GENERAL DISCUSSION
Results of the present study clearly demonstrate the influence of environmental factors on the life cycle of the silkworm, *Bombyx mori* from egg hatching to adult eclosion. The influence was quite perceptible on the growth parameters of the 5th instar, biochemical constituents as well as economic aspects. This investigation is expected to pave way towards a greater understanding of the intricate interactions between the silkworm and the environment, with special reference to photoperiodism, temperature and relative humidity. A review of the results on all aspects examined in the present work reveals the following salient features.

**Hatching:** In the present study, all the three silkworm races (PM, NB4D2 and PM x NB4D2) have expressed their peak activity of hatching in the early hours of the day, *i.e.*, just after lights-on, thus implying a diurnal pattern of hatching. The occurrence of hatching during early hours of the day might be due to the relatively low temperature and high humidity in the micro/macro environment, which minimizes 'the risk' of desiccation, as demonstrated for *Drosophila* eclosion (Pittendrigh, 1966). Under low temperature (25°C) and high humidity (80%), all the three races showed hatching on a single day. Except NB4D2, the other two breeds/hybrids (PM and PM x NB4D2) showed hatching for two consecutive days, the duration between the two peaks being around 24 hours. The occurrence of hatching for two consecutive days in PM and PM x NB4D2 and the two observed peaks being 24 hours apart from each other in the present study strongly suggest the gating phenomenon of hatching. This is further supported by long hatching durations (of around 24 hours). The situation with NB4D2 offers a different argument that the breed being a temperate bivoltine one (Jolly, 1987) cannot withstand high temperatures and low humidities of tropical region. The hypothesis is strongly supported by less hatching percentage under high temperatures and low humidities. The observations on the unhatched egg
percentage again supports the above that the hatching in NB4D2 is very low under high temperatures and low humidities, perhaps the environmental conditions induce mortality of the incubated eggs, allowing no living egg to utilize the next gate(s). Murai (2000) reported that high temperature coupled with low humidity inhibits the egg development in *Thrips tabaci*. Similarly, Katayama (1997) reported very low hatchability in *F. accidentalis*. In other words, the tropical multivoltine breed (PM) and the hybrid (PM x NB4D2) can withstand high temperatures and low humidities to a certain extent to utilize consequent gate(s) for their hatching activity. The best hatching percentage, low hatching durations and low number of unhatched eggs are observed only at 25°C temperature and 80% relative humidity, perhaps following the phenomenon of minimizing 'the risk' of desiccation, as demonstrated for *Drosophila* eclosion (Pittendrigh, 1966).

Thus, the present study demonstrates that incubation of silkworm eggs at 25°C temperature and humidity of 80% results in the best economical (Krishnaswami, 1986) hatching of over 90%. Temperature (and humidity?) did not alter the hatching rhythmic pattern in *B. mori*, as already viewed by Saunders (1982). However, it prolonged the hatching in the commercial silkworm, which is uneconomical (Krishnaswami, 1986). Therefore, extreme temperatures and humidities seem to exert synergetic effects on the silkworm, *Bombyx mori* through low, prolonged and uneconomical hatching, and hence are not desirable in the commercial silkworm rearing.

**Moulting:** In the present study, the observed patterns of rhythmicity in larval ecdysis, SM and CM under LD 12:12 conditions and the persistence of rhythmic pattern under all temperatures and humidity (experimental conditions) are worth noting. Sivarami Reddy (1993) and Sivarami Reddy *et al.* (1990) reported that the ecdysial rhythm under LD 12:12 condition persisted under all other photoperiodic conditions including continuous conditions. From the present study, it is clear that the rhythmicity of ecdysis persists under constant temperature and humidity.
conditions also. Considering the reports of Truman (1972), review of Beck (1980) and views of Sivarami Reddy (1993) and Sivarami Reddy et al., (1990), the present results indicate that moulting phenomenon could be a gated phenomenon (after fixed hours of PTTH release) in *Bombyx mori*. Most significantly, Truman (1972) found that though the release of PTTH is a gated event, the secretion of growth hormone (ecdysone and juvenile hormone) occurring subsequent to the release of PTTH in the moulting cycle was not gated. However, the synchrony of larval ec dysis appeared to be dependent solely on the gated secretion of PTTH. In the present study, the synchrony of CM appears to be dependent solely on SM. However, the subject whether the SM in *Bombyx mori* is the immediate response of PTTH or not would be of much interest (Sivarami Reddy, 1993).

