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Influence of group density and sex ratio on the immune response in the tilapia *Oreochromis mossambicus* (Peters)

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We report here the modulatory influence of overcrowding and sex ratio on humoral response to bovine serum albumin in the fish *Oreochromis mossambicus*. Significant enhancement was found in overcrowded groups compared to the control group and the enhancement was directly proportional to the degree of overcrowding. When the sex ratio and the group density were investigated, it was observed that there were differences in antibody titres among the different sex ratio groups. Except equal sex ratio group, all the groups showed enhancement of immune response as a function of overcrowding compared to the control. No immunomodulation due to overcrowding was observed in the case of equal sex ratio group. These results indicate a possible psychoneuroimmunological basis for the immunomodulation related to sex ratio and group density.

THE immune system is a delicate one, whose response is found to change quantitatively on exposure to modulatory factors such as overcrowding, environmental pollutants, etc. Generally, overcrowding is considered as a stressor having suppressing effects on the immune response[1]. In the present study we investigated the effect of overcrowding and the sex ratio on the immune response in *O. mossambicus*.

For the experimental animal, the tilapia *O. mossambicus* (Peters), a common fresh and brackish water cichlid fish was used. Fish weighing 25–30 g were used. Uncontrolled ambient water temperature of the fish tanks was 28 ± 1.2°C. Since the daily fluctuation of water temperature was negligible, the temperature was not controlled. Water was renewed daily to avoid possible stress due to excretory ammonia.

