ity can be enhanced by making suitable derivatives since it posesses exocyclic methylene double bond of lactone ring and a cyclopentenone moiety in its skeleton which provide an excellent site for structural modifications[5].

1. Duke, S. O., *Rev. Weed Sci.*, 1986, **2**, 15–44.
2. Towers, G. H. N., Rodriguez, E., Bennett, F. D. and Rao, P. V. S., *J. Sci. Ind. Res.*, 1977, **36**, 672–684.
3. Kohli, R. K. and Daizy Rani, *Res. Bull. Panjab Univ.*, 1994, **44**, 105–149.
4. Pandey, D. K., *J. Chem. Ecol.*, 1996, **22**, 151–160.
5. Saxena, D. B., Dureja, P., Kumar, B., Daizy Rani and Kohli, R. K., *Indian J. Chem.*, 1991, **30**, 849–858.
6. Rizvi, S. J. H., Mukerji, D. and Mathur, S. N., *Agric. Biol. Chem.*, 1981, **45**, 1255–1256.
7. Heisey, R. M., *Am. J. Bot.*, 1996, **83**, 192–200.
8. Duke, S. O., Vaughin, K. C., Croom, E. M. Jr. and Elsholy, H. N., *Weed Sci.*, 1987, **35**, 499–505.
9. Chen, P. K., Polatnick, M. and Leather, G., *J. Agric. Food Chem.*, 1991, **39**, 991–994.
10. Macais, F. A., Torres, A., Molinillo, J. M. G., Varela, R. M. and Castellano, D., *Phytochemistry*, 1996, **43**, 1205–1215.
11. Kohli, R. K., Daizy Rani and Verma, R. C., *Biol. Plant.*, 1993, **33**, 567–576.
12. Batish, D. R., Kohli, R. K., Saxena, D. B. and Singh, H. P., *Plant Growth Regul.*, 1997, in press.
13. Tomaszewski, M. and Thimann, K. V., *Plant Physiol.*, 1966, **41**, 1443–1454.

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Failure of brown oak (*Quercus semecarpifolia*) to regenerate in central Himalaya: A case of environmental semisurprise

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The brown oak (*Quercus semecarpifolia* Sm.), a late successional species is the greatest forest-forming species above 2400 m altitude in the Himalaya. The failure of brown oak to regenerate in central Himalaya is a case of environmental semisurprise. Presence of saplings along roadsides and their absence in shaded habitat of forest interior suggest that this oak requires not only canopy gaps but also exposed soil for regeneration. It seems that in the current chronic form of disturbance, gaps formed are too small to enable brown oak to establish seedlings, and in large gaps grazing/browsing does not allow regeneration to progress up to tree size.

ENVIRONMENTAL degradations of global and regional scales generally have an element of surprise[1]. Many of the present front-rank problems, such as global warming and tropical deforestation were not even mentioned in the Stockholm conference on the environment held only two and one-half decades ago. Indeed true surprises, by definition, are beyond the scope of our research, but the likely candidates for semisurprises, that have potential to become outsize problems need to be identified at an early stage[2]. There are a few preliminary studies which suggest that the failure of regeneration of the brown oak (*Quercus semecarpifolia*), the most extensively distributed Himalayan oak, to regenerate at a regional scale may be regarded as one such likely candidate[3–5]. In this article based on population structure of the Himalayan brown oak (*Quercus semecarpifolia*) at a number of sites in Garhwal region, we have addressed questions: (i) Is this oak failing to regenerate in its forests in all sizes of canopy gaps? (ii) What are the factors responsible for its poor regeneration at a regional scale?

The brown oak, a late successional species is the greatest forest-forming species of the entire east-to-west arc, above 2400 m altitude in the Himalaya. Much of brown oak zone and adjacent alpine meadows have been historically under the influence of grazing of migratory livestock such as sheep and goats. It is used for firewood, charcoal, fodder and agricultural implements, and now in tasar sericulture.

