CONCISE SUMMARY ~

Reproduction is an energetically expensive phenomenon. In majority of birds, it is periodic in nature *i.e.* when the proper season arrives birds seem to put all there physiological reserves into an intense and concentrated effort to produce the next generation. The act of leaving offspring to succeeding generation is the most important aspect of any animal life. Successful reproduction demands proper channelization of energy for breeding activities in relation to mate and environmental resources of energy in terms of food. Thus, majority of birds show a distinct annual reproductive cycle and the reproductive activities coincide with a season that is most advantageous for producing offspring (Murton, 1975). Reproduction demands increase in daily dietary energy intake, which may vary quantitatively and qualitatively during different reproductive phases, and during this period superfluous time and energy are required which remain after the basic daily metabolic requirement of growth and maintenance of a bird (Murton & Westwood, 1977). Various adaptations are shown by birds to combat increased energy demands and which depend on different patterns of energy procurement during breeding season. Energetics associated with breeding activities is achieved by synchronization of the endogenous controlling mechanisms by a variety of environmental cues to which a particular species has evolved a response. These cues give predictive clues (proximate factors) like change in temperature, weather, nest-site, nest material, food supply etc. to the bird and act as promoters or repressors (Welty, 1990).

Photoperiodism also plays an important role in breeding. It is a physiological mechanism that brings seasonality in birds. Animals synchronize their internal rhythm of body functions with the external chronology of the day and night cycle (Murton and Westwood, 1977) and other environmental stimuli. Increased photoperiod also provides increased time for procurement of food for the increased demand of energy.

In the present study the energy metabolism and utilization of some of the major metabolites of the body, over the reproductive cycle, in two closely related long day breeding passerines belonging to family sturnidae are investigated. The species selected for the study are, the Bank myna *Acridotheres ginginianus* and Brahminy myna (Black Headed myna) *Sturnus pagodarum* commonly seen in the area.

Both the species are hole nesters; Bank myna is a colonial nester and builds nests in earthern riverine banks, sides of wells, under bridges and is seen to be nesting in broken walls in the vicinity of water body (Ali, 1979; Narang and Lamba, 1984; Khera and Kalsi, 1986; Personal observations). It is a gregarious bird with communal roosting habits exhibited throughout the year. Only during the breeding season the incubating and brooding females sleep solitarily in the nest during night hours (Khera and Kalsi, 1986). Otherwise both the sexes take part in incubation and feeding the young. Being an omnivore Bank myna feeds on fruits, grains and insects (Ali, 1979) and are easily found on municipal garbage dumps and railway stations in large numbers and are also found feeding on oily human left over. Unlike Bank Myna, though an omnivore Brahminy myna is a solitary hole nester with selective omnivore habits. They feed on fruits of Lantana, Zizyphus, Wild Figs, nectar of Erythrina etc. and insects like grasshoppers, moths, caterpillars etc (Ali and Ripley, 1983; Narang and Lamba, 1984).

These birds prefer taking to the nest holes of other birds; sometimes they even drive away the Barbets from their nests, (Panicker, 1980). They are also seen nesting successfully in nest boxes. They roost in association with other mynas. This species being a selective omnivore feeds whereas Bank myna is known to feed on whatever is available. This aspect was also taken into consideration during the present investigation as food is important source of dietary energy and energy metabolism depends on the type of food ingested. Colonial nesting and solitary nesting species differ from each other with respect to their energetics involved for different activities that also includes their sojourn of reproduction. In colonial species the cost of reproduction is lower as compared to solitary nesting species as all the members of the colony share many of the reproductive activities. Whereas in solitary nester both the male and female have to equally share all the reproductive activities starting from territoriality, nest guarding, feeding the young etc.

