~ DISCUSSION ~

GLYCOGEN

All physiological activities involve energy expenditure and breeding activity is no exception. Seasonal breeders that perform all activities related to breeding like territory establishment, nest building, egg laying, incubation and nurture of young during short favourable season of the year require tremendous energy and hence show metabolic adaptations. These metabolic adaptations are programmed so as to meet the exigencies of gonadal activation and general body functioning during breeding (Ramachandran and Patel, 1987). Metabolic reserves of body are generally built up prior to seasonal breeding (Perrins and Birkhead, 1983). Carbohydrates are the immediate and most important metabolic reserves utilized by the animal for various activities of which the glycogen is a stored polysaccaride form. The fate of dietary carbohydrate is either to get metabolized to carbon dioxide and water, or to get polymerized and stored or to be converted into lipoid moieties in liver (Hazelwood, 1986). Though glycogen stores of extra hepatic tissues can sustain small-scale requirements, hepatic glycogen stores are the major glycogen pools of energy and are catabolised by a cascade of reactions acted upon by different enzymes of carbohydrate metabolism. These should get depleted during large-scale protracted periods of seasonal breeding activities. In the present study carbohydrate metabolism over various phases of reproductive cycle in three tissues, liver, intestine

and kidney of both the sexes of two omnivore species of birds Bank Myna, *Acridotheres tristes* and Brahminy Myna, *Sturnus pagodarum,* is studied. For the convenience of discussion glycogen content is taken first followed by different enzymes of carbohydrate metabolism.

Breeding activities should bring about alterations in hepatic and extra hepatic tissue glycogen stores. In the present study, the males of both the species of mynas unveil accumulation of hepatic glycogen (Table 1a,b) during the pre - breeding phase which is used up during the breeding phase, similar changes also occur in pigeon, *Columba livia* (Ramachandran and Patel, 1987; Patel *et al.,* 1988). This bear testimony that onset of breeding activities place heavy demand on energy provisions of the body, leading to utilization of stored glycogen. Male individuals of both the species also exhibit lowering of body weight (Table 11), which is highly significant in Bank myna compared to Brahminy myna. Testosterone is reported to influence hopping activity and food intake with decrease in body mass in White Crowned Sparrows *Zonotrichia leucophrys gambelii* (Wikelski *et al.,* 1999) and in male Bank myna and Brahminy myna also, during breeding phase, when testosterone levels are high (Sapna, 2002) body weight showed a decline. Here testosterone levels were higher in Bank myna compared to Brahminy myna, supported by highly significant decrease in body weight of Bank myna compared to Brahminy myna, which had steady increase as well as decline in plasma testosterone level and non-significant decline in body weight over the cycle. In a social

breeder Jungle babbler *Turdoides striatus* having long period of readiness to breed, testosterone titers are still lower with no significant difference in the body weight of breeding and non-breeding birds (Bharucha, 2002). 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 and 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 and body weight (Table 11) is also depleted. The total lipid content of liver, kidney and intestine also exhibit supporting changes (Table 9b). The female Bank myna, which feeds on human leftover oily food (Parasara *et al.*, 1990; personal observation) with insects, exhibits increase in lipid content whereas Brahminy myna, which exclusively feeds on insects, with seasonally, feeding on plant matter, during breeding (Simwat and Sidhu, 1974; Narang and Lamba, 1984) exhibited significantly low lipid contents.

Thus the difference in glycogen content of the two species may be associated with their nesting as well as feeding habits. Brahminy myna is a solitary hole nester, feeding purely on insects and fruits whereas Bank myna along with insects and fruits devours human left over oily food and hence the difference in the energetics associated with breeding activities are amplified. This species being a colonial nester, the flock defends the colony. Female of Bank Myna has been

reported to spend maximum time in foraging during laying with minimum priority to nest building, resting and self maintenance while during the incubation period they spend least time for foraging and more time for incubation (Simwat and Sidhu 1974; Khera and Kalsi, 1986). The increase in foraging time is to compensate the increased energy demands for producing eggs of fairly large sized clutch during the laying period. The male spends less time for incubation and maximum time for foraging, resting, maintenance activities, and agonistic behaviour, during this phase. Accumulation of glycogen during breeding phase in female Bank myna can be the result of resting, as resting and incubation are considered behaviourally and energetically similar. Maxon and Oring (1980) suggested that, the bird could rest while sitting on nest for incubation. Energy utilized during incubation by bird is equal to time spent for resting during other phases of their life cycle under similar environmental conditions (Custer and Pitelka, 1972; Siegefired *et al.*, 1976; Walsberg and King, 1978; Mugaas and King, 1981; Grant and Wittow, 1983). A decline in hepatic glycogen by 28% and 15%, in male and female birds of Bank myna respectively, during the next consecutive post - breeding season, suggest extensive use of glycogen reserves for post breeding activities like nurture of the young. At the end of the cycle when energy demands decrease, with the cessation of breeding activities glycogen content in both the sexes are almost maintained to that recorded during post breeding seasons. In both the sexes of Brahminy