The forest sites sampled occurred between 2400 and 3200 m altitude in Akashkamini Catchment (30°28'–30°31'N lat. and 79°13'E long.) of Garhwal region of Himalaya. The main criterion of site selection was gap size because of its importance in regeneration[6], however, the study sites also differed in altitude and distance from edge of the forest (Table 1). The forest edge site (site 1, Table 1) resulted from road construction about 30 years ago. Its ground was disturbed due to cuts and debris deposits. The site with unbroken canopy (site 2) with about 80% crown density (measured with densiometer) represented the forest interior. In the site with large canopy gaps (>0.06 ha, Table 1) livestock grazing had recently begun, which is why its forbs were from both forest as well as meadows. The forest meadow site had large (site 5) meadow patches, with domination of forbs of alpine meadows, such as *Trachydium roylei* and *Potentilla* spp.

At each site, all individuals of brown oak were counted in 5–16 randomly distributed 100 m^2 circular quadrats. Individuals up to 150 cm tall were divided into 50 cm height classes, and those larger than them were divided into 60 cm cbh (circumference at breast height,

Table 1. Regeneration status in relation to site characters. Values in parentheses indicate number of sample quadrats, each of 100 m^2 area, distributed between 2400 and 3400 m altitudes[a]. In first three sites grazing is occasional. [b]Meadow formation indicates a relatively longer history of livestock grazing that leads to spread of forbs of alpine meadows

Sites	Type of population structure	Seedlings	Saplings	Age of youngest trees (yr)
1. Forest edge along roads, old trees with canopy at 25 m (n = 6)	Transition between I and II	Present	Present	>150
2. Unbroken canopy at low height (about 15 m) (>80% crown density) (n = 5)	Type III	Absent	Absent	48
3. Small gaps (<0.06 ha), at 15–20 m canopy height (n = 5)	Type III	Absent	Absent	70
4. Large gaps (>0.06 ha) at 15 m canopy height within forest (n = 6)	Type III	Absent	Absent	50
5. Forest meadows[b] with old trees with canopy at about 25 m (n = 8)	Type II	Present	Absent	75

Table 2. A summary of characters of *Quercus semecarpifolia* relating to regeneration. Comparison with *Q. floribunda* (referred to as *Q. fl.*) is given as this oak is its common associate at lower elevations (2400–2600 m) and regenerates well (based on 3, 4, 12, 13 and our field observations in the study area)

Character	Description	Ecological implications
Minimum tree age of seed production	About 50 yr (compared to about 20 yr for *Q. fl.*)	Poor colonizer, a later successional species character.
Seed fresh weight	7.5 g, among the heaviest (*Q. fl.* 3 g)	Seedlings likely to be adapted to deep shade.
Duration and time of seed fall	About a month during July–August (in *Q. fl.*) throughout three months of rainy season	Establishment of seedlings may be affected by the delay in commencement of monsoon.
Germination	10 days of seed viability, viviparous, high (95%) seed germination capability (*Q. fl.* similar but has a longer viability).	Germinating seed assured of moisture, humidity and warm temperature; but seedling establishment of viviparous seeds poor.
Gap size requirement for seedling establishment	A well lit gap with overhead light (*Q. fl.* endures a deeper shade)	Contrary to the expectation based on large seeds.
Initial growth rate	Very slow, stem thick (in *Q. fl.* thick but comparatively less)	Thick stem may survive damages of litter and snow fall.
Ability to coppice	Exists (but far less than in *Q. fl.*)	Coppicing inadequate to replace older individuals if they die.

1.37 m) classes. Percentages in each size classes were plotted for each site (based on average of sample quadrats) to depict population structure and regeneration trend.

At each site approximate tree age of the lowest cbh was estimated based on relationship between age and cbh derived from the tables given in ref. 3. That gave an approximate estimate of length of time over which the brown oak had been failing to regenerate at the study sites. The impact of livestock grazing on seedlings was measured at the forest meadow site (site 5, Table 1), by counting their number periodically during the entire stay of graziers on the site (summer and rainy season).

A short period of seed viability combined with vivipary and intolerance of seedlings to shade are the characteristic features of the brown oak (Table 2). A short period of seed viability is unlikely to limit its regeneration, as most of seeds germinate in warm and humid environment while still on tree crown (Table 2). As the tissues of germinated seeds are no more dormant, the viviparous seeds fallen on the ground are not protected by seed coat. In a normal terrestrial habitat, vivipary appears to be disadvantageous also because seeds germinated before reaching ground cannot again use favourable moisture condition, once they desiccate after falling in a dry microsite.