The silkworm, *Bombyx mori*, being a poikilothermic insect, depends largely on the quality of food (mulberry leaf) and environmental conditions. The mulberry fed to the silkworm, in the present study, was of uniform in quality, i.e., all the silkworm batches received similar and uniform type of feed. Therefore, the observations reported need not be attributed to the feed. The productivity of the silkworm in terms of cocoon crop depends on several factors that operate within and outside the body of the silkworm. The main influencing factors being photoperiod (Sivarami Reddy, 1993) and temperature (Benchamin and Jolly, 1986, Shiva Kumar et al., 1997). The moulting durations and the moulting percentage, especially in the bivoltine silkworm breed (NB4D2), were more influenced by temperature and the interactions between temperature and humidity. Therefore, it can be concluded that the silkworm moulting patterns, moulting durations and moulting percent (otherwise called survival during moulting) are more influenced by temperature and humidity. The interactions between temperature and humidity have profound effects on the bivoltine silkworm, NB4D2.
Pupal eclosion: In the present study, the observed rhythmic patterns in larval ripening and pharate pupal formation and pupation under LD 12:12 and at all temperatures and humidity suggest the endogenous nature of the rhythmicity. The endogenously free-running nature and circadian control over the phenomenon have been demonstrated in *Bombyx mori* (Sivarami Reddy et al., 1993). All the three developmental marker events of the pupal ecdysis studied exhibited diurnal predominance. Hatching in *B. mori* was reported diurnal (Sivarami Reddy et al., 1984; Sivarami Reddy and Sasira Babu, 1990). Larval ecdysis was reported to be instar-dependent (Krishnaswami et al., 1973; Sivarami Reddy et al., 1984, 90; Krishnaswami, 1986). In the present study, the pupal ecdysis is diurnal, probably as a resultant of a long fifth instar stadium (around 8 days) (Truman, 1971; Sivarami Reddy, 1993; Sivarami Reddy et al, 1993).

Insects generally require an adequate level of environmental conditions in order to keep their physiological system normal (Mellors et al., 1984; Emori, 1976; Bauer, 1976; Hoy, 1975; Tsurumaki et al., 1999). Among the environmental conditions, temperature, relative humidity and photoperiod are the most important. In the present study, however, three temperatures (25, 30 and 35°C) and three relative humidities (60, 70 and 80%) were imposed based on the climatic conditions of the region. Pure Mysore (PM) breed gave a considerably higher pupation rate (%) compared to bivoltine breed (NB4D2). The productive bivoltine breeds are reported to be susceptible to high temperature and low humidity (Kato et al., 1989). This is further supported by the observation that pupation rate in NB4D2 is significantly influenced by both temperature and humidity. The pupation rate in the hybrid, PM x NB4D2 was still higher than that of the two pure breeds (parents). Suresh Kumar and Yamamoto (1995) have reported that the hybrids are more tolerant than the pure races.

Moth eclosion: Eclosion (%) has considerable importance in commercial sericulture. This is particularly important in seed organization, since the seed
production efficiency mainly depends on eclosion maxima. The multivoltine breed PM is indigenous to our country and more adapted to tropical conditions of India. Therefore, one can expect greater eclosion percent in this breed. The bivoltine breed, NB4D2 is mainly best suited to temperate countries like China and Japan, and they cannot sustain the conditions of high temperature and low humidity. Therefore, the eclosion is very low. The significant effects of both temperature and humidity and their interaction effects on eclosion clearly vindicate the hypothesis.

**Growth and biochemical constituents:** The results indicate that variations exist in the silkworm larval and silk gland growth and growth rates. In addition, biochemical constituents in the silk gland, like total proteins, total free amino acids and total carbohydrates varied to the maximum extent. These variations were categorized into variations among breed/hybrids and variations between rearing conditions. Sivarami Reddy (1993) reported shorter growth period coupled with highest growth rate for *Bombyx mori* under short-day condition, especially under LD 11:13. In the present study, however, only one photoperiodic condition, viz., LD 12:12 has been selected, which many workers consider as the short-day condition. In such a case, the observed differences in growth in terms of larval and silk gland weight and biochemical constituents of the silk gland cannot be attributed to photoperiod alone.

The variations in the 5th instar silkworm larval growth among the breed/hybrids such as PM, NB4D2 and PM x NB4D2 can be ascribed to their voltinism. Thus, PM being a multivoltine breed registered lower larval growth, whereas the bivoltine breed NB4D2 registered the highest growth. The hybrid, PM x NB4D2 registered intermediate growth in between PM and NB4D2. The silk gland growth also is viewed on similar lines. The important aspect is the differences in attaining peak weights in larval and silk gland weights. In the three cases, the larvae attained their maximum growth on the 6th day of 5th instar,
whereas the silk gland on the 7th day of 5th instar. This situation offers the logical possibility that the silkworm larva as a whole attains its highest weight much earlier than the silk gland. The silk gland is a specific organ for silk protein synthesis, and attainment of its maximum weight can generally be expected much delayed compared to the silkworm body weight. Therefore, the observed variations in body weights and silk gland weights can be attributed to the effects of temperature and humidity.