Myna, no such difference was noted, suggesting that this species, being a solitary hole nester, accumulates glycogen pools during pre - breeding and utilize them for different breeding activities over the rest of the cycle.

Increase in intestinal and renal glycogen content in males of Bank myna during breeding 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. Kidney is now recognized as an important organ for inter-organ glucose metabolism and renal glucose release is reported to be of the same order of magnitude as splanchnic glucose release during post absorptive period (Cano, 2001). The decrease in renal glycogen during post – breeding can be attributed to the change to omnivorous diet of these birds after breeding is over. In the female of this species, which spends less time in foraging, while incubating intestinal carbohydrate deplete during breeding, and the females probably feed occasionally on lipid rich diet resulting in activation of gluconeogenic pathway leading to accumulation of glycogen in liver and kidney. At the end when fledgling leave the nest energy demand decreases hence, no accumulation of glycogen content in all tissues of male and female Bank myna is seen.

The depleting intestinal and renal glycogen content during breeding in both the sexes of Brahminy Myna, with simultaneous non significant increase in lipid (Table 9b, Figure 9b) and protein (Table 8b; Figure 8b) content indicates dependence on proteinaceous and lipid rich foods with higher involvement of kidney in releasing glucose by gluconeogenic pathway too, to provide energy for increased monogamous breeding activities. In this species accumulation of hepatic glycogen in female, is higher compared to the female of Bank myna, further reflecting the difference in their nesting habits. Omnivore species have been reported to feed on plant matter during non-breeding season and switch over to insectivorous diet during breeding phase (Mathew, 1976; Anderson, 1972; Mathew, 1986; Toor and Saini, 1986; Parasara, *et al.,* 1990; Patel *et al.*, 1992). The Bank myna feeds on anything and everything available throughout the year but decreases feeding in large flocks near railway stations and restaurants during breeding season (Parasara rt al., 1990) thus increasing animal matter in the diet in the form of insects. Brahminy myna is reported to include plant matter in their diet during a period that coincides with breeding season (Narang and Lamba, 1984). Hence, diet of Bank myna during breeding season is rich in protein, which may be serving as precursor for gluconeogenesis. This machinery is usually switched on during the starvation or when the diet contains low carbohydrate (Mehta, 1985). After this phase the glycogen levels are depleted and are maintained till non-breeding

indicating cessation of gluconeogenic pathway and switching over back to omnivorous food habits after breeding.

GLYCOGEN PHOSHORYLASE

The glycogen phosphorylase (GP) is a key enzyme for degradation of glycogen *i.e.* glycogenolysis. It is an important extramitochondrial cytoplasmic enzyme that catabolizes the initial reaction in glycogenolysis. There are two types of phosphorylase *viz.* muscle phosphorylase and liver phosphorylase. Muscle phosphorylase is immunologically and genetically distinct from that of liver (Mayes, 2000) and both the type of phosphorylase exist in active as well as inactive forms. As GP is a rate-limiting enzyme in glycogenolysis, several studies on significance of carbohydrate metabolism have been carried out with the help of fluctuations in the activities of enzymes concerned. (Susheela & George, 1966; Patel *et al.*, 1983; Joseph and Ramachandran, 1992; Bollen *et al.*, 1998). Glycogen phosphorylase metabolizes glycogen, producing glucose-1-phosphate (G-1-P), which can be used for ATP production. (Biron & Graves, 2000).