For both the species of mynas breeding season coincides with long days with the onset of monsoon and increases in insect population. Insects are rich source of protein and fat, which are the other sources of energy after carbohydrates. It aids in speedy development of the young. Both the bird species are known to feed their nestlings predominantly with animal matter (mainly insects) that is 95.59% by frequency in Bank myna (Parasara *et al.*, 1990) and 81.57% by frequency in Brahminy myna (Patel *et al.*, 1992). Hence, The reproductive cycle of both the species of mynas in Baroda (Longitude 72.3°13'E, Latitude 22° 18'N) is considered in four phases *viz.* Feb-Apr as pre breeding (Pre-Br), May-Jul as breeding (Br), Aug-Oct as post breeding (Po-Br) and Nov-Jan as non breeding (Non-Br) seasons as per Padate (1990) and Sapna (2002). Variations in certain biochemical parameters related to carbohydrate metabolism were carried out in three tissues *viz.* liver, intestine and kidney.

Liver plays a key role to in different metabolic activities like glycogen synthesis, HMP shunt pathway, glycolysis, lipogenesis, lipolysis, protein synthesis, and gluconeogenesis. During a specific phase of reproduction, as and when, an essential metabolite is needed it is mainly synthesised in the liver. Liver plays an important role in the assimilation and treatment of absorbed food, and shows metabolic and enzymatic adaptations. As depending on the diet and physiological condition of the bird, metabolism in liver is adjusted; liver is one of the tissues studied. The second tissue studied is the intestine, the principle organ where chemical digestion of food takes place. It is a part of digestive system that functions both as a secretary organ as well as digestive chamber (Ziswilar and Farner, 1972). It plays an important role in absorption and transfer of nutrient materials from lumen into the blood. The capacity of the intestine to absorb nutrients appears to be influenced by the pattern of feeding and caloric intake of the animal (Reiser, 1976). Hence, intestine was considered for the study. The third tissue studied, the kidney, plays an important role in general metabolism due to its involvement in glucose homeostasis through gluconeogenesis (Shen and Mistry, 1979, Christensen *et al.*, 1999; Yorita *et al.*, 1987), a mechanism where glucose is produced from non-carbohydrate precursors like proteins, fats etc. This is usually switched on during starvation or when diet lacks carbohydrates (Mehta, 1985). Hence, the role of kidney in combating the increased demand of energy during reproduction is looked for.

The energy demands during the different reproductive phases should depend on the metabolic changes based on shift in rates of synthesis of enzymes, their degradation, quantum and nature of factors that activate the whole enzyme cascade. The present study aims to deal with variations in enzyme activities mainly related to carbohydrate metabolism and involvement of some of the important metabolites in energy metabolism in relation to reproductive cycle in three metabolically important tissues mentioned earlier in two closely related omnivore species of birds which show difference in food preferences.

Carbohydrates are the dominant and preferred form of dietary substances providing about 80% energy by degrading processes of carbohydrate metabolism, which release energy required for various activities including the sojourn of reproduction. The second important source of energy is the dietary fats, the lipids. Lipids are found in all cells and play an important role not only in providing structural support but also in diverse physiological processes *viz.* energy production, reproduction, migration etc. The Total lipid includes four principal forms: triglycerides, phospholipids, cholesterol and free fatty acids of which, triglycerides or neutral fats are fatty acid esters of glycerol, which serve as a major store as well as source of energy. Fat intake varies according to the eating habits of a species. Carbohydrates and proteins serve as a source material for lipogenesis. Thus, absorption of fat from intestine and its synthesis from non-lipid compounds are two different pathways of lipid accumulation. Cholesterol, a principal form of lipid present in tissues and plasma lipoproteins (as free cholesterol or cholesteryl esters) playing vital role in fatty acid transport, acts as a precursor for production of steroid hormones and bile acids too. Liver is an organ for synthesis and degradation of cholesterol and intestine is another site for cholesterol synthesis. Pathways of lipid and cholesterol metabolism meet at a common intermediate "Acetyl Co-A", and glycerol of fats are capable of joining the reversible pathway of carbohydrate metabolism. These facts indicate a close integration of lipid and carbohydrate metabolism (Patel, 1982).