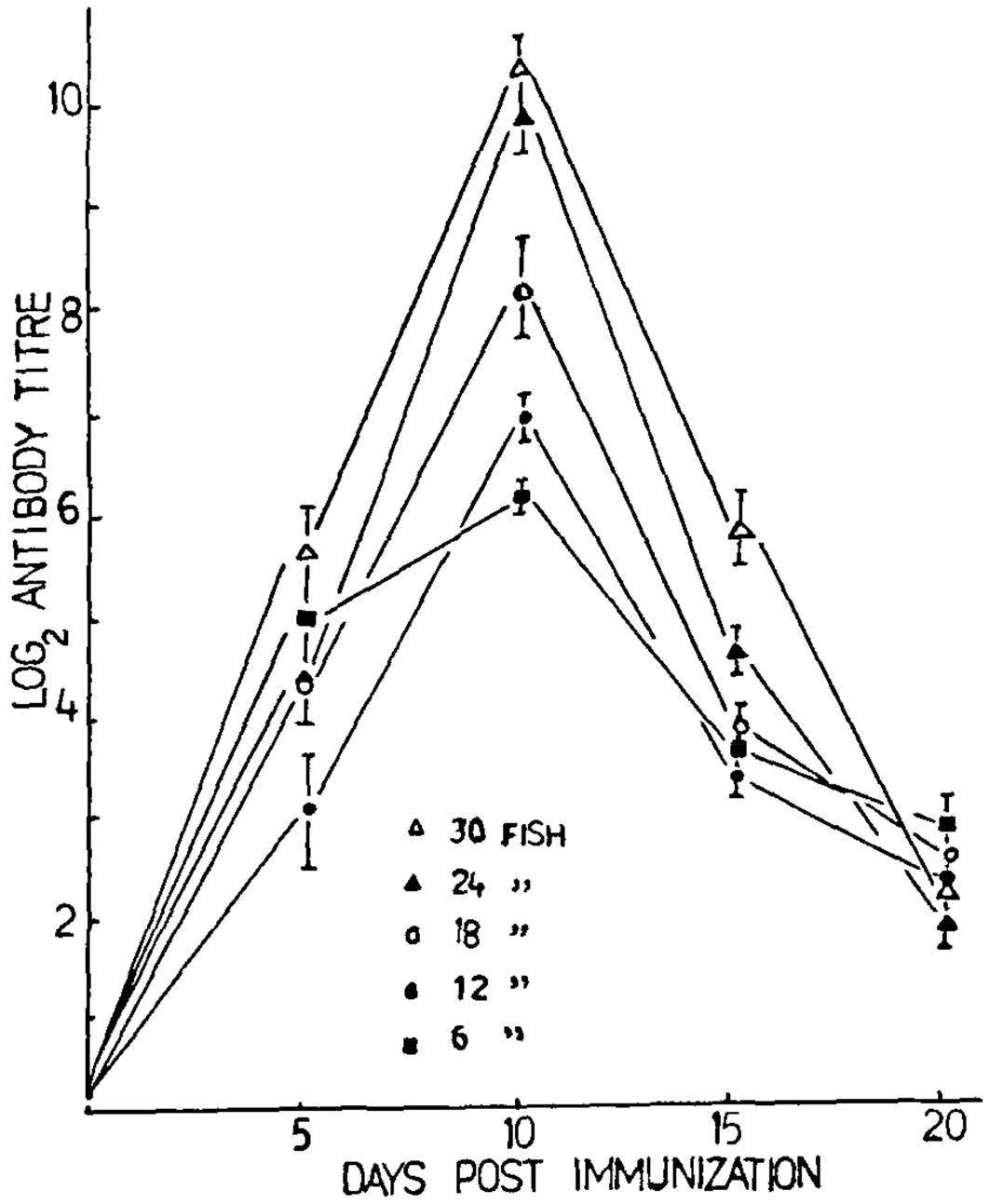

Figure 1. Effect of group density on humoral immune response to S-BSA. Fish were maintained in different group densities of 6, 12, 18, 24 and 30 and immunized with 5 mg of S-BSA. Antibody titres were quantified by passive haemagglutination assay. Each point represents mean ± S.E. of 6–8 fish.