The glycogen metabolizing enzymes have properties that enable the liver to act as a sensor of blood glucose and to store or mobilize glycogen according to the peripheral needs (Bollen *et al.*, 1998). Liver responds directly to changes in circulating glucose concentration with

reciprocal changes in glucose production, which will indirectly affect glycogenolysis. An increased GP level indicates increased glycogenolysis (Cahill *et al*, 1957) resulting in release of glucose for various energy needs. In the present study, the increased hepatic GP activity (Table2a) in male Bank Myna, during breeding phase indicates increased glycogenolysis corroborated by decreased glycogen pools (Table 1a, Figure 1a) during same period for the extra hepatic tissues, to meet the general energy requirements associated with breeding activities. The energy stored during pre – breeding, is utilized by male in establishing territory & selection of mate as well as nest site. However in the other species *i.e.* male Brahminy Myna also hepatic GP showed increase, and the increase here was more pronounced than in the Bank myna along with pronounced decrease in glycogen level during breeding phase. Metabolic adaptation and the concentration of enzyme in liver are greatly influenced by the dietary preferences (Shah *et al.*, 1972). The solitary nester has to defend the territory more vigorously than the colonial nester and hence more energy is required. Occasionally Brahminy myna has also been observed competing with House Sparrow, *Passer domesticus,* for nesting site. Males of both the mynas exhibit similar pattern in the hepatic total lipid content (Table 9a, Figure 9a) with accumulation of lipids during breeding season. The percentage increase is higher in Bank myna compared to Brahminy myna. Both the species are omnivore but Bank myna feeds on

everything available whereas Brahminy myna prefers either frugivorous or insectivorous diet.

The female Bank Myna showed different pattern of hepatic glycogen metabolism by exhibiting different GP activity, compared to male Bank myna with 28% decrease in GP and 4% increase in glycogen content. As said earlier resting and incubation are behaviorally and energetically similar and 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. Total lipid (Table 9a; Figure 9a) and cholesterol (Table 10a; Figure 10a, b) content also increased in females of Bank myna during this period. Peak in hepatic total lipid and cholesterol may be ascribed to the reproductive hormones, testosterone, estrogen and progesterone that are known to enhance lipid synthesis in liver (Griminger, 1986) and elevated levels of testosterone and progesterone occur in female Bank myna during this phase of breeding cycle (Sapna, 2002). Further, in female birds yolk lipids are exclusively synthesized in liver and transported to developing ova in the ovary (Bell and freeman, 1971; Griminger, 1986; Moran, 1987). The breeding season of Bank myna coincides with the onset of monsoon, increasing the insect population extensively and as the diet of Bank myna keeps on changing with the change in seasons, crop rotation, fruiting season, emergence of various groups of insects available over the year (Narang and Lamba, 1984). They feed on more insect matter during this period.

Protienaceous diet also increases the cholesterol synthesis in liver and intestine of young chicken (Grimminger, 1986). This also could be the reason for increase in hepatic and intestinal cholesterol content influencing increase in total lipid content. In the other species, *i.e.* Brahminy myna, sharing all the reproductive activities with the partner almost equally, no sex specific difference is noted except variation in magnitude of GP activity and glycogen content. However, increase in intestinal cholesterol whereas decrease in hepatic cholesterol of this species supports the consumption of mixed diet with increase in plant matter during this period of the year (Narang and Lamba, 1984).

Though male Bank myna is known to spend more time in feeding (Khera and Kalsi, 1986) during breeding, it may not be consuming more quantity, which is reflected by non-significant decrease in intestinal GP and with significant increase in intestinal glycogen content. While female needs more nourishment for production of egg, hence, probably consumes more food in short spells of feeding, indicated by increase in intestinal GP activity with decline in intestinal glycogen, to provide energy for increased peristaltic movements of the intestine. In the Brahminy myna male, there is opposite trend where male also needs more energy to defend the territory and thus probably consumes supplementary food. Female Brahminy myna also shows similar trends to that of male but to a lesser magnitude, as both the sexes support each other in breeding activities. The clutch size of Bank

myna is 3 to 5 eggs per brood whereas in Brahminy myna it is 3 to 4 eggs per brood.