Proteins, the third source in order of preference by the body, provide the remaining energy only after meeting the requirement for dietary amino acids. Proteins play a functional role in supplying energy during their course of degradation (Griminger and Scanes, 1985). Energy from protein is considered as detrimental because of cost, heat increment and nitrogen toxicity. Nevertheless, it does provide energy in the time of need. Excess of amino acids are not excreted instead they are converted to precursors of glucose, fatty acids and ketone bodies, hence can be called as metabolic fuel (Voet *et al.*, 1998). Taking these facts into consideration variations in glycogen, protein and lipid levels, with cholesterol were studied in three tissues of both the species and sexes of mynas over the reproductive cycle.

TCA cycle, an important metabolic process in carbohydrate metabolism for liberation of free energy is also common for metabolism of fat and proteins. The efficiency of energy retention is more in fat compared to protein (Boekholt *et al.*, 1994).

Glycogen is an important polysaccharide involved in carbohydrate metabolism. The fate of dietary carbohydrate is either to get metabolised to carbon dioxide and water with release of energy or to get polymerised and stored or to be converted to lipoid moieties. Glycogen is present in almost all tissues, liver being the richest storage organ where it is stored as a readily available source of energy for extra-hepatic tissues and mobilized according to the peripheral needs. In the present study, glycogen content was found to be low during the breeding phase in both the species of mynas, except for the females of the Bank myna, which exhibited high glycogen content during the same period. This bear testimony that onset of breeding activities place heavy demand on energy provisions of the body leading to utilization of stored glycogen and lowering of the body weight. During this period, in the females of both species, a different trend supports the difference in breeding habits. In Bank myna, colonial nester, which spends more time in foraging (Khera & Kalsi; 1986) at the time of egg laying, hepatic glycogen is maintained, while in female Brahminy myna, the individual nester, spending equal time with the male, in breeding activities, hepatic glycogen is depleted along with body weight. The intestine and kidney also exhibited species-specific differences in the glycogen content. Increase in intestinal and renal glycogen content in males of Bank Myna during breeding season when the female is busy incubating eggs, may be due to the fact that males spend maximum time in foraging, resting and maintenance (Khera and Kalsi, 1986). This is reflected by consumption of more carbohydrate and protein, accumulated in kidney too, for later use when hepatic sources deplete. The depleting intestinal and renal glycogen content during breeding in both the sexes of Brahminy myna suggests the role of these tissues in energy metabolism and energy is expended for increased monogamous breeding activities. Thus the difference in two species may be associated with their nesting as well as feeding habits.

Degradation of glycogen (Glycogenolysis) is accomplished by a rate-limiting enzyme, phosphorylase or Glycogen phosphorylase (GP) that plays a strategic role in glycogenolytic pathway (Mayes, 2000). It is the initial catalytic reaction in the chain of chemical events that lead to the phosphorylative degradation and utilization of glycogen (Stetten *et al.,* 1960). Increase in the phosphorylase activity can be considered as an index of extent of glycogen depletion, that indicates increased glycogenolysis (Cahill *et al.,* 1957). The increased GP activity of the tissue denotes increased energy demands for the various activities and also the use of carbohydrate as the chief fuel for instant energy and a rise in GP activity exhibited in liver of male Bank myna and in both the sexes of Brahminy myna, evince glycogen mobilisation/utilisation as the onset of breeding activities are expected to place heavy demand on energy provisions of the body, wherein, the females of the former species showed a decline indicating decreased energy demands as the females of this species spend maximum time in incubation which has been compared with resting as resting and incubation are behaviourally and energetically similar. A bird can rest while sitting on the nest for incubation (Maxon and Oring, 1980). Thus, conservation of energy by this sex of the species is indicated. Intestinal GP activity maintained from pre-breeding to breeding in Bank myna and elevated in Brahminy myna during breeding phase indicate the difference in the qualitative and quantitative feeding habits along with the energy requirement of the colonial and the solitary nester. Renal GP followed the same trend as that of liver in Brahminy myna whereas depletion in the enzyme level was recorded in both the sexes of Bank myna. The kidney, probably contributed to the accelerated need of glucose by increasing glycogenolysis in Brahminy myna whereas in Bank myna the energy released from liver is probably sufficient for the bird to carry out different breeding activities.

