maximum during March which is a possible indication that Stage IV oocytes developed into Stage V oocytes without a change in the stage of development of the ovary. Females with Stage V – VI ovaries were not found during any of the sample months. Stage VII ovaries were found occasionally but not used for analyses since it was very difficult to distinguish between residual and ripe oocytes.

Ovarian mass, circumference and the GSI values reached maxima during August and September (Tables 2 & 3). The fluctuations of the GSI and ovarian circumference/ovarian

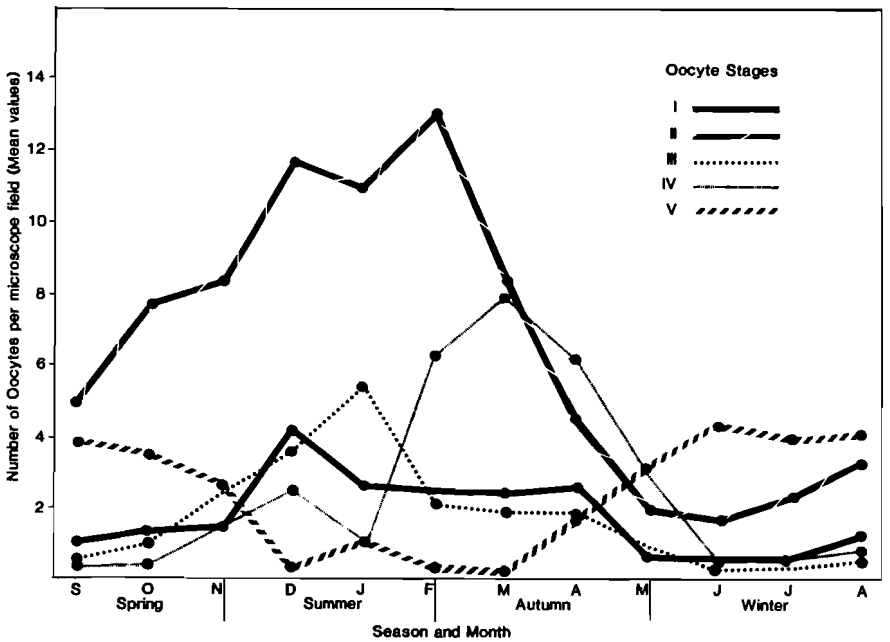

Figure 2 Seasonal occurrence of different oocyte stages of *C. carpio* in fish ponds at Marble Hall, Transvaal.

Table 2 Monthly body mass, fork length and GSI of *C. carpio* (Mean ± SE), $n = 10$

Season	Month of sampling	Body mass (g) $\bar{X}$	± SE	Fork length (cm) $\bar{X}$	± SE	GSI (%) $\bar{X}$	± SE
Spring	Sept.	1309	55,46	38,8	0,47	11,88	0,69
	Oct.	1483	111,91	39,9	0,94	6,23	1,31
	Nov.	1178	90,32	38,8	0,93	5,04	0,07
Summer	Dec.	1189	67,98	38,9	0,71	2,77	0,30
	Jan.	1035	75,04	38,9	1,12	3,67	0,69
	Feb.	1204	60,36	40,5	0,75	3,72	0,41
Autumn	March	1196	88,45	40,7	0,99	4,93	0,36
	April	1149	74,66	41,0	0,92	6,36	0,45
	May	1155	86,34	36,1	1,95	8,76	0,50
Winter	June	1205	53,26	39,8	0,82	8,68	0,65
	July	907	73,29	36,8	1,07	9,11	0,89
	August	1423	139,82	40,6	1,00	13,45	0,71

length ratio followed a similar pattern with the exception of August and September. The ovarian circumference/ovarian length ratio declined from October to December which coincides with the onset of the breeding season and consequent release of eggs from the ovaries. A drop in the GSI was also found during the same period. The lower values obtained for ovarian mass and consequently fecundity during July may be the result of the sampling of females in a less advanced stage of ovarian development. The increase recorded in the fecundity in December from a low in November may be the result of late maturation of female fish which prepared to spawn during late summer.

Discussion

Members of the Teleostei produce eggs with marked seasonal periodicity (Tokarz 1978). Gonadal activity has a cyclic pattern and there is a variation in the length of the breeding cycle. This breeding cycle may also be influenced by environmental factors such as photoperiod, temperature and other physio-

Table 3 Mean monthly values for ovarian mass, length, circumference and the ovarian circumference/ovarian length ratio as well as fecundity of *C. carpio* (Mean ± SE), $n = 10$

Season	Month of sampling	Mass (g) $\bar{X}$	+ SE	Length (cm) $\bar{X}$	+ SE	Circumference (cm) $\bar{X}$	+ SE	Circumference/length ratio	Fecundity $\bar{X}$	+ SE
Spring	Sept.	120,8	6,90	14,3	0,29	10,2	0,20	0,71	155812	12162
	Oct.	96,2	16,71	14,9	1,18	9,0	0,78	0,62	99561	16593
	Nov.	62,1	8,25	14,1	0,29	7,3	0,18	0,53	6532	816
Summer	Dec.	33,6	3,40	13,5	0,19	6,0	0,12	0,45	40476	5120
	Jan.	35,8	4,90	12,8	0,28	5,5	0,19	0,42	46827	5536
	Feb.	42,6	4,10	14,5	0,28	6,1	0,15	0,41	103800	10401
Autumn	March	60,4	4,76	15,0	0,40	6,7	0,26	0,45	172134	14344
	April	72,7	5,28	14,6	0,41	7,7	0,19	0,53	147309	12267
	May	102,7	6,95	15,2	0,19	8,4	0,11	0,55	131398	7411
Winter	June	105,7	6,86	15,4	0,30	9,5	0,21	0,62	140481	7981
	July	89,2	8,96	14,2	0,90	8,4	0,61	0,59	97193	10816
	August	191,4	11,56	18,3	0,78	12,0	0,65	0,66	173773	12735

chemical conditions of the habitat (Hoar 1969; Richter *et al.* 1982). It can be concluded that there is a measurable reproductive rhythm which may be triggered by environmental factors. The breeding cycle of fish is regulated by hypophyseal activity which, in turn, is under direct control of a genetic rhythm. Light and temperature play a vital part during the breeding cycle where seasonal fluctuations of abiotic factors may occur (Hoar 1969; de Vlaming 1972). *C. carpio* has a breeding season that coincides with the summer owing to longer photoperiod and subsequent warmer water — especially in the district of Marble Hall, where water temperatures exceed 20°C. (Information supplied by Fisheries Staff).

A definite seasonal breeding cycle was found in *C. carpio* and the marked increase in fecundity (November to March, see Table 2) indicated that environmental factors such as temperature and photoperiod probably bring about changing secretion rates of hypophyseal gonadotropic hormones at various seasons of the year (Chaudhuri 1976). Spawning in spring is followed by the onset of the next reproductive cycle. The period of spawning coincided with the increase of photoperiod, rise in temperature and change in conductivity just after the winter at the beginning of the rainy season. The greater gonadal mass recorded from August to October (coinciding with the end of winter and the spring period in South Africa), may, amongst other factors, be the result of final oocyte maturation and hydration of the ovaries preceding spawning (Babiker & Ibrahim 1978).

Based on the actual observation of the stage of ovary development in the samples collected from August to the height of summer (December to February) it seems possible that spawning commences from the end of winter to continue throughout spring and summer. The larger number of Stage II ovaries present during January could possibly serve as an indication of the onset of the breeding cycle. The Stage II peak was followed by a Stage III maximum and the absence of Stage II ovaries from February to June, and the reappearance thereof in October, November and December served as an indication that there was no latent phase in the ovaries. All the females used in the present investigation were mature with the exceptional occurrence of Stage I oocytes and the latter was therefore presented together with Stage II oocytes in Figure 1. A specific sequence in the occurrence of maturing (Stage II) and developing (Stages III & IV) ovarian stages was found which explained the specific pattern of the respective increase and decrease of ovarian stages during the breeding season. Furthermore it was evident that the time needed by the ovary to develop to the next stage differed from one stage to another. The absence of Stages V and VI ovaries is possibly the result of a fast change in the ovary from Stage IV to Stage VII during which eggs become fully hydrated and deposited (Table 1, Figure 1.)

The maximum values obtained for ovarian mass, circumference and GSI can be ascribed to vitellogenesis and the concomitant hydration of the maturing follicles which reached a peak during early spring (Babiker & Ibrahim 1978). The fluctuations of the GSI and ovarian circumference which followed a similar pattern strongly indicated that an increase in ovarian mass was accompanied by diametrical expansion of the ovary (Table 3). Increased mass and greater ovarian diameter should however not be confused with the notion that these changes are brought about by an increase in the diameters of all of the oocytes present. In this regard the rising fecundity values indicated a rapid increase in the number of Stage III+ oocytes from November to March although this was not at the time accompanied by increasing ovarian diameter and GSI (Figure 2) during the same period.

Although the Stage III oocytes decreased numerically from March to July, GSI and ovarian diameter still increased over the same period. Resorption of a large number of Stage III oocytes evidently therefore occurred while the remainder of oocytes showed an increase in diameter to such an extent that ovarian expansion was found (Babiker & Ibrahim 1978). During the present investigation, peak values for GSI during August coincided with a comparable increase in fecundity. Completion of vitellogenesis and hydration of maturing oocytes during this time may explain this phenomenon (Babiker & Ibrahim 1978). Increasing fecundity, however, may also indicate further development to maturity of Stage III oocytes. The decline in GSI indicated the commencement of spawning as from early spring to early summer.

Fecundity increase in early summer (December) prior to the recorded GSI, may be due to the development of a new generation of Stage III oocytes. Maxima observed for the ovarian circumference/ovarian length ratios in September, when values for GSI and fecundity had already commenced declining, may have been the result of the increasing hydration and concomitant expansion of the ripening follicles.

The reasonably direct relationship between the numerical occurrence of Stages I and II oocytes which is apparent from Figure 2, indicates that Stage I is rapidly converted to Stage II oocytes, especially so during the height of the breeding activity. From January to May the number of Stage IV oocytes increased, indicating the commencement of maturation of the then present immature oocytes (Stages II & III) within the ovaries. At the time when the maximum number of Stage IV oocytes were present in the ovary, the number of Stage V oocytes started to increase which was possibly due to the beginning of final maturation of the oocytes.

The decrease in the number of these oocytes from September to December, may be ascribed to the final stages of oocyte maturation which is followed by spawning (Figure 2). Some fish may, however, already have commenced final maturation of Stage V oocytes present during September (early spring). Thus atresia may not necessarily be involved in the numerical decrease of these oocytes at that stage of the breeding cycle. It was further obvious that Stage V oocytes (Figure 2) may be present in some fish during January with spawning still possible. Atresia of these oocytes could also occur, since they were found at the end of the spawning cycle.

In conclusion, it is clear from the results in the present study that *C. carpio* has an extended spawning period under temperate conditions, which can commence, depending on environmental conditions, between late winter (August) to late summer (February).

By changing environmental conditions such as temperature and photoperiod under controlled conditions and the administration of reproductive hormones, it might be possible to induce spawning during August. Stage V oocytes are already present in large numbers in the ovaries and the hydration and final maturation would possibly be achieved with the artificial application of hormones and lengthening of daylight. More information is needed to fully understand the development and ripening of oocytes in the ovaries of *C. carpio*. The role of reproductive hormones should be investigated before definite conclusions can be made.

Acknowledgements

The authors wish to thank the staff of the Provincial Fisheries Station at Marble Hall for assistance and provision of fish.

The financial support of the CSIR and the RAU are gratefully acknowledged.

References

BABIKER, M.M., & IBRAHIM, R. 1978. Studies on the biology of reproduction in the ciclid *Tilapia nilotica* (L.): gonodal maturation and fecundity. *J. Fish Biol.* 14: 437 – 448.

BAGGERMAN, B. 1972. Photoperiodic responses in the stickleback and their control by a daily rhythm of photosensitivity. *Gen. Comp. Endrocrinol.* 3: 466 – 476.

BROMAGE, N., WHITEHEAD, C., ELLIOT, J., BRETON, B. & MATHY, A. 1982. Investigations into the importance of day length on the photoperiodic control of reproduction in the female rainbow trout. In: Reproductive Physiology of Fish, (eds) Richter, C.J.H. & Coos, H.J.Th., pp. 147 – 150. Centre for Agricultural Publishing and Documentation, Wageningen.

CHAUDHURI, H. 1976. Use of hormones to induce spawning in carps. *J. Fish. Res. Bd Can.* 33: 940 – 947.

DE VLAMING, V.L. 1972. Environmental control of teleost reproductive cycles: A brief review. *J. Fish Biol.* 4: 141 – 140.

DE VLAMING, V.L. 1974. Environmental and endocrine control of teleost reproduction. In: Control of sex in fishes, (ed.) Schreck, C.B. pp. 13 – 83. Virginia Polytech. Inst. and State Univ., Blacksburg, Va.

EGAMI, N., & HOSOKAWA, K. 1973. Responses of gonads to environmental changes in the fish, *Oryzias latipes*. In: Responses of fish to environmental changes, (ed.) Chavin, W. pp. 279 – 301, Charles C. Thomas, Springfield.

FRANGIONI, G. & BORGIOLI, G. 1979. Polystyrene embedding: A new method for light and electron microscopy. *Stain Technology* 54(4): 167 – 172.

HANYU., ASAHINA, K. & SHIMIZU, A. 1982. The roles of light and temperature in the reproductive cycles of three bitterling species, *Rhodeus ocellatus, Acheilognatus tabira* and *Pseudoperilampus typus*. In: Reproductive physiology of fish, (eds) Richter, D.J.J. & Goos, H.J.Th., pp. 147 – 150. Centre for Agricultural Publishing and Documentation, Wageningen.

HENDERSON, N.E., 1963. Extent of atresia in maturing ovaries of the eastern brook trout, *Salvelinus fontinalis, J. Fish. Res. Bd Can.* 20: 899 – 908.

HOAR, W.S. 1969. Reproduction: In: Fish physiology, (eds) Hoar, W.S. & Randall, D.J. Vol. III, pp. 1 – 59. Academic Press, New York.

HUMASON, G.L. 1979. Animal tissue techniques. 4th edn, pp. 3 – 650. W.H. Freeman and Company, San Francisco.

KESTIVEN, G.L. 1960. Manual of field methods in fisheries biology. F.A.O. Manuals in Fisheries Sciences, No. 1 152 pp. F.A.O., Rome.

MALHOTRA, Y.R., JYOTI, M.K. & GUPTA, K. 1978. Ovarian Cycle and spawning season of *Ophiocephalus punctatus*, inhabiting Jammu Waters, India. *Japan. J. Ich.* 25: 190 – 195.

REISMAN, H.M., & CADE, T.J. 1967. Physiological and behaviou-ral aspects of reproduction in the brook stickleback, *Culaea inconstants. Am. Midl. Nat.* 77: 257 – 295.

RICHTER, C.J.J., EDING, E.H., LEUVEN, S.E.W. & VAN DER WIJST, J.G.M. 1982. Effects of feeding levels and temperature on the development of the gonad in the African catfish, *Clarias lazera* (C&V). In: Reproductive physiology of fish, (eds) Richter, C.J.J. & Goos, H.J.Th., pp. 147 – 150. Centre for Agricultural Publishing and Documentation, Wageningen.

SUNDARARAJ, B.I., & VASAL, S. 1976. Photoperiod and tempe-rature control in the regulation of reproduction in the female catfish *Heteropneustes fossilis. J. Fish. Res. Bd Can.* 33: 959 – 973.

TOKARZ, R.R. 1978. Oogonial proliferation, oogenesis and folliculogenesis in nonmammalian vertebrates. In: The vertebrate ovary, (ed.) James, R.E., pp. 145 – 179. Plenum Press, New York and London.