Another vital organ of the body, the kidney, probably contributed to the accelerated need of glucose by increasing glycogenolysis in Brahminy myna only during breeding. However, Bank myna exhibited decline in renal GP level, it can be suggested that in Bank mynas, the energy released from liver, in the form of glucose, by enhanced glycogenolysis is sufficient for the bird to carry out different breeding activities, hence an opposite trend to that of Brahminy myna in GP activity and glycogen content is noted during this phase. However, in the subsequent phase when there is glycogen depletion in liver, kidney probably joins the liver and provides glucose by glycogenolysis, indicated by elevated renal GP activity during post – breeding in both the sexes of Bank myna. In Brahminy myna, kidney probably contributed to the accelerated need of glucose in both the sexes exhibiting similar trends in GP activity to that of liver all throughout the breeding cycle. In both the sexes of Brahminy myna, kidney could be supplementing some amount of glucose by gluconeogenesis. In birds kidney is known to be gluconeogenic organ (Watford *et al.*, 1981; O'Neil and Bannister, 1986; Yorita *et al.*, 1987; Watford, 1989; Christensen *et al.*, 1999) next to liver. Evaluation of organ specific glucose release showed that renal glucose release is of the same order of magnitude as splanchnic glucose release during post absorptive period (Cano, 2001) and a recent findings of the same

author says that renal gluconeogenesis substantially participates in post absorptive glucose production, and that its role in glucose homeostasis is of first importance (Cano, 2002).

GLUCOSE-6-PHOSPHATASE (G-6-Pase) ~

Glucose-6-phosphatase (G-6-Pase) is an important endoplasmic reticular enzyme, which hydrolyses or dephosphorylates glucose-6-phosphate (G-6-P) to glucose and phosphate, releasing the former into the blood stream, and is an important enzyme in intermediary metabolism. G-6-Pase catalyses the terminal step of both glycogenolysis and gluconeogenesis (Mayes, 2000). It occurs mainly in glycogenic tissues such as liver, where it plays an important role in synthesis of glucose, the immediate source of energy and a carbohydrate essential for tissue functioning.

Significantly high G-6-Pase (Table 3a, 3b; Figure 3a, 3b) along with increased GP (Table 2a, 2b; Figure 2a, 2b) and diminished glycogen (Table 1a, 1b; Figure 1a, 1b) content during breeding phase in the males of both the species, *viz.* Bank myna and Brahminy myna, evince increase in body metabolism and activity in conjunction with reproductive functions. High percentile (83%) rise in Brahminy myna

compared to Bank myna (60%) may be due to their non-identical nesting habits. The former being a solitary hole nester in which energy expenditure for different breeding activities including defense of nest, egg and young one is expected to be high. Feeding on high fat diet and high protein intake stimulates G-6-Pase activity and lowers glycogen levels (Rosebrough and Begin, 1976; Garfield and Cardell, 1979; Donaldson and Christensen, 1991) and both the species of mynas studied are also known to consume good amount of protein rich diet along with carbohydrates. Hence, intense G-6-Pase activity in both the male mynas. Decline in G-6-Pase activity after breeding in male Brahminy myna may indicate replenishment of exhausted metabolic resources of the body. Whereas the continuous rise in hepatic G-6-Pase in male Bank myna from breeding to post - breeding may be ascribed firstly, to its feeding habit, as it is seen to be feeding on oily human left over and also seen feeding near restaurants on the railway stations in large number before and after breeding seasons (Parasara *et al.*, 1990). Secondly, could be due to elevated energy requisite for post - breeding activities.

Low G-6-Pase activity in female Bank myna, during breeding might be due to diminished glycogenolysis, which is confirmed by low GP (Table 2a) activity too. As the females of this species spend maximum time in incubation and hence energy expenditure is reduced to minimum. A converse swing, in female of the other species was observed compared to Bank myna female but in accordance to that of

the male of the same species, indicates that in the Bank myna, the colonial nester, post – breeding activities are probably prolonged compared to Brahminy myna which tries to finish breeding activities earlier (Padate, 1990) which may reduce the pressure of predation on the young (Welty, 1986) of the individual nester.

SUCCINATE DEHYDROGENASE (SDH) AND ADENOSINE TRIPHOSPHATASE (ATPase)

Changing energy demands associated with seasonal reproductive cyclicity, should involve alterations in the operations of oxidative pathways coupled with energy metabolism. To understand the degree of elicitation of energy metabolism, a study on the seasonal changes in activities of Succinate dehydrogenase (SDH), the key enzyme of Krebs cycle and Adenosine Triphosphatase (ATPase) the hydrolytic enzyme were carried out. SDH, an important mitochondrial marker enzyme, catalyses the stereo specific dehydrogenation of succinate to fumarate in Krebs's cycle. Shifts in energy balances and synthesis of different macromolecules are often result of synchronized and controlled activities of dehydrogenase in a living system and SDH being one of them is an important enzyme to evaluate the energy