Another enzyme which catabolizes the final step of glycogenolysis and gluconeogenesis is glucose-6-phosphatase (G-6-Pase) enzyme complex. It occurs mainly in glycogenic tissues such as liver, where it plays an important role in releasing glucose into the blood stream by hydrolyses of glucose-6-phosphate to glucose and phosphate, (Mayes, 2000; Plewka *et al.,* 2000). This enzyme is active when the blood glucose levels are low and triggers the glycogenolytic pathway. Significantly high G-6-Pase during breeding phase, in the males of both the species *viz.* Bank myna and Brahminy myna and the females of the later, evince increase in body metabolism and activity in conjunction with reproductive functions. In female Bank myna, low G-6-Pase activity during breeding might be due to diminished glycogenolysis, which is confirmed by low GP activity. As said earlier, females of this species spend maximum time in incubation and hence energy expenditure is reduced to minimum.

Another enzyme studied is Succinate Dehydrogenase (SDH), a key enzyme in TCA cycle, which plays a pivotal role in metabolism and is an important mitochondrial marker enzyme (Mayes, 2000). The activity of SDH can be an index of the oxidative metabolism and production of ATP molecules in a tissue and an active TCA cycle. It replenishes the supply of energy rich ATP molecules for the enzyme Adenosine Triphosphatase (ATPase), involved in high-energy phosphate metabolism responsible for splitting of ATP to ADP and high-energy phosphate in the form of utilizable free energy (Mayes, 2000). Thus, operations of TCA cycle and ATP utilization can be deduced from the activities of SDH and ATPase. Both these enzymes have been studied together in the present work and they show sex specific and species specific trends to that of GP in all the three tissues over the reproductive cycle. Elevated hepatic SDH and ATPase levels during breeding phase in the male mynas of both the species are suggestive of enhanced oxidative metabolism resulting in active synthesis of ATP to be hydrolysed to ADP by the enzyme ATPase to release energy. Species-specific differences are noted depending on the type of food consumed during breeding.

Though both the species of birds are omnivores, they show difference in food preference and also change the diet seasonally depending on the availability of food (Narang and Lamba, 1984; Simwat and Sidhu, 1973). Hence, another group of enzymes that is taken up for the present investigation are non-specific acid phosphatase (AcPase) and alkaline phosphatase (AlkPase). They are known to play significant role in secretion of digestive enzymes and absorption of digested food (Shah *et al.*, 1975). This group of enzymes help in transport of metabolites across the membrane and hydrolyses of phosphate esters (Patel, 1978). These enzymes according to their presence at a particular site, are involved in variety of cellular activities like, absorption, cellular phagocytosis, phosphorylation, protein synthesis, carbohydrate metabolism (Rosenthal *et al.*, 1960), phosphate transfer in DNA metabolism (Rogers, 1960). Their role in liver of birds with different feeding habits has been found to vary considerably (Shah *et al.*, 1972). Hence, by studying AcPase and AlkPase, an attempt is being made to find, if there exists any, species-specific differences in diet consumed. Higher AcPase activity in the intestine of both the species and sexes supports the role of these enzymes particularly in absorption and transmembrane transport in response to increased protein synthesis and secretary activities (Shah *et al.*, 1975; Majumdar *et al.*, 1988) especially during breeding season in female birds. The other enzyme AlkPase also exhibited similar trends. Increase in hepatic AcPase has been associated with carbohydrate diet, glycogen deposition and lipogenesis (Pilo *et al.*, 1978) in graminivores. However an opposite trend is noted in the omnivore species in the present study. Renal AcPase and AlkPase activities probably show difference in relation to species-specific differences in their nesting and feeding habits. In both the sexes of Brahminy myna, the individual nester where both the partners take equal part in breeding activities, kidney is probably gluconeogenically more active during breeding with its high AcPase and AlkPase activities when protein consumption increases. In the other species, Bank myna, this situation probably occurred during post-breeding season when both the parents start taking care of the young and simultaneously feeding in larger flocks on non-carbohydrate food.

