CHAPTER V

SUMMARY AND CONCLUSIONS
The work included in this thesis entitled, “Some Aspects of Endocrine Regulation of Surfacing Behaviour in an Air-Breathing Catfish, *Clarias batrachus*” has been divided into five chapters. Chapter I contains review of literature of studies conducted on surfacing behaviour, air-breathing physiology and related areas. Chapter II deals with fabrication of IR system which was used to monitor surfacing frequency. Chapter III has been divided into two sections. Section I concerns with the air-breathing nature of *C. batrachus*. Section II deals with multifrequency rhythms in surfacing behaviour of *C. batrachus*. Attempt has been made to validate circadian time structure in surfacing behaviour and also to study free-running properties of this rhythm. Efforts have also been made to study the circannual rhythm in surfacing behaviour of *C. batrachus*. Chapter IV concerns with the effects of metabolic hormones on surfacing behaviour. Chapter V deals with the summary of each of the previous chapters. This also includes conclusions and recommendations drawn from this doctoral dissertation.

5.1 Brief Review of Literature

Ethology encompasses studies on animal behaviour. The award of the Nobel prize for medicine in 1973 to Karl Von Frish, Konrad Lorenz and Niko Tinbergen testifies its maturity, assumed in early Seventies. Causation and ontogeny are two important areas of enquiry, inducted by Tinbergen (1963) as additional dimensions of behavioural biology apart from those of function and evolution conceptualized by Darwin (1872). The study of causation is nothing but an attempt to relate generation of behaviour to some underlying mechanism(s), whereas ontogeny of any kind of behaviour characterizes dramatic change in behavioural pattern in an animal during its development. Now ethology is being considered as one of the most interesting and important areas of modern biology.

This review concerns behavioural studies conducted in Pisces. In teleosts various kinds of behaviour have been studied. The locomotor behaviour has been most extensively studied (Gray, 1933; Welty, 1934; Gibson, 1958; Lighthill, 1960; Hafeez, 1970; Kapoor and Kleerekoper, 1970; Kapoor, 1971; Schwassmann, 1971; Blight, 1976; Timms, 1976; Van Veen, et al., 1976; Craig, 1977; Helfman, 1978; Thorpe, 1978; Eriksson and Van Veen, 1980; Webb, 1984; Vide1er, 1985; Fuiman, 1986; Webb and Weihls, 1986; Fuiman and Webb, 1988; Wardie and Vide1er, 1993; North, 1996).

Many species of fishes have been known to exhibit schooling behaviour. In fact schooling results from interactions in which an individual controls its movements in relation to neighbours and coincidentally has an influence on its neighbours (Aoki, 1982). Behavioural properties of schooling species have been explored from various angles, such as by limiting a sensory clue, reactions to stationary and moving objects and analysis of individual movements within the school (Shaw, 1960; Pitcher et al., 1976; Partridge et al., 1980; Aoki, 1982, Koike, 1985; Koike and Matsuike, 1987; Levin and Belmonte, 1996).

In piscine group of vertebrates various kinds of behavioural studies, such as fanning behaviour in *Gasterosteus aculeatus* (De Ruiter et al., 1986), social behaviour in *Fundulus heteroclitus* (Kavaliers, 1980), cleaning behaviour in *Laboides dimidiatus* (Lenke, 1982), phototactic behaviour in *Anguilla anguilla* (Van Veen et al., 1976) and *Nemacheilus evezardi* (Pradhan et al., 1989), light oriented swimming behaviour in *Cheirodon pulcher* (Levin et al., 1989), burying behaviour in *Nemacheilus evezardi* (Biswas et al., 1990a), air-gulping behaviour in *Plecostomus pumilus* (Gradwell, 1971), *Ophiocephalus striatus* (Vivekanandan, 1977a, b, c) and *Nemacheilus evezardi* (Biswas et al., 1990b), huddling behaviour in *Clarias batrachus* (Varghese and Pati, 1997) have been conducted.