competence of tissues and processes. The activity of SDH is an index of oxidative metabolism which involves production of energy rich ATP molecules, requisite for synthetic processes *viz.* glycogenesis, lipogenesis etc. Thus, TCA cycle replenishes the supply of energy rich ATP molecules as substrate for the enzyme ATPase to act upon and release utilizable free energy. ATPase is a group of enzymes localized in various cell organelles functioning at different pH optima. It is actively involved in driving catabolic and energy utilizing reactions with involvement in high-energy phosphate metabolism. An active synthesis of ATP and its enzymatic hydrolysis normally run parallel to each other in an organ or tissue that is engaged in synthetic processes (liver), active transport (Intestine and kidney) and reabsorption (kidney and intestine).

Elevated hepatic SDH (Table 4a, b) and ATPase (Table 5a, b) 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. The liver is main organ involved in the synthesis of essential metabolites and liver metabolism has to show varying degrees of adjustment to meet the exigencies of energy generating (TCA cycle) pathway. As during breeding phase energy utilizing reactions are operative increase in hepatic ATPase and SDH is the result. Carbohydrate rich food intensifies TCA cycle in the liver (Patel *et al.*, 1976; Patel *et al.*, 1979). Bank myna is known to feed on mixed

diet all throughout the year whereas Brahminy myna increases plant matter in their diet during this phase (Narang and Lamba, 1984). This is reflected by higher ATPase and SDH levels in the males of Brahminy myna during breeding phase. Accumulation of lipid (Table 9a, 9b) noted in both the species during this period indicated dependence on carbohydrate metabolism for energy releasing processes. Birds have been reported to evaluate the importance of a specific feeding schedule as a zeitgeber, either from temporal information on duration and type of daily food access or from energetic consideration (Hau and Gwinner, 1996).

During post - breeding phase species-specific differences are noted between the two species. Here Bank myna, the colonial nester with bulkier size and larger brood shows higher ATPase and SDH activities compared to the Brahminy myna male. Young ones of Bank myna probably depend on the parents for longer duration than the young ones of Brahminy myna. Brahminy myna, the individual nester, is probably more susceptible the environmental hazards including predation hence the young ones develop faster and become independent earlier than the young ones of Bank myna. Natural selection has shortened the nestling period for species that are most susceptible to natural hazards (Welty, 1990) and hence the difference in the energy expenditure by the parents and differences in energy metabolism of parents of the two species.

A parallel trend in hepatic SDH and ATPase levels in both the sexes of Brahminy myna indicates equal participation of both the sexes of solitary nesters in breeding activities. Increased SDH and ATPase activities in all the three tissues studied during the breeding phase indicates involvement of all the tissues in energy metabolism with intestine involved in increased digestion as the amount of plant matter consumed increases (Narang and Lamba, 1984). The demand of energy increases for absorption of food and active transport in intestine and in liver for general metabolism whereas in kidney for enhancing the elimination of metabolic end products. This can be envisaged by decrease in glycogen pools (Table 1b) during the same phase.

The females of Brahminy myna, exhibited a similar trend in the enzyme activity of liver to that of the males. The only difference being in the total lipid content (Table 9b) that exhibits depletion along with the glycogen content, which probably might be utilizing the lipid reserves for the increased energy demands for egg formation and laying. According to Ricklefs (1974) energy cost of egg production may impose considerable nutritional demands on the female birds and they meet these demands in various ways. The Pintail ducks (*Anas acuta*) utilize body fat and eat high protein diet (Wihttow, 1986). This view was contradicted by Durant *et al.* (2000), who stated that the nutrients and energy requirements during egg formation could be obtained without modification of daily food intake. However, these

authors indicate increase in energy demand during egg formation. The metabolizable energy is also significantly high during egg laying in Zebra Finch (Whittow, 1986). Probably in female Brahminy myna the total lipids that are being used as a source of energy, undergo lipolysis to produce fatty acids, which undergo β-oxidation to form the Acetyl-CoA, that enters the TCA cycle to generate more energy rich ATPs to release free energy on hydrolysis. The above mentioned pathway may also be operative during the post - breeding phase in both the sexes of Bank myna, for the high cost of energy expenditure during post - breeding activities *viz.* feeding and taking care of the fledglings (Muggas and King, 1981; Ettinger and King, 1980) The SDH and ATPase activities are high despite of low hepatic glycogen content in (Table1a) Bank myna female. This probably suggests active oxidative metabolism, through lipolytic pathway, resulting in high-energy phosphate for metabolism by the enzyme ATPase.