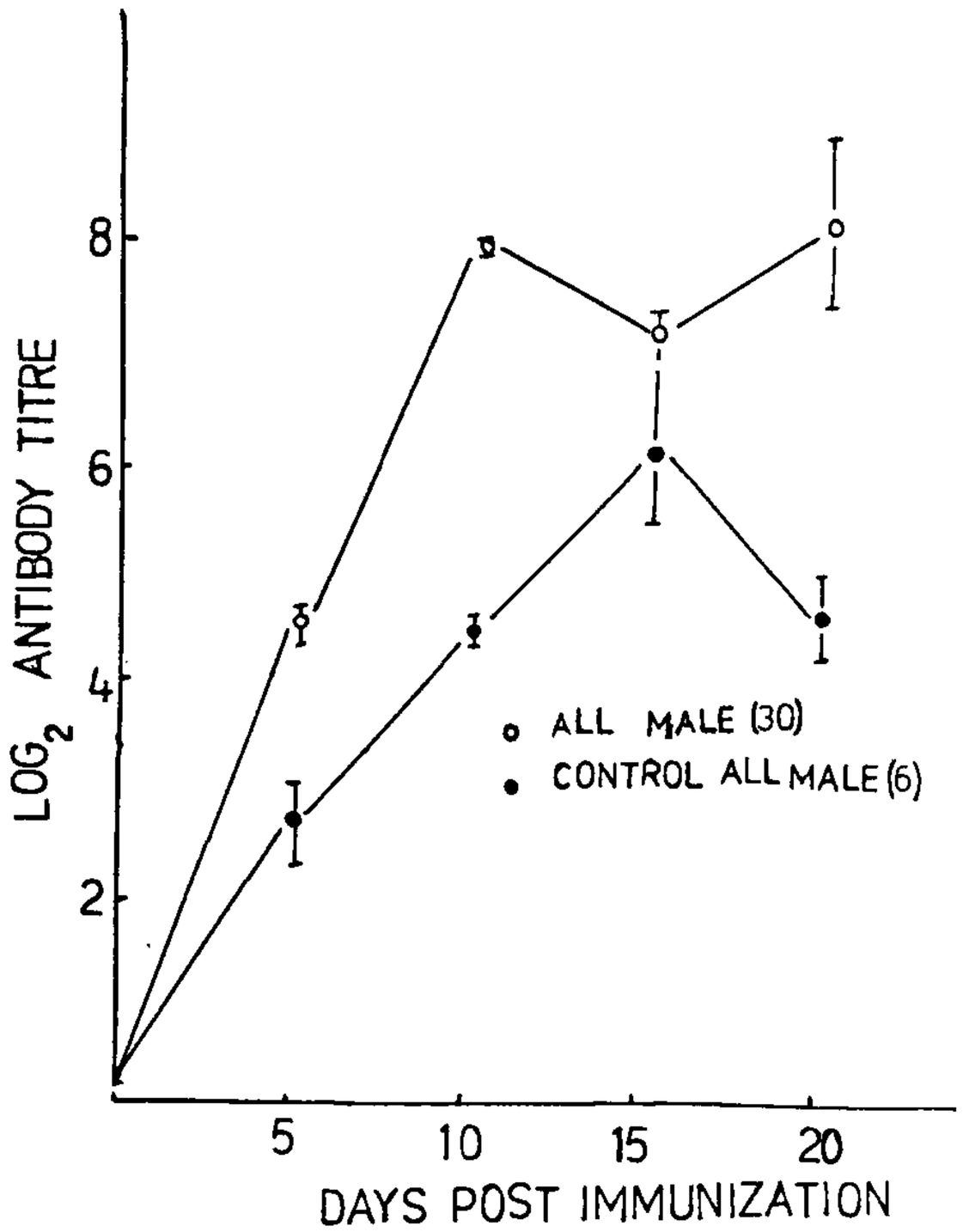

Figure 2. Effect of sex ratio and group density on humoral immune response to S-BSA. Male fish alone were maintained with group densities of 6 or 30. All fish were immunized with 5 mg of S-BSA and antibody titres were quantified by passive haemagglutination assay. Each point represents mean ± S.E. of 6–8 fish.