Of the five types of forest sites sampled, only forest edge had both seedlings and saplings, samples with large gaps and meadow patches had only seedlings and the remaining sites had only individuals of tree size. In

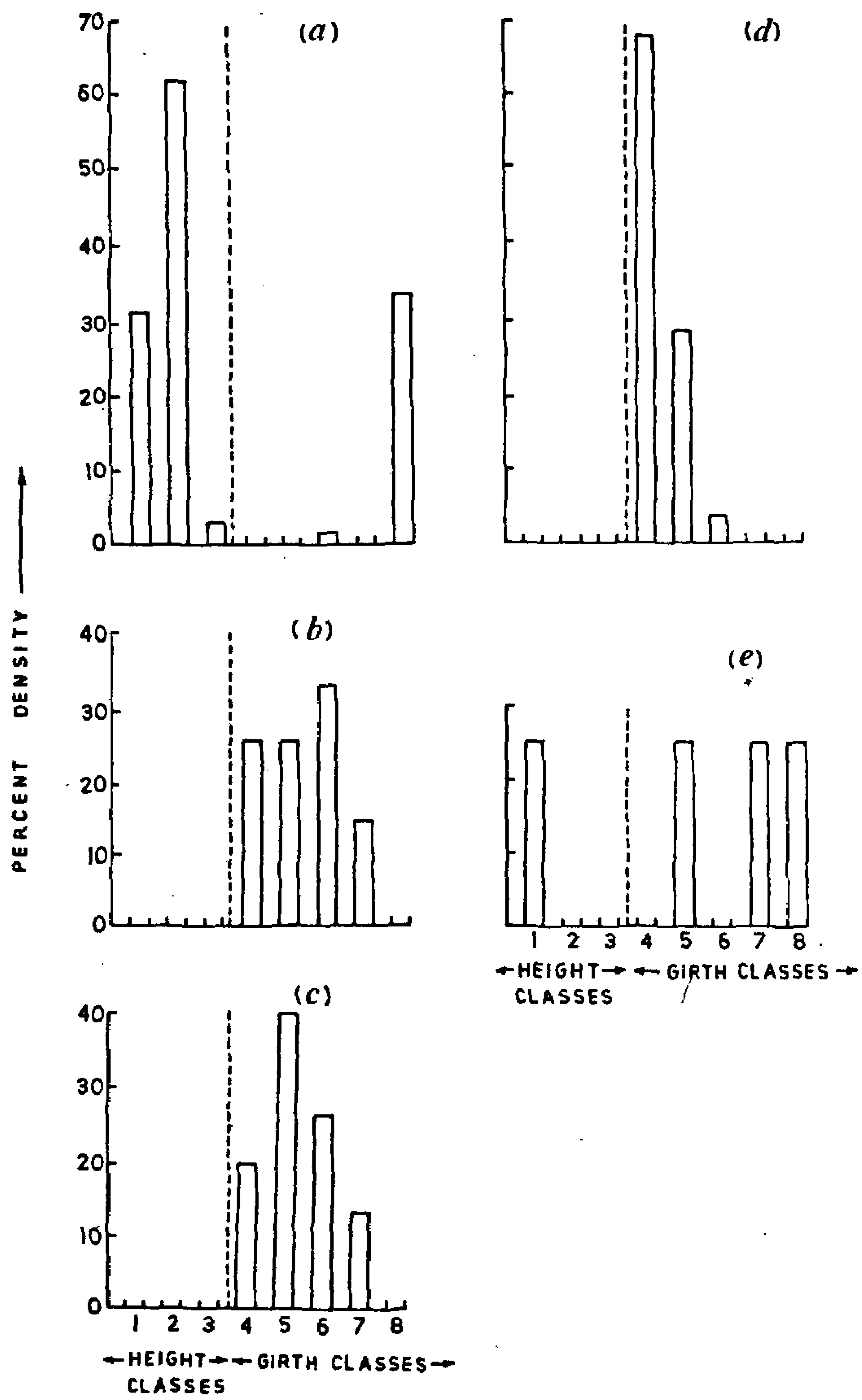

Figure 1. Representation of the size class distribution of *Quercus semecarpifolia* population in Akashkamini catchment (Garhwal Project area) for sites 1–5; on the X-axis, classes 1–3 are the height classes of seedlings and saplings (1st height class is for seedling <50 m tall, 2nd and 3rd height classes are for saplings, 50–100 cm and 100–150 cm tall, respectively). Fourth to 8th classes are girth classes (girth is circumference of tree at breast height (cbh) or 1.37 m) for trees (4th girth class = 30–90 cm cbh, 5th = 91–150 cm cbh, 6th = 151–210 cm cbh, 7th = 211–270 cm cbh and 8th >270 cm cbh).

the forest meadow site, only one quadrat around an isolated old tree had seedlings, all below 30 cm height. Their survival was low due to grazing/browsing and trampling of animals. It was observed that during two months grazing the mortality of seedlings was about 75%.

In none of the sites was the population stable, self-maintaining (Type I), characterized by a greater number of small individuals than large individuals and a more or less constant reduction in individuals from lower to higher size class (referred to Type I by Peters[7]). The sampled populations had either structure with peaks and troughs in the size class distribution indicating irregular seedling establishment (close to Type II), or that with many more individuals in higher than in lower size classes (Figure 1), indicating failure to regenerate for quite some time (Type III). Distribution of Type II is common among late secondary species that depend on conspicuous canopy gap for regeneration[7]. The study samples showing similarity to Type II population have troughs in population distribution caused through lack of individuals, specially in small size classes, reflecting failure of regeneration that has been continuing for quite long time.

Type III in which individuals are in a limited number of size classes, is a characteristic of light-demanding, early pioneer species having a pool of dormant seeds in soils[7]. However, when this type is exhibited by a primary, or late successional species, such as the brown oak, it reflects failure to establish seedlings for a long time. The above three size class distribution types are of a single sequence through which population passes on its way to extinction (from Type I to Type III), and the populations of brown oak already under such a transition.