However, interestingly surfacing behaviour exhibited by some fish species has not drawn due attentions of the ethologists. There are only a few papers on the surfacing behaviour of fishes (Gradwell, 1971; Vivekanandan, 1977a-c). Usually surfacing behaviour in these species is associated with air-gulping above the surface of the water. Since air breathing in fishes has been considered as an interesting phenomenon, surfacing behaviour assumes considerable physiological significance (Carter, 1957; Gradwell, 1971; Vivekanandan, 1977a).

Evidence suggests that in some species, for example in *Plecostomus* the inhaled air oxygenates the blood inside the stomach (Gradwell, 1971). This could be termed as a phenomenon of gastrointestinal mode of respiration. Indian fresh water catfish, *Clarias batrachus*, unlike *Heteropneustes fossilis*, has been reported to be an obligatory air-breathing fish (Thakur and Das, 1986). However, nothing more is known of this interesting behaviour. Therefore, investigation to elucidate the underlying mechanism of surfacing behaviour in *C. batrachus* would be worthwhile.

5.2 Fabrication of IR system

An infrared system was designed to record the frequency of surfacing in Indian catfish, *Clarias batrachus*. This system uses IR transmitter (Tx) and receiver (Rx). The frequency of interruptions of the IR beam caused by surfacing activity is recorded by a receiver that relays signals to a 4-digit electronic counter. Thus the frequency of surfacing over a specified time scale is expressed in number of interruptions per unit time. The sensitivity of this system was tested. With a sensitivity of about 94% the present IR system appears to be an excellent research tool for recording surfacing activity in Indian air-breathing catfish.
5.2.1 Design of the aquarium

Specific glass aquariums with narrow mouths at the top were used for experimentation. The dimension of the mouth of the aquarium was 7 x 7 cm. The area available for surfacing was further reduced by putting removable packing materials in two sides of the mouth of the aquarium.

5.3.1 Facultative or obligatory air breather: An attempt to resolve controversy

Air-breathing fishes are either facultative or obligatory (Das, 1940; Johansen, 1970; Munshi et al., 1976; Singh, 1976). Unlike Thakur and Das (1986), several scientists have described the Indian walking catfish, *Clarias batrachus* as a facultative air breather (Singh and Hughes, 1971; Munshi et al., 1976; Singh, 1976). In the present study, an attempt was made to resolve the controversy regarding the air-breathing nature of *C. batrachus*. Two series of experiments were conducted to observe the opercular frequency of *C. batrachus*. Opercular frequency was recorded in *C. batrachus* under surfacing-prevented and -allowed conditions with intact accessory respiratory organs and without left side accessory respiratory organs. A statistically significant elevation (P<0.01) in the rate of opercular frequency was recorded in the fishes without access to air in intact and operated fish. The opercular frequency under prevented condition was much more higher in the operated fish (without left arborescent organ, AO) as compared with the intact. However, interestingly unilateral removal of AO did not alter the intensity of opercular activity under surfacing-allowed condition. Thus, it appears that fish when prevented from surfacing compensates oxygen debt by increasing the rate of water flow over the gills through the enhancement of buccal pressure and activity of opercular suction pump. Results of present study clearly reconfirm the finding of Munshi *et al.* (1976) and Munshi and Ghosh (1994) who have identified *C. batrachus* as a facultative air breather.

5.3.2 Multifrequency rhythm in surfacing behaviour

The diurnal pattern of surfacing activity was examined under a LD 12:12 photoperiod at 2-hour intervals over a period of 48 hours in a group of adult *C. batrachus* of mixed sexes. The inter air-gulping interval in minutes between two consecutive bouts was also recorded four times at 06:00, 13:00, 17:00, 01:00 over the above time scale. The data were analyzed by cosinor rhythmometry. A statistically significant diurnal rhythm was validated for the rate of surfacing activity and inter air-gulping interval in *C. batrachus*.