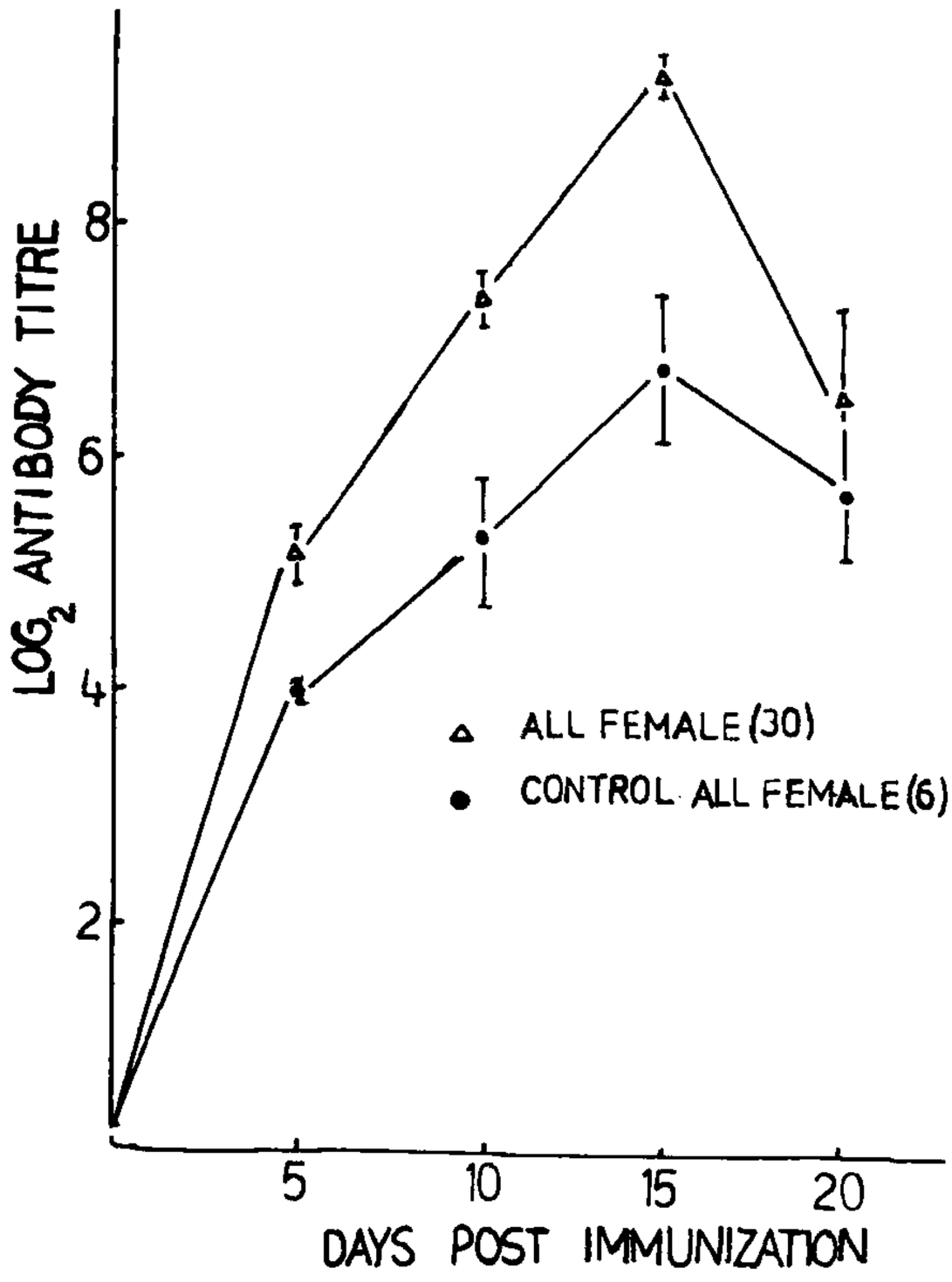

Figure 3. Effect of sex ratio and group density on humoral immune response to S-BSA. Female fish alone were maintained with group densities of 6 or 30. All fish were immunized with 5 mg of S-BSA and antibody titres were quantified by passive haemagglutination assay. Each point represents mean ± S.E. of 6–8 fish.

Bovine serum albumin (BSA) (fraction V powder, Sigma, USA) was the antigen used and was given to fish through intraperitoneal route with a concentration of 5 mg BSA/0.2 ml saline.

Fish were bled serially from the common cardinal vein[2] using 2 ml glass syringe and 24 gauge needle at 5 day intervals after immunization. Blood from the syringe was transferred to serology tubes (10 mm dia, 75 mm long) and kept at room temperature for 5 min and then overnight in the refrigerator. The serum was separated by spinning down the clot at 3000 rpm for 15–20 min and kept at 57°C in a waterbath for 30 min to inactivate complement (classical pathway) and stored at –20°C for further use.

Anti-BSA titres were determined by passive haemagglutination assay. BSA was coupled to sheep erythrocytes (indicator cells) by chromic chloride as described by Michael[3].

Two-fold serial dilutions of the antiserum (50 µl/well) were made with saline in microtitre plates (Laxbro, Pune). To each well 25 µl of 1% BSA coupled SRBC in saline was added. The plate was hand-shaken for

