Chapter - 4
Discussion
4.1 Behaviour

It is well known that the environmental conditions have tremendous influence on activity of any animal. Generally favorable condition enhances the activity while the adverse climatic condition have negative or retarding effects on the activity, this applies both to land and freshwater pulmonates (Burch, 1955; Webley, 1964; Newell, 1966; Cameron, 1969, 1970; Lewis, 1969; Pinder, 1969).

Nocturnal activity of the slug is the most common feature of all the species found all over the world. Most species of slug are active between 2nd hr after sunset and 2nd hr before sunrise (Barnes and Weil, 1944, 1945). Our observations are consistent with these.

It is most likely that the nighttime falling temperature in the fields must be an important stimulus contributing towards the nocturnal activity. Further more, during night the slug can protect themselves from their nocturnal enemies like carabid beetles and birds etc.

In the present investigation, it was noticed that high temperature (summer) caused reduction in the feeding activity as well as appearance of the slug, *Semperula maculata* on the soil surface. They were buried inside the soil throughout the day except very rarely early in the morning. Likewise low temperature (rainy and winter) favored the feeding activity and appearance of the slug. They were seen crawling on soil surface and feeding during most of the day except mid
noon hours and when there was heavy rain. Their appearance is mostly on the soil surface after the showers of rain. Slugs actively feed on garden Spinach, Coriander, Ferugreek, Marigold, Gourd. Slugs are nocturnal but seen active during rainy and cloudy days. Similar results were found in the slug *Semperula birmanica* (Panigrahi, 1995), in slug *Laevicaulis alte* (Bodhankar, 1984), in the snail *Cerastus mousonianus* (Choudhary, 1998).

Temperature greatly affects the slug, *A. reticulatus*, which responds quickly to small changes in temperature (Dainton, 1954). He further found that the activity of slugs and snails increases as a result of a fall in temperature below 21°C and by a rise in temperature over a range of 20°C and 30°C; the increase in activity is however, less marked at higher temperature. At a constant temperature of 18°C and constant darkness the slug, *Deroceros reticulatum* shows diurnal rhythm of activity being active at night. This rhythm is lost after 4 or 5 hours. Field experiments were conducted by Ricou (1964) in areas where maximal temperatures lay between 15.3°C and 25.2°C showed that *Deroceros agreste* and *D. reticulatum* are very active and thus cause considerable damage at temperature between 17.5°C and 20.5°C. Mellanby (1961) found that even at 0.8°C *D. reticulatum* fed very actively on the young shoots of grain plants. This slug shows amazing resistance to low temperature and even at 0°C the animal do not remain
completely immobile whereas the slug, *Milax budapestensis* is inactive and another slug *Arion hortensis* hardly moves at 5°C. Field slugs have in fact been found to survive a temperature of 8°C for several hours and several days after being returned to a temperature of 18°C (Godan, 1983). Gates (1959) reported that *D. laeve* can tolerate subfreezing temperature while both *D. reticulatum* and *A. circumscription* are killed in these temperature. *Arion circumscription* required more moist situation for activity than did *Deroceros laevae* and *Deroceros reticulatum*.

Miles et al., (1931) found that feeding habits appears to be broadly characteristics of a particular slugs species and certain preferences for plant species and part of the plant are exhibited, *Agriolimax agrestis* is usually a leaf and stem feeder and shows distinct preference for feeding above the soil surface. *Milax sowerbyi* on the other hand is of subterranean feeding habit and confess its attacks largely to the under ground parts of the plants.

Gebauer (2002) most of the field slug *Deroceras reticulatum* preferred to feed on a mixture of seeds, but the highest activity was recorded on the pea seeds. Giant slugs are common in flower gardens at Mangpoo Darjeeling district, west Bengal. They were active in Manson (April - September) and pass in hibernation in winter (October - March) in the climatic condition at mangpoo. They are hermaphrodite and
nocturnal in nature. During daytime, they hide in contracted quiescent state under some suitable protective such as stones, wooden logs, fallen leaves, inside loose moist soil and crevices in the stone made walls. Slug come out of the hiding pockets and complete feeding on succulent leaves of the gardens close to soil and leaf litter in several spells. A number of 1-5 individual hibernated in one pocket (*Austenia annandalei God Winausten, 2000*).