A study was conducted to assess if the diurnal rhythm in surfacing activity is indeed endogenous. Initially animals were maintained under LD 12:12 photoperiod and entrainment
kinetic of the rhythm was studied. Thereafter, animals were exposed to DD for a period of 8-10 days in order to elucidate the free running period of the rhythm. The rhythm in surfacing activity did not clearly free run under constant conditions. Only two of the six animals showed a faint free-running pattern in the rhythm of surfacing behaviour. However, more extensive investigations are required in order to forward an emphatic conclusion as regards persistence of the surfacing rhythm under DD, LL and other constant conditions.

An attempt was also made to observe the annual rhythm in surfacing behaviour. A small population of fishes of average body weight 60-80 g were kept under artificial LD 12:12 (Light on at 06:00) for 15 months. The rate of surfacing activity was examined in each individual two times every month over the entire tenure of the study. A statistically significant annual rhythm in surfacing activity was observed in C. batrachus. In addition, a statistically significant 6-monthly rhythm was also detected. The ecophysiological significance of these rhythms was discussed.

5.4 Metabolic hormones and surfacing behaviour

Attempts were made to examine the effects of metabolic hormones on surfacing behaviour. Five experiments were conducted independently for studying the effects of hormones, namely epinephrine (Experiment-1), norepinephrine (Experiment-2), triiodothyronine (Experiment-3), thyroxine (Experiment-4) and hydrocortisone (Experiment-5). Hormones were administered by two routes to assess if mode of administration of the same amount of hormone has any effect on surfacing activity. Each experiment included two studies. In study-1 injections were given by intraperitoneal route whereas in study-2 the injections were given by intracranial route. Surfacing activity was recorded after 0.5, 1.0, 2.0, 4.0 and 6.0 hours post-treatment. Data obtained from each experiment were analyzed by using ANOVA (Bruning and Kintz, 1977) to evaluate effects due to dose, time-lag and their interaction. Statistically significant differences between group means were validated by Duncan’s multiple-range test (Duncan, 1955; Snedecor and Cochran, 1994).

Results of ANOVA indicate a statistically significant dose effect, irrespective of the hormones used and their mode of administration. However, there was an exception. Norepinephrine failed to modulate surfacing activity when given either as an intraperitoneal or intracranial injection.

In general, while epinephrine and norepinephrine declined surfacing activity, triiodothyronine, thyroxine and hydrocortisone elevated it. A more or less similar pattern of hormonal effects on surfacing behaviour was observed in Heteropneustes fossilis (Maheshwari and Pati, 1998). However, unlike C. batrachus, H. fossilis appears to be less sensitive to hormonal treatments as regards surfacing behaviour. The physiological significance of the role of hormones in surfacing behaviour has been discussed.
5.5 Recommendations

The optoelectronic device designed, developed and fabricated for this study, appears to be an excellent tool to monitor surfacing activity in catfish. *Clarias batrachus* is a facultative air breather. It exhibits multifrequency rhythms in surfacing behaviour. Catecholamines suppress the rate of surfacing activity. In contrast, thyroid hormones and the glucocorticoid elevate the rate of surfacing activity. Data obtained, in the present study, may help others in understanding surfacing behaviour and respiratory physiology in this and other air-breathing species.

It is recommended that *Clarias batrachus* is an excellent laboratory model for studying surfacing behaviour and respiratory physiology. The optoelectronic device developed during the course of the present investigations appears to be a low cost, but sensitive system that can be used for monitoring surfacing activity. It is also recommended that with certain minor modifications the proposed device could also be used to monitor swimming activity, accesses to food, emergence from an artificial burrow, phototactic behaviour and many other behavioural activities in a number of fish species. Last but not the least, the proposed IR system could be used as an excellent equipment for conducting number of physiological and behavioural experiments in graduate and postgraduate laboratories of Biology departments in Colleges and Universities.