Intestinal SDH and ATPase levels of both the species exhibit a parallel trend in both the sexes of Brahminy myna and opposite trend in both the sexes of Bank myna during the breeding phase. In Bank myna dependence seems to be more on stored energy of liver during pre- breeding phase whereas during breeding season on the food available as probably food consumption increases with increase in motility of intestine needing more energy and hence increased SDH and ATPase activities. Male Bank myna is known to forage for longer duration during this period (Khera and Kalsi, 1986) compared to

female. This is envisaged by increase in lipid content in male birds during breeding season. The extra amount of energy consumed is probably converted to lipids (Table 9a). Padate (1990) has shown increase in body weight also during this period, which is significant in female birds. However in female birds also increase in hepatic and intestinal lipids occur (Table 9a). Yolk granules are synthesized in liver and transported to ovary (Bell and freeman, 1971; Griminger, 1986; Moran, 1987). Female Bank myna spends less time in foraging during breeding season hence less energy required for motility of intestine and resulting in decrease in SDH and ATPase activities indicating poor energy demand. In female Bank myna the free energy is derived from stored metabolites in liver during breeding phase and not during pre-breeding as noted for the male birds. During breeding phase female Bank myna spend more time for incubation that can be considered equal to resting (Maxon and Oring, 1980). As the cycle ends male and female individuals of Bank myna show parallel trend from post-breeding to non- breeding seasons.

The renal tissue shows species-specific differences but sex specific similarities. During breeding phase of the reproductive cycle in Brahminy myna where more energy is required kidney probably functions at enhanced rate in excreting the metabolic wastes for which more energy is utilized and the trend continuous during post – breeding phase. In the colonial nester comparatively the energy requirement is lower generating less metabolic waste and resulting in

TCA cycle operating at a lower rate and slow hydrolysis of ATP by ATPase when compared to solitary nester.

ACID PHOSPHATASE (AcPase) AND ALKALINE PHOSPHATASE (AlkPase)

Phosphatases are involved in various aspects of cellular metabolism. They are classified into phosphomonoesterase, phosphodiesterase and pyrophosphatases of which phosphomonoesterases *viz.* acid phosphatase (AcPase) and alkaline phosphatase (AlkPase) have wide distribution and known to occur in almost all tissues. As early as 1946 Moog reported the involvement of AcPase and AlkPase as phosphomonoesterases subserving variety of processes requiring the mobilization of phosphate radicals or entail dephosphorylation as steps in metabolism and transport of ions. Since they hydrolyse number of phosphate esters, they are termed as non-specific phosphatases. They are associated with several prominent functions but because of their non-specificity it is intractable to ascertain specific roles played by either acid or alkaline phosphatase in a particular tissue or cell. AcPase is active at an acidic pH whereas the

AlkPase is active at an alkaline pH. The later is membrane bound enzyme, stably anchored at the cell surface by covalent linkages and is involved in the transport of metabolites across the cell membrane acting as both transferase and hydrolase according to the site of localization. Non-specific phosphatases are known to be versatile in their functional association with many biological processes *viz.* absorption, secretion, cellular phagocytosis, protein synthesis etc. Their role in nutrient transport across the mucosa of alimentary canal of White rock cockerels (Majumdar *et al.*, 1988), carbohydrate metabolism (Rosenthal *et al.*, 1960), and in many phosphorylating reactions have been reported. In the present study quantitative estimation of nonspecific AcPase and AlkPase in three tissues *i.e.* liver, intestine and kidney have been carried out with a view to understand the changes in the pattern of enzyme activity in the two omnivore species with difference in food preference and nesting habits over their reproductive cycle. The enzymatic adaptations are some of the prerequisites for change in tissue metabolism during reproduction.

In the present study both the phosphatases exhibited parallel trends with species-specific differences in all the three tissues studied. The sex specific differences being only in amplitude of both the enzymes. 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. This enzyme involved in calcium metabolism (Dupuis *et al.*, 1990) and carbohydrate metabolism (Rosenthal *et al.*, 1960), exhibited sex specific prominent variations in the magnitude in the colonial nester with larger clutch whereas in the individual nester with smaller clutch these differences were of lower magnitude.