Relationship between size and age indicates failure of brown oak to regenerate for last 6–10 decades.

Absence of saplings in shaded habitat of forest interior and their presence along roadsides with disturbed ground surface suggest that the brown oak requires not only canopy gaps but also exposed soil for regeneration. Presumably, well-lit microsites with exposed mineral soil, resulting from tree fall and landslides constituted natural habitat for regeneration of this oak. However, such sites were also preferred by graziers and their herds, resulting in expansion of gaps[8].

Though road construction promoted recruitment and growth of oak seedling and saplings by disturbing canopy and forest floor, it also led to temporary human settlements and to concomitant increase in demand of firewood and fodder. This in turn, damaged sapling and young trees.

It seems that in the current chronic form of disturbance, gaps formed are too small to enable brown oak to establish seedlings, and in large gaps grazing/browsing does not allow regeneration to progress to tree size.

In the chronic form of forest disturbance, common in developing countries, only a small fraction of forest biomass is removed at given time (e.g., collection of leaves and cutting of twigs for fodder and firewood[9]) resulting in a small increment in canopy gaps. By the time the creeping harvest of biomass forms a gap sufficiently large for species to regenerate, the gap habitat is substantially modified and is very different from that resulting from an acute disturbance, such as cutting of a whole tree or a forest stand. Modification of habitat may include compaction of soil, replacement of forest herbs with grasses of alpine meadows, and consequent increase in root growth, impeding tree seedlings to establish[10]. They may combine (synergism) to cause an irreversible decline in regeneration of brown oak.

The ban on tree cutting clamped about two decades ago has, in part also contributed to failure of regeneration of brown oak, as well as of most tree species, except the shade-enduring (*Quercus floribunda* Lindl.[4]) by restricting formation of gaps of an adequate size.

The failure of regeneration would not come to public notice as long as old trees persist. Reports of failure of regeneration of brown oak in three separate areas (viz., Naini Tal, Pindari and the present area)[3,10] located more than 300 km apart, need to be taken as a serious warning, warranting action before the problem assumes an element of surprise.