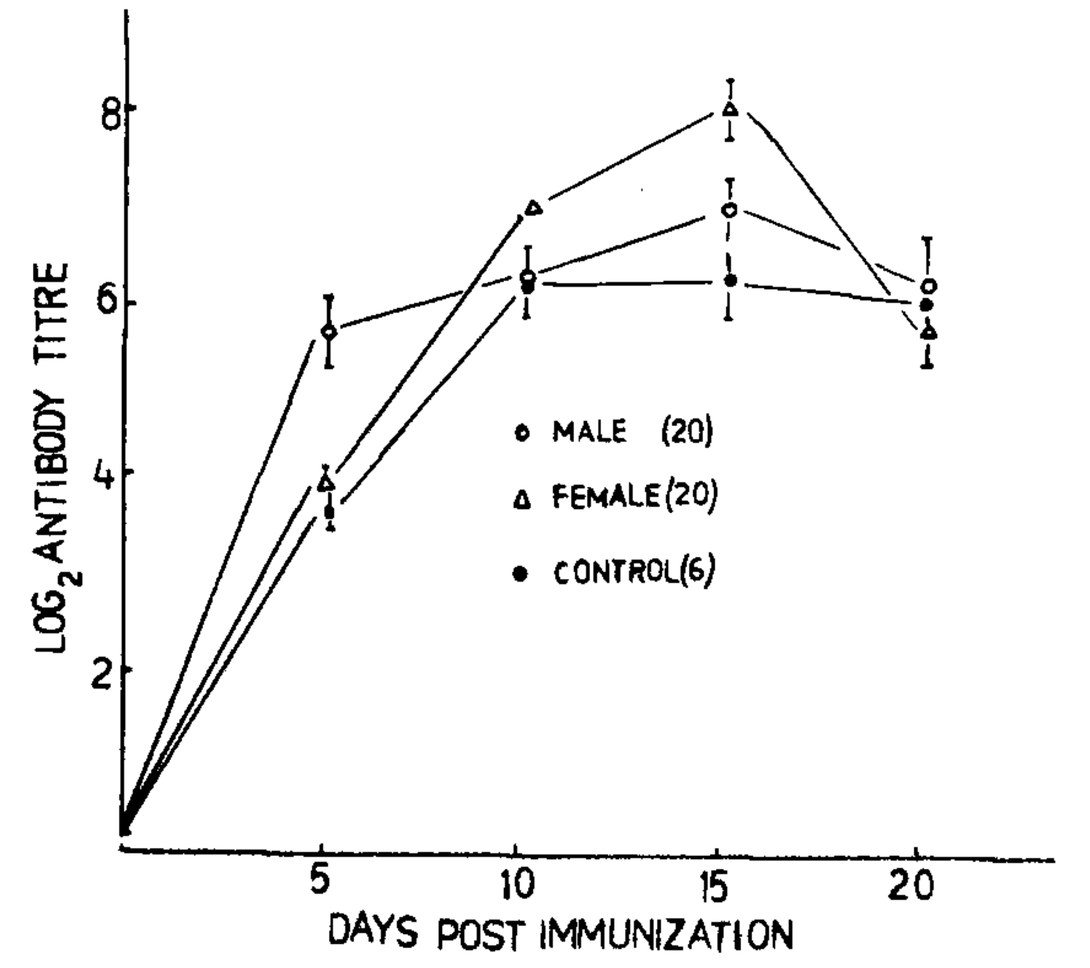

Figure 4. Effect of sex ratio and group density on humoral immune response to S-BSA. Fish were maintained in female dominant ratio (female:male = 2:1) with group densities of 6 or 30. All fish were immunized with 5 mg of S-BSA and antibody titres were quantified by passive haemagglutination assay. Each point represents mean ± S.E. of 6–8 fish.