Liver is the major organ for inter-conversion or synthesis of metabolites as well as storage of fat. A very low activity of AlkPase occurs in mammalian liver (Wachstein, 1963). However among birds the omnivores with gall bladder show presence of moderate AlkPase activity in the peribiliary zones of liver and lower AcPase activities compared to AlkPase in only parenchymal and periportal zones (Shah *et al.*, 1972) whereas quantitatively higher AcPase activity compared to AlkPase activity has been reported in developing avian liver (Moog, 1965). In the present study also both the enzymes were low in liver when compared to intestine, however AcPase was higher than AlkPase. Sex specific differences noted for Bank myna in hepatic AcPase and AlkPase were higher levels during post-breeding season in females. Female is probably compensating for the energy expenditure or low feeding activity of the previous season. Increase in 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 also with reference to lipids, (Table 9a, 9b) and glycogen, (Table 1a, 1b) levels showing more dependence on non-carbohydrate diet. Brahminy myna consumes more plant matter in their diet along with insects during breeding season (Narang and Lamba, 1984), hence influx of carbohydrates, and increased activity of AcPase is recorded. Ayyar (1987) has also reported higher AcPase in liver of breeding pigeons, a graminivore species. Continuous decline in hepatic AcPase from post breeding to non breeding in this species may be attributed to increase in protein and fat in diet, the fact also reported by Shah *et al.*, (1975) in Wagtail and this is supported by rise in protein content (Table6b) and total lipid content (Table 9b) of Brahminy myna during non breeding season.

Kidneys are fairly rich sources of AcPase and AlkPase enzyme (Moog, 1946). High concentration of AlkPase in mammalian kidney has been associated with reabsorption of sugar from the tubules by phosphorylated mechanism (Moog, 1946; Bradfield, 1950). Whereas AcPase that is also concerned with intracellular digestion of protein (Mortimore and Poso, 1984) seems to supply gluconeogenic avian renal tissue with the supply of amino acids to produce energy from non-carbohydrate sources. Kidney is a major gluconeogenic organ for highly metabolically active organisms like birds (Watford *et al.*, 1981; O'Neil and Bannister, 1986; Yorita *et al.*, 1987; Watford, 1989;

Christensen, *et al.*, 1999). Between the two species studied, 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 as well as intestinal AcPase and AlkPase levels also increases. In the other species, Bank myna, this situation probably occurred during post-breeding season when both the parents started taking care of the young and simultaneously feeding in larger flocks on non-carbohydrate food. This is supported by variation in the levels of protein content in the kidney too (Table 8a).

No sex specific differences in AcPase and AlkPase were recorded over the reproductive cycle in both the species of mynas.

PROTEIN, TOTAL LIPID AND CHOLESTEROL

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. The 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 also serve as source materials 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 major organ for synthesis and

degradation of cholesterol and intestine is another site for cholesterol synthesis. Pathways of carbohydrates, 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.

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, 1986). 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). TCA cycle, an important pathway in carbohydrate metabolism for liberation of free energy is also common for fat and protein metabolism. The efficiency of energy retention is more in fat compared to protein (Boekholt *et al.*, 1994).

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). As these hormones enhance synthesis of liver lipids (Griminger, 1986) and cholesterol being a precursor for steroid hormones, both exhibit elevation during breeding season. In female Brahminy myna decreased hepatic total lipid and cholesterol (non-significant) during breeding may be attributed in addition to hormone synthesis of steroids, increased energy demands for egg production as well as for supporting males in nest guarding. In the other species the nest guarding is collective reducing the energy expenditure by all the individuals of a nesting colony. In female Brahminy myna the additional energy required may be supplied by the degradation of lipids. The declined cholesterol content, in female Brahminy myna although is non significant, is puzzling at this juncture. Female birds of both the species show parallel testosterone and progesterone levels (Sapna, 2002) and according to Padate (1990), female birds show late recrudescence compared to male birds. Bank myna male show sudden rise and fall in testosterone levels whereas in Brahminy myna male this rise is slow and subdued (Sapna, 2002). A further decline in all the metabolites in the successive phase is exhibited as the females of this species need the extra energy by probably playing a major role in feeding the fledglings (Padate, 1990) and males with higher levels of testosterone levels are still busy in defensive activities. Testosterone is known to be responsible for aggressive behaviour in birds (Hegner and Wingfield, 1986; Schlinger and Callard, 1990; Wingfield *et al.*, 2001). Probably

the evaluation of estrogen levels in male and female birds of both the species may throw more light on this difference. Both the sexes of Bank myna also exhibit a similar pattern in hepatic total lipid content with decline during post breeding phase. Bank myna are known to increase their feeding activities at railway station during this phase by feeding on oily food wastes (Parasara *et al.*, 1995) hence, this can be one of the probable reasons the decline as increased fat in diet decreases lipogenesis (Rosebrough and Steele, 1985; Griminger, 1986; Rosebrough *et al.*, 1999) by reducing the precursors for fat synthesis.

The fall in intestinal lipid reserves in male Bank myna during the breeding phase is probably due to the change of diet as its feeding activities are mainly restricted in the neibourhood of nesting colony and the numbers seen near railway station and food vendors decreases during this period (Parasara *et al.*, 1995). The increased cholesterol content in intestine reflects the availability 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, with the protienaceous insectivore diet which is not as high as in fats, and incorporation into protomicrons (Griminger, 1986) for subsequently laying down in yolk material. In the male Brahminy myna, preserved total lipid and cholesterol content during post-

breeding and non-breeding phases are probably result of being a solitary nester spending more energy for breeding activities, and thus accumulation of any of the metabolites is not recorded, as their synthesis and utilization are as per energy needs and are parallel. In the females of this species the significant decline in intestinal total lipid content during breeding phase may be attributed to speedy utilization of lipoid reserves for the transport in developing ova whereas high cholesterol may be due to higher rates of synthesis by the intestine. And, as this species is reported to start its breeding activities late as compared to the Bank myna (Padate, 1990), utilization of the precursor material is reflected in the declined intestinal cholesterol content during breeding phase.

The role of kidney in excretion of steroid hormones as well as cholesterol metabolites is a well-established fact. Parallel trends in renal total lipid content over the reproductive cycle but with species-specific differences in amplitude of total lipid content suggests the accumulation of lipids in the kidney of Bank myna female during pre-breeding and breeding season. This supports the earlier discussions suggesting female Bank myna spends more time in incubation, which can be considered resting, and thus needs comparatively less amount of energy towards various breeding activities. Both the species are known to feed on plant matter as well as insect matter rich in carbohydrate, proteins and fats during breeding. Kidney is an important organ involved in glucose homeostasis (Cano, 2002) and

kidney may take up the compensatory role in metabolic homeostasis according to the requirement. Increase in protein and lipid rich diet increases rate of gluconeogenesis (Mehta, 1985). The fall in total lipid content during the post-breeding phase may be due to utilization of renal lipid content for energy production as this phase is energetically costly as it includes feeding and taking care of the fledglings. The cholesterol content of both the sexes of Bank myna exhibits similar pattern to that of the total lipid whereas variations in Brahminy myna in renal cholesterol level are not parallel to those of lipids. This can be due to different food preference where Bank myna consumes more lipid rich food, which may show some accumulation in kidney too, to be utilized for post breeding activities as said earlier. The continuous decline in renal cholesterol content from breeding to post breeding season in Brahminy myna, may be ascribed to synthesis and simultaneous utilization as a precursor for steroid hormones and hence, not available for the kidney to be excreted in large quantities while in the other species which starts its breeding activities early as compared to Brahminy myna (Padate, 1990) the utilization of the precursor for the active synthesis of steroid hormones might have subdued and increased cholesterol synthesis due to increase in protienaceous food and for maintaining the hormone level throughout the breeding phase. In the consecutive post-breeding phase decrease in cholesterol content in both the sexes of Bank myna and Brahminy myna suggests utilization of the cholesterol for subdued hormone

metabolism (Sapna, 2002) which is maintained during non-breeding too.

Proteins exhibit species-specific oscillations in all the three tissues over the reproductive cycle in the males and females of both the species. Increased protein content during breeding can be attributed to cumulative increase in various enzyme levels and dietary proteins intake as discussed in earlier part of this study. 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. In mammals high protein diet is known to increase glomerular filtration rate (GFR) and cause renal hypertrophy (Bouby, 1988; Kayson, 1989). In birds, kidney is not only highly efficient excretory organ, excreting highly concentrated nitrogenous wastes in the form of uric acid but also a very efficient gluconeogenic organ (as discussed earlier with reference to glycogen phosphorylase). Hence, when there is high demand of energy, increase in uptake by kidney for gluconeogenic supply of energy resulting in decreased protein content during the subsequent post-breeding phase. That can be summarised as change in kidney function rate during high-energy demand as also reported in rats by Hammond and Janes, 1998.