Garden snail *Peas gracile* (Hutton) were most active in Manson period (June - September) and pass in aestivation state during rest months of year. They fed on leaves of Marigold, Amaranth, garden spinach and also semi decomposed plant materials especially at night times. They sheltered under some protective and moist soils at boundary line of the gardens during aestivation. Final awakening was made following first shower of manson (*Panigrahi, 1998*).

Slugs are most active in response to a falling temperature in the range 4°C to 20°C, it is not known whether there is specific surface thermoreceptor or whether the responses result from changes in temperature of the nervous system. But environmental changes as small as 0.1°C are perceived (*Dainton, 1954*).

Pulmonates are generally negative to light as might be anticipated from their nocturnal habits (*Bodhankar, 1984; Panigrahi, 1995; Hommay et al., 1997; Chaudhary, 1998*). Activity starts in the late
afternoon and reached a peak between midnight and 3am. Hence most of the snails active life was take place in the dark with the onset of daylight, activity gradually declines and the snail retired under some object and passed into a quiescent state. The temperature affects behavior, decrease survival in the test animals. Effects of temperature on the rate of growth, reproduction, and behavior have been noted in *Lymnaea stagnalis* by Vaughn (1953), in *Australorbis glabratus* by Michelson (1961), in *Bulinus* (Physopsis) *globosus* by Shiff (1964), in *Biomphalaria pfeifferi* by Sturrock (1966) and Appleton (1977), in *Biomphalaria glabrata* by Chernin (1967), Sturrock and Sturrock (1972) and Vianey Liaud (1982), in *Biomphalaria pfeifferi* by Shiff and Garnett (1967), in *Bulinus truncatus* and *Biomphalaria alexandrina* by El-Hassan (1974) and in *Indoplanorbis exustus* by Raut et al., (1992).

Kulkarni and Nagabhushanam (1973) reported that the bare lands and the extremes of temperature and other natural factors hindered the population of *Laevicaulis alte*. When the food was scare the slugs were found to be feeding on any part of the plants or even on dead and decaying material that was present in the soil.

In the laboratory, when slugs *Semperula maculata* were kept in glass or plastic tough, they were attached to the sides of the tough and also on the muslin cloth covered on tough. Such tendency was also
observed by Choudhari (1998) in snail *Cerestus moussonianus*. Pulmonates gathered on the darker side of a container, although some were positive to light at times such as *Lymnea stagnalis* and *Helix pomatia*.

4.2 Temperature tolerance

Terrestrial animals are subjected to much greater fluctuation in the temperature. The lethal temperature is alterable according to acclimation temperature. The lethal temperature is influenced not only by acclimation temperature and genetic background but also by age, diet, size and environmental factors such as photoperiod, oxygen and salinity. *Mytilus* in low salinity were less tolerant to high temperature (Schliper et al., 1958). A variety of effects of dietary lipids on temperature tolerance in poikilotherms have been reported. A diet of highly saturated fat increased their heat tolerance (House et al., 1958).

Evidence also exists to the fact that adaptation to a higher temperature implies an upward shifting of the lethal temperature. Acclimation to high temperature, with resulting increase in the high temperature tolerance of the species, has been demonstrated by Mc Leese (1956); Edney (1953); Iwanyzki and Mc Cauley (1993).

Huntsman (1924) showed that lobster larvae raised at temperature between 20°C and 25°C were more heat resistant than those raised at 15°C. The experiments performed on *Cryptozona*, had shown
that 24hr. median heat tolerance limit to 35 ± 0.5°C acclimatized snails was 43.6°C. While those of the control snails, which were subjected to the normal room temperature (27°C - 28°C), the 24hour median heat tolerance limit was 40.3°C. Thus acclimatization to higher temperature increased the heat tolerance of Cryptozona by nearly 3.3°C Mantale (1970). Grant (1953) noted in Earthworm Pheretima that an increase in heat tolerance by 0.3°C per 1°C rise in conditioning temperature. Cocking (1959) also observed 1°C rise of lethal temperature per 3°C of acclimatization in Rutilus.