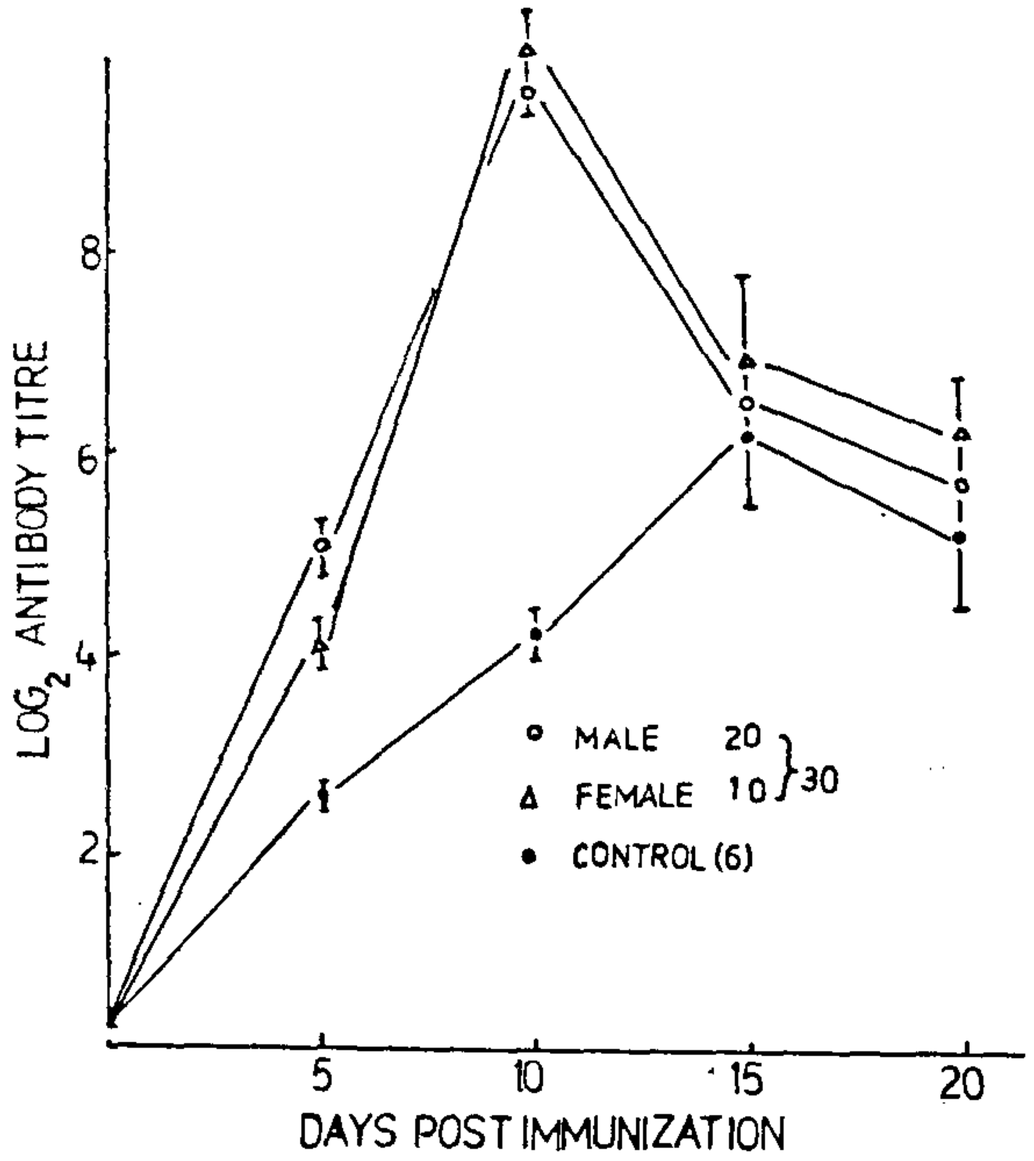

Figure 5. Effect of sex ratio and group density on humoral immune response to S-BSA. Fish were maintained in male dominant ratio (male:female = 2:1) with group densities of 6 or 30. All fish were immunized with 5 mg of S-BSA and antibody titres were quantified by passive haemagglutination assay. Each point represents mean ± S.E. of 6–8 fish.