Proteins play central role in cell function, as enzymes and cell structure. It plays a functional role in supplying energy during the course of their degradation (Griminger and Scanes, 1985). The bulk of cell's amino acids are incorporated into proteins and excess of dietary amino acids are not excreted, instead they are converted to precursors of glucose, fatty acids and ketone bodies, hence, can be called as metabolic fuels (Voet *et al.*, 1998). Increase in protein content was recorded during the breeding season in both the sexes of Bank myna and Brahminy myna. Increased protein content during breeding can be attributed to cumulative increase in various enzyme levels and dietary protein intake. Both the species being omnivore feed on both carbohydrate rich plant matter and protienaceous insect diet that is reflected in increased hepatic and intestinal protein content. Increase in renal total protein during breeding suggests increased activity of kidney for energy yielding gluconeogenic processes.

Lipids are another major group found in all cells and play an important role in diverse physiological processes such as energy production, reproduction, migration etc. Cholesterol, a principal form of lipid present in tissues and plasma lipoproteins (as free cholesterol or cholesteryl esters), playing vital role in fatty acid transport, acts as a precursor for production of steroid hormones and bile acids too. Fat intake varies according to eating habits of the species. Variations are seen in lipid content during different phases of reproduction in both the species of mynas. The parallel trend for hepatic total lipid and cholesterol content in male mynas of both the species and female Bank myna with maximum total lipid during breeding may be testosterone and estrogen mediated. Elevated plasma testosterone levels have been reported during breeding season for male and female individuals of both the species in our laboratory (Sapna, 2002). In female Brahminy myna decreased hepatic total lipid and cholesterol (non significant) during breeding may be attributed, in addition to hormone synthesis of steroids, to the increased energy demands for egg production as well as for supporting males in nest guarding. The fall in intestinal lipid reserves in male Bank myna during the breeding phase is probably due to the change of diet and increased cholesterol content reflects the synthesis of the precursor for steroidogenesis, as intestine is also a site for cholesterol synthesis (Griminger, 1986). A non-significant rise in intestinal total lipid of the opposite sex of this species suggests its role in uptake of dietary fats, along with the protienaceous insectivore diet decreasing the proportion of fats in diet, and incorporation of the former into protomicrons (Griminger, 1986) for subsequently laying down in yolk material. Parallel trend in renal total lipid content during breeding in both the sexes of Bank myna and Brahminy myna is probably due to similar pattern of diet during the breeding phase. The cholesterol content of both the sexes of Bank myna exhibit similar pattern as that of total lipid whereas species-specific differences are recoded for Brahminy myna.

The data taken together, suggests that different pathways of carbohydrate metabolism are in an active state during breeding contributing to energy economy of the bird. All the tissues studied were found to be contributing to the species specific and sex specific energy demands during the various phases of the reproductive cycle. Liver and kidney are involved in release of glucose, liver mainly by glycogenolysis and kidney by getting gluconeogenically active. Whereas the intestine is actively involved in secretion, digestion and absorption of the carbohydrate, fat and protein rich food. The type of food consumed during different phases of reproduction was also found to be influencing the carbohydrate metabolism, fulfilling the energy requisite of the birds. The present study also supported the increased energy demands of a solitary nester as compared to the colonial breeder, which is advantageous in terms of energy conservation.