Among the environmental factors, temperature and humidity are reported to be the most important ones affecting the growth and development of insects. Murai (2000) reported that high temperature and low humidity inhibit egg development in *Thrips tabaci*. A low hatchability has been reported at 35°C for *F. accidentalis* (Katayama, 1997). There are reports on the nutritional efficiency of silkworm (Anantha Raman *et al.*, 1993). Reports are also available on feed efficiency (Anantha Raman *et al.*, 1994), effect of season and mulberry variety on the feed conversion efficiency (Anantha Raman *et al.*, 1995), ingestion, digestion and conversion efficiencies of silkworm (Benchamin and Jolly, 1984; Kanika Trivedi and Nair, 1999) etc. They indicate that silkworm varieties differ among themselves in terms of growth and development based on rearing temperature and humidity. Based on the numerous reports on the effects of temperature and humidity on *Bombyx mori*, Krishnaswami (1986) suggested a temperature of 25°C coupled with high humidity of 85% as optimum for the commercial silkworm rearing. Therefore, low growth under higher temperatures and low humidities and vice-versa are justified.

Sivarami Reddy (1993) reported that proteins in the silk gland did not exhibit dial variations, but cyclical variations spread over the entire length of the 5th instar, the peak being on the 7th day of 5th instar. The results on protein content in the silk gland reported in the present chapter are of 7th day of 5th instar, and day-to-day variations are not reported. Highest values of total protein content
in the silk gland of bivoltine (NB4D2) on the 7th day of 5th instar clearly indicate not only cyclical fluctuations, spread over the 5th instar period, but also its enhanced synthesis and thus accumulation of total proteins in the silk gland (Tazima, 1978). This again supported fluctuations in free amino acid and carbohydrate contents. Free amino acids in the silk gland serve as pool for silk protein synthesis (Prudhomme et al., 1985; Sehnal and Akai, 1980; Siva Prasad and Murali Mohan 1990; Sivarami Reddy, 1993). Carbohydrates might be used for energy purposes (Aschoff, 1964; Pavan Kumar et al., 1981; Sivarami Reddy, 1993), for synthesis of amino acids (Molloo et al., 1974) or for polysaccharides and carotenoid formation in the inner and outer layers of silk fibre, the sericin (Sehnal and Akai, 1990). Interestingly, lower levels of carbohydrate were observed under low temperature and humidity conditions. This suggests that more carbohydrates are needed for larvae under extreme high temperature and high humidity conditions for energy purposes or for amino acid biosynthesis to support the active silk synthesis.

Many environmental factors have been reported to show their influence on the growth and development of insects. Of these, photoperiodism has been extensively studied. Photoperiodism was shown to have profound effects on the growth and development of insects. In the commercial silkworm Bombyx mori also, photoperiodic effects were reported on larval growth (Hirasaka et al., 1969; Hirasaka and Koyama, 1970, 72; Sivarami Reddy et al., 1984) and on overall economic characters (Sivarami Reddy, 1993).

Economic aspects: The macro data on economic aspects of silkworm, like cocoon weight, shell weight, shell ratio, filament length etc. were analysed for E. l. values. Maximum values of E. l. (more than 50) were observed for all the silkworm breeds/hybrids reared at 25°C, irrespective of the relative humidity to which they were exposed. Index values for individual traits of different breeds/hybrids varied from -5.467 (for cocoon weight of NB4D2) to 162.362 (for
The inconsistency with regard to computed index value of individual economic traits indicated that the overall 'evaluation index' may agree, but the component trait may or may not (Table 6.6). The present findings revealed that not even a single breed/hybrid scored an index value of above 50 for all the economic traits. Upon computing for average E. I. values, however, all the breeds/hybrids reared at a temperature of 25°C, irrespective of relative humidity, alone recorded E. I. value of over 50 and emerged as the best performing breeds/hybrids. The other temperatures (as well as humidity conditions) can be differentiated as non-suitable environmental conditions for profitable silkworm rearing.

Knowledge on hormonal regulation of insect development and silk gland function is well utilized for controlled silk yield and fibre qualities. Use of juvenile hormone (Akai, 1982; Akai and Kobayashi, 1971; Akai et al., 1973, 85, 87, 88; Sehnal and Akai, 1990) and moulting hormone (Chou Wei-Shan, 1980) has been well documented. Synchronized egg hatching (Sivarami Reddy and Sasira Babu, 1990; Sivarami Reddy, 1993), larval-to-larval moulting (Sivarami Reddy, 1993; Sivarami Reddy et al., 1984, 90), pupation (Sivarami Reddy et al., 1984, 93a), moth emergence (Sivarami Reddy et al., 1984, 93b) larval and silk gland growth, protein accumulation in the 5th instar silk gland (Sivarami Reddy et al., 1984) were reported. In the present study (Chapters 1 to 6) the possibility of photoperiodic and optimum environmental conditions (temperature and humidity) were examined for economic silkworm rearing and to define the rearing...
conditions for Andhra Pradesh region in general and Anantapur area in particular.