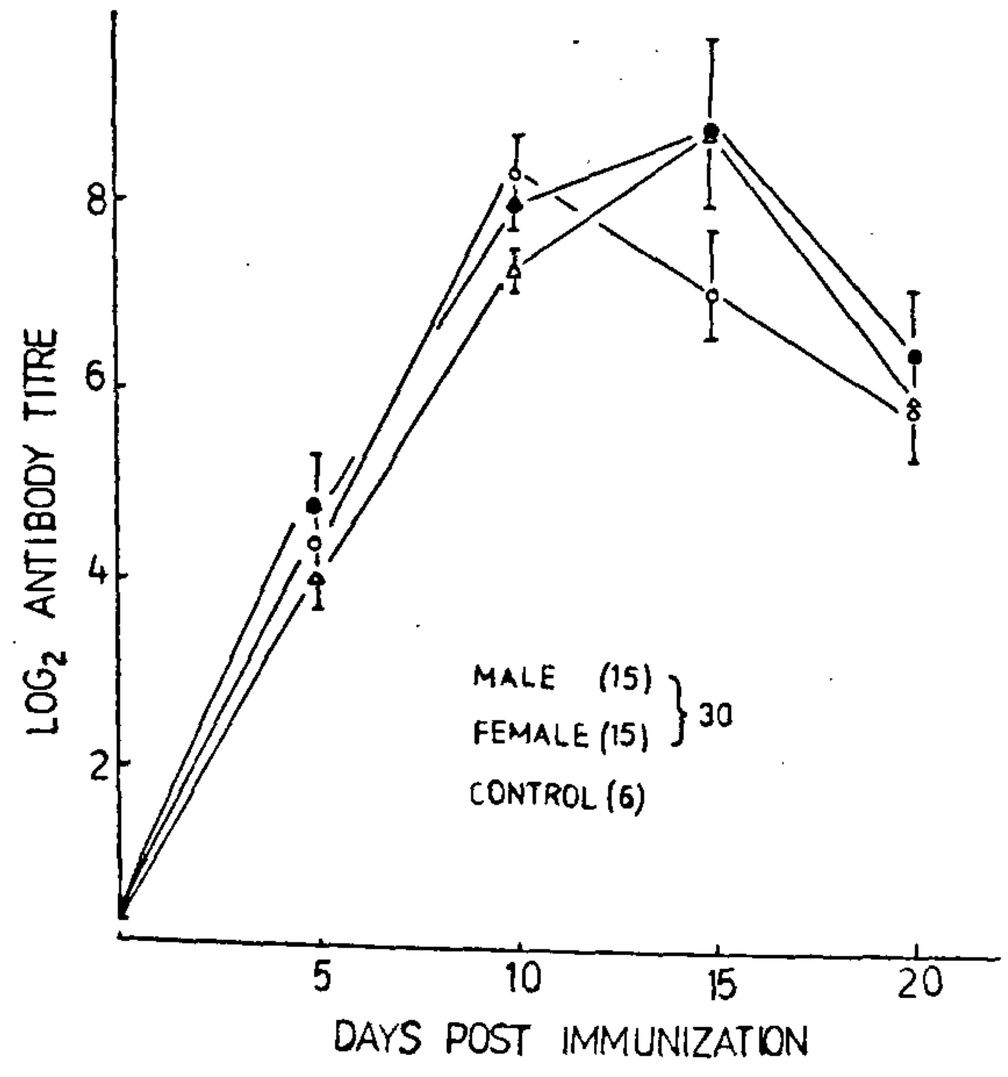

Figure 6. Effect of sex ratio and group density on humoral immune response to S-BSA. Fish were maintained in equal sex ratio with group densities of 6 or 30. All fish were immunized with 5 mg of S-BSA and antibody titres were quantified by passive haemagglutination assay. Each point represents mean ± S.E. of 6–8 fish.

effective mixing of reagents and incubated for 1 h at 37°C and for another hour at 10°C. The maximum dilution of serum samples, which showed detectable agglutination was recorded and expressed as $\log_2$ antibody titre of the serum.

To study the modulatory effect of overcrowding on humoral response in *O. mossambicus* to S-BSA, groups of fishes were maintained in increasing order of group density, 6, 12, 18, 24 and 30 fish respectively in 5 separate tubs (vol. 170 l). A group consisting of 6 fish was considered as the control group.

Investigation on modulation of humoral response in *O. mossambicus* by sex ratio and group density was done by maintaining fishes in 5 different sex ratios at two extreme categories of density namely 6 and 30. Different sex ratios are, all male, all female, female dominant (female and male 2:1), male dominant (female and male 1:2), and equal sex ratio combinations.

Figure 1 indicates that antibody response was enhanced by overcrowding of fish. The enhancement was significant ($P < 0.005$) and directly proportional to the increase in group density.

Figures 2, 3, 4, 5 and 6 indicate that overcrowding or increased group density results in enhancement of immune response in all sex ratio groups except the equal sex ratio combination. In equal sex ratio combination, the overcrowding does not show any modulatory effect on all days tested ($P > 0.005$).

The present observations, i.e. occurrence of enhancement in overcrowding situations, do not agree with earlier reports, which have indicated overcrowding as a stressor, having suppressive effect on immune response. Perlmutter *et al.*[1] reported that maintenance of fishes in overcrowded situations resulted in immunosuppression. Similarly. McLeay[4] observed a lymphopenic response in fish exposed to crowding stress. The interpretation of the contradictory finding in the present study perhaps lies in terms of psychoneuroimmunology[5,6], which is a new branch of science dealing with reciprocal interactions among nervous, endocrine and immune systems. The occurrence of enhancement of immune response is possibly due to certain pheromones exchanged among the fish.

Evidence for the existence of such piscine pheromones was given by Pfuderer *et al.*[7]. They have extracted and purified a crowding factor produced by fishes in crowded conditions. Pheromones have also been shown to modulate the fish immune response[1]. Cohen *et al.*[5] have also provided evidences of such pheromones in mice, which caused enhancement of immune response in syngeneic animals.