Evidences on thermal relations of poikilotherm display that lethal temperature of a species is not an absolute entity but is anchored to the immediate thermal history of the organism. Consequently, median cold tolerance is not significant unless acclimation temperature is also cited. Moreover, acclimation influences not only the upper and lower incipient lethal temperature but also the duration of exposure to a lethal temperature that an animal can withstand (McWhinnee, 1967). These subtleties are confirmed through the data chained in the present investigation on the slug, Semperula maculata.

During present work, in control (26°C - 28°C) slug, Semperula maculata the median heat tolerance for 12hrs and 24hrs was 38.6°C and 38.4°C. On cold acclimation this was decreased to 36.4°C, Whereas on warm acclimation the thermal resistance was increased to 39.9°C.
For lower temperature, the control slug (26°C - 28°C) median cold tolerance for 12hrs and 24hrs was 9.6°C and 9.8°C. On cold acclimation they could sustain up to 7.5°C (cold resistance increased) but in warm acclimation could not continue life beyond 19.0°C, that is cold resistance decreased. It means that the slug, *Semperula maculata* exhibits a simple pattern of acclimation effects in relation to lethal temperatures (Median heat and cold tolerance). Firstly, decline in heat and cold tolerance appreciably greater following cold and warm acclimation respectively as opposed to the increase in temperature tolerating capacities after thermal acclimation. Secondly, the effect of high temperature rises both upper (both physiological and numerical increase) and lower (lethal temperatures, whereas lowers both). Thirdly, the fully acclimated state, whether to high or low temperatures, seems to be approached asymptotically as is the case with the isopods *Porcellio laevis* and *Armadilidium vulgare* (Edney, 1964) and achieved in about 10 to 12 days.

*Miles* (1963) has recorded the upper lethal temperatures of 33.3°C for *Eisenia foetida* and 25.7°C for *Allobophora terrestris*, which were acclimated to 15°C for few weeks. However, temperature tolerance are based on a 12hours exposure, leaving 80% of the animals still alive after the end of experimentation (LT-20). The slug, *Laevicaulis alte* possessed the 24hr. median heat tolerance of 34.5°C which was
condition to (25°C to 30°C) and when animals acclimated to higher temperature then 24hr. median heat tolerance was 37.5°C (Nagbhushnam and Kulkarni 1970). Edney (1964) has recorded the upper lethal temperature (after 30 minutes exposure) at about 38.5°C for *P. laevis* and 39.5°C for *A. vulgare* maintained at 20°C. In this context 24hours median tolerance was 38.4°C of *Semperula maculata* speaks for its capability to sustain higher temperature for a long period.

On the contrary, 24hrs cold tolerance of *Semperula maculata* was 9.8°C which reflects its ability to endure the cold as compared to the terrestrial isopods *P. laevis* and *A. vulgare* which having low lethal values of about 0.6°C and 1.6°C respectively (Edney, 1964). Kale and Rao (1973) have mentioned that lowest temperature that could be tolerated by *P. excavatus* is 9 ± 1°C.

Broekhuysen (1940) and Sandison (1967) found a graded adaptation to temperature in a number of species of African snails when submerged which corresponded almost exactly to their intertidal zonation. Southward (1958) was able to relate vertical distribution to tolerance of high temperature in top shells and in barnacles common in the Plymouth area. On the other hand Evans (1948) reported no such thermal tolerance relationship between a number of different gastropods and they are rather wide zones of vertical distribution.

The heat coma and lethal temperatures of *Thais* and *Littorina sp*
were observed (Gowanloch and Hayes, 1926; Hayes, 1929; Manigault, 1932; Evans, 1948; Frankel, 1960, 1961). Most of these observations have been made on submerged snails *L. neritoides*.

Evans (1948) provided the most complete data on temperature tolerance on snails from Cardigan Bay, Wales, and a comparison between the heat coma and lethal temperature of submerged snails from Cardigan Bay and Post Seton. The temperatures lethal to *Thais* and *Littorina spp* from Cardigan Bay were 4-7°C higher than those which were lethal to Port Seton snails; similarly Cardigan Bay snