MATERIALS AND METHODS
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With the initiation of the All India Co-ordinated Research Project on composite fish culture during 1971 in different states of our country, comprising different agro-climatic zones, fish farm at Hadapsar, Pune in Maharashtra State was chosen to represent the western zone. The experiments were conducted at Hadapsar fish ponds at Pune, Maharashtra, for composite fish culture from 1971 to 1985. Hadapsar fish farm is located in the suburbs of Pune city in Maharashtra state at Pune-Solapur road, 10 Km away from the city. It is located at 17.5° to 19.2° north latitude and 73.2° to 75.5° east longitude. Each pond (area 0.32 ha), having inlet and outlet, is fed by a left-bank irrigation canal carrying water from Khadakvasla dam. The area of farm is 4 hectares out of which 2.5 hectares is the water-spread area. It consists of 6 stocking ponds each of 0.32 ha in area with depth of 2 meters and 21 nursery ponds each of 0.02 ha with depth of 1 meter (Plate 2). The experimental Kachha pond was stocked with fry / fingerlings of 6 species viz., catla, rohu, mrigal , common carp, silver carp and grass carp
Plate - 2

Showing stocking ponds at Hadapsar Fish Farm.
with a stocking density of 6,000 numbers/ha. The pond was dewatered before the start of the experiment and was cleared of all unwanted fishes. Liming was done @ 500 kg/ha. Organic manuring was done @ 6,000 kg/ha/year, of which 1/6th of the total quantity was applied before stocking and the rest in equal quantity every fortnightly alternating with inorganic manuring @ 740 kg/ha/year. The manuring was suspended at the time of algal blooms. Fishes were fed daily with 1:1 mixture of groundnut oil cake and wheat/rice brain @ 1-2% of body weight. Grass carp was fed with aquatic weeds like *Hydrilla*, *Potamogaeton*, *Ceratophyllum* and terrestrial grasses as per availability.

The average water depth in a pond for composite fish culture is an important factor. This, generally depends on various factors like rainfall, lifting of water for irrigation, etc. (Sinha, 1990). It is also important to note that with intensive fish culture, heavy accumulation of metabolites takes place at the bottom of such ponds and made deplete oxygen in the medium during low water depth, adversely affecting the fish growth.
Since the stocking ponds at Hadapsar, with a water depth of 2 meters with stocking density with fingerlings of 60-70 mm size and 1875 in numbers per pond, it was essential to transfer the fishes from this stocking pond to another ponds after three to six months to reduce the stocking density and proper management of water quality.

From the introduction of fingerlings of silver carp in the pond in October 1988 till the completion of 2nd year life and exploitation for commercial purpose, the water was analysed for physico-chemical characteristics following the standard methods of A.P.H.A. (1985) and portable water analysing kit CK710 Century. Parameters like temperature of water (°C), pH, dissolved oxygen (ppm), carbon dioxide (ppm), total alkalinity (ppm), and transparency (Cm) were measured every fortnightly.

From the time of introduction of fingerlings in October 1988, the samples were collected fortnightly and measured for their total length. A total of 1331 fishes were measured on a measuring board to the nearest millimeter during the period
October, 1988 to July 1990. The data of the total length were grouped in 10 mm class interval from 60 to 460 mm size group and converted into percentage frequency distribution as per Petersen's method (1891).

A total of 574 specimens collected from the pond at Hadapsar near Pune ranged from 98 to 470 mm in total length and were weighed to the nearest gram. After this, fishes were released back into the pond. Males and females were treated separately. Males ranged from 310 to 450 mm and were grouped in 10 mm interval, whereas females ranged from 300 to 460 mm also and were also grouped into 10 mm interval. The average lengths and weights were calculated for each of the above groups for both the sexes. The review of literature reveals that in fishes the growth pattern follows the cube law (Broady, 1945; Lagler, 1952) but the actual relationship departs from this cube law (Le Cren, 1951) either due to the impact of environmental factor/s or the reproductive/the feeding condition of the fish. Hence, here Le Cren's equation has been applied and the relationship has been expressed by using the equation \( W = c L^n \). The lengths and weights of the
fish were converted into logarithmic form to use the values in the formula as above, where \( W = \) weight in gram, \( L = \) total length in mm and 'c' and 'n' are constant. The values for correlation coefficient (r) of the above fishes between the variables length and weight were calculated using the method of Bailey (1959).

The relative condition (Kn) as an indicator or robustness of the fish was determined from the above fishes by using the equation \( Kn = \frac{\bar{W}}{\bar{W}} \) where \( \bar{W} \) = observed weight and \( \bar{W} \) = calculated weight.

170 fishes were individually weighed soon after the collection and dissected for the entire gut. The gastro-somatic index was calculated by using the formula -

\[
G.S.I. = \frac{\text{Weight of the gut (g)} \times 100}{\text{Weight of the fish (g)}}
\]

To study gonado-somatic index (Gn.S.I.) the fishes used in above G.S.I. determination were subjected to find out the weight of gonads of individual fish (nearest to mg). The data was subjected for calculation using the formula -

\[
\text{Gn.S.I.} = \frac{\text{Weight of the gonad} \times 100}{\text{Weight of the fish}}
\]
The size at first sexual maturity was determined by collecting the fishes of the 1st year and 2nd year size groups (as in the growth rate study) at intervals of 10 mm from March to August (i.e. during the maturation to the spawning period). The gonads of undifferentiated sexes and both the sexes belonging to different size groups were fixed in Bouin's fluid and subjected to histological details, for the observation of sperms and mature ova.

To study the ova-diameter, maturity and spawning, monthly collected samples were dissected for the ovaries and were preserved in 5% formaline for the maximum period of 48 hours. These were then subjected for the measurement of around 500 ova from ovaries. The measurements were done under research microscope. The method followed by according to Clark (1934) and Prabhu (1956). A middle fraction of each ovary was teased on the slide and then the measurement of ova were done. The percentage frequency for each size-group of ova (at 5 X 0.027 mm interval) was calculated monthly to determine the growth of ova and spawning period.
To study the fecundity aspect, the ovaries of gravid females were weighed and preserved in 5% formaline for the maximum period of 48 hours and the total length and weight of the fishes were noted. The number of mature ova were counted from anterior, middle and posterior region. From this the total number of ova in each ovary was estimated according to Lagler (1956). The fecundity of 15 fully gravid female fishes was determined during the entire study. The relationships between the fecundity and the total length, weight of the fish ovary length and ovary weight were determined by least square Log $F = a \log X^c$, where $F$ = number of ova in thousand, $X$ = total length of fish in mm or weight of fish in grams or length of the ovary in mm or weight of the ovary in mg, and 'a' and 'c' are constants. The fecundity factor (relative fecundity) was calculated by dividing the total number of eggs by total weight of the individual fish.

The samples collected for growth rate study were extended to determine the sex ratio. The fishes from 300 to 479 mm in length were observed for the relative abundance of males and females.
Chi-square analysis was used to evaluate the probability of sex differences. The analysis of data is as a test of heterogeneity.

The gonads of the fishes from the time of introduction in the pond (undifferentiated sexes) till to the completion of 2nd year life were fixed in Bouin's fluid for histological preparations. The gonads of the juveniles were fixed along with the peritoneum for studying the first stage of male and female differentiation, whereas in the specimens wherein the gonads were differentiated the middle portion was studied. All the sections were cut at 6-7 microns (μm) and stained with Mallory's triple.

Since no significant difference ($P < 0.05$) was found between the size of oocytes in different regions of the left and right ovaries measurements of oocyte diameters, which were recorded to the nearest 1 μm using a micrometer and Zeiss Photomicroscope II, were made only on oocytes in the mid-part of the left ovary. The diameter of each oocyte and its coamponents was recorded as the mean of the longest and shortest diameters, with measurement only being made on those oocytes in
which a prominent nucleus was present (except the fully mature oocyte) (Foucher and Beamish, 1980). Each of the means for a particular oocyte stage was based on at least 10 measurements from each of 3 to 5 different ovaries from fish of varying size. Similar technique was used while studying the histological details of male gonads.