Further, the differential response observed in different sex ratio groups maintained in overcrowded conditions is possibly due to the action of (sex) pheromones on the immune system. But conclusive evidence for the role of pheromones in the present finding can be given only

when the nature of psychoneuroimmune interaction in fish is delineated when we get more information on the fish pheromones and their mode of action on immune system.

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Lamellar angiography, a novel method for gill respiratory area measurement: SEM of gill corrosion vascular replica

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Measurement of lamellar dimensions of the swamp catfish, *Chaca chaca* has been made using corrosion vascular replica. Advantage of corrosion vascular cast for sampling lamellae for their measurements is discussed in the light of data obtained from Bouin's fixed materials.

FISH gill lammellae are the sites for gaseous exchange and their measurement is subjected to various methodological errors because of the heterogeneity in the dimensions of a large number of lamellae[1]. It is impossibly difficult to measure the area of all the gill lamellae immediately after their removal from the anaesthetized fish to obtain the absolute lamellar values. Formalin- and Bouin's-fixed gills are commonly used for surface area measurements[2]. In recent years, Bouin's-fixed gills have been used for lamellar measurements because it fixes and stains as well. Shrinkage due to fixation is one of the greatest sources of error. The other source of error is the inclusion of nonrespiratory pillar cell system in the lamellar measurements of fresh and fixed gills. To get rid of these sources of errors, an attempt has been made to sample lamellae of *Chaca chaca* injected with methyl methacrylate resin for estimating only the lamellar blood channels, which are the actual functional sites for gaseous exchange.

Chaca chaca belongs to the family Chacidae of the order Siluriformes and is well adapted to hypoxic swamp (2 mg O_2/l) infested with macrovegetation and decaying organic matter. Live specimens of *C. chaca* were collected from the swamp near Purnia (Bihar), and maintained in plastic tanks (40 l) in the laboratory. Live specimens ($n = 3$) of *C. chaca* were anaesthetized by MS 222, ventral aorta was cannulated and phosphate-buffered Ringer's solution with 100 USP/ml heparin, was infused at physiological pressure of 30 mm Hg to fill the lumen of the cardiovascular system by replacing blood. Methyl methacrylate (Mercox) was mixed with catalyst and infused also at 30 mm Hg (ref. 3). At the onset of polymerization, the ventral aorta was clamped and the fish was placed in 60°C tapwater for 2 h to ensure complete polymerization. The vascular replica was obtained after treating the carcass with 20% NaOH, water and 3% HNO_3. Vascular replicas of gill filaments of the sampled fishes were mounted on an SEM stub with silver paste, gold sputtered and examined with P-SEM 500 scanning electron microscope.

Scanning electron micrographs (SEMs) of the sampled lamellae ($n = 15$) from base, middle and top of the filamentar vascular replica were projected on mm^2 rectilinear grid to measure their dimensions. The data were compared with those from Bouin's-fixed gills of *C. chaca* (62 ± 2 g) body weight. Paired t test was employed to test the level of significance between the mean lamellar area values of the lamellae sampled from vascular replica and those from Bouin's-fixed gills.

Respiratory and nutritive vascular systems are discernible in the angioarchitecture of the gill filament (Figure 1). The marginal channel and the central vascular network of a lamella constitute its respiratory part, whereas the network of blood channels of the vascular replica of the gill filament is its nutritive part. The former takes care of the gaseous exchange between the O_2 present in the ambient water and the blood that circulates through the marginal and the central network of the lamellar channels and the latter provides nourishment and O_2 to the underlying tissues of the gill filaments. The afferent and efferent sides of the lamellar vascular replica are differentiated by narrow and wider profiles respectively (Figure 1 *b*). Flow of blood from afferent to efferent ends through the vascular network and the up-inclined marginal channel results in lower velocity of blood flow, which allows the haemoglobin greater O_2 loading from the counter-current flow of ventilating water from leading to trailing edges of the inter-lamellar spaces. The total vascular area of an average lamellar replica is about 0.11328 ± 0.01011 mm^2. This value is significantly ($P < 0.005$) higher (63.298%) than the