1. Myers, N., *Science*, 1995, **269**, 358–360.
2. Faber, M. R., Manstetten, R. and Prupps, J. L. R., *Environ. Values*, 1992, **1**, 217.
3. Troup, R. S., *The Silviculture of Indian Tree*, Clarendon Press, Oxford, 1921, vols I–III.
4. Singh, J. S. and Singh, S. P., *Forests of Himalaya: Structure, Functioning and Impact of Man*, Gyanodaya Prakashan, Naini Tal, India, 1992.
5. Adhikari, B. S., Ph D thesis, Kumaun University, Naini Tal, 1992.
6. Brokaw, N. M., *Ecology*, 1985, **66**, 682–687.
7. Peter, C. M., Sustainable harvest of non-timber plant resources in tropical moist forest: An ecological primer, Biodiversity Support Program, Washington, 1991, p. 45.
8. Negi, G. C. S., Rikhari, H. C., Jeet Ram and Singh, S. P., *J. Appl. Ecol.*, 1993, **30**, 385–394.
9. Singh, J. S., Pandey, U. and Tewari, A. K., *Ambio*, 1984, **13**, 80–87.
10. Singh, S. P., Singh, R. P. and Rawat, Y. S., Final Technical Report, Ministry of Environment and Forest, Govt. of India, New Delhi, 1990.

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Primary cell culture from explants of heart tissue of Indian major carps

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Heart tissue of Indian major carps was explanted in minimum essential medium supplemented with 15% fetal bovine serum. Fibroblast-like cells emerged from the explant and formed a monolayer within a week. The cell monolayer could be harvested for passage by either trypsinization or scraping.

FISH cell lines are indispensable in fish virology besides their importance in toxicology, biomedical research, biotechnology and many other basic studies[1]. Most of the established fish cell lines are derived from cold water fish[2,3]. Very few cell lines have been developed from the tissues of warm water fish cultured in Asia.

In India, a cell line from the gill tissue of mrigal, *Cirrhinus mrigala* has been developed[4]. Primary cell cultures from kidney of stinging catfish, *Heteropneustes fossilis*[5] and from caudal fin of rohu, *Labeo rohita*[6] have also been developed.

Indian major carp culture has been established on a commercial scale in India. The growing carp culture industry has suffered due to several incidences of mass mortality of carps in culture systems suspected due to microbial diseases, particularly of viral etiology[7]. Besides, mortality due to increased aquatic pollution is also on the rise. Hence, there is a need to develop cell lines for the major culturable varieties of fish in India. This paper describes the development of primary cell culture from the heart tissue of the Indian major carps, catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*).

Major carp fry (5–8 g) were obtained from the State Government Fish Farm, Gajanur, Shimoga and reared to fingerlings in 25 m^2 cement tanks at the fish farm of College of Fisheries, Mangalore. The fish were fed daily with artificial feed[8]. The fingerlings (12–15 g) were transferred to laboratory and maintained for 5 days in aquaria containing tap water treated with $KMnO_4$ (5 ppm). These fish were then transferred to sterile water overnight. They were killed instantly by a hard blow on the head, disinfected in calcium hypochlorite (500 ppm) for 5 min, washed in sterile water and swabbed with 70% ethyl alcohol. The heart was removed aseptically from several fingerlings using sterile scissors and forceps and pooled in a vial containing cold phosphate buffer saline (PBS). Pooled heart washed twice in PBS were squeezed to remove blood cells and washed again with PBS. The organ was cut into small fragments of approximately 1 mm^3 and washed twice at 1000 rpm in PBS.

The pellet consisting of small pieces was resuspended in 2–3 ml of PBS containing antibiotic–antimycotic solution (penicillin, 250 IU/ml; streptomycin, 0.25 mg/ml and amphotericin B, 0.625 µg/ml Sigma, USA) and further washed twice in a sterile petri dish. The tissue pieces were transferred to a 25 cm^2 tissue culture flask (Tarsons, India) and then distributed uniformly by flooding with 1 ml PBS supplemented with antibiotic–antimycotic solution as above. Excess PBS was then pipetted out to the last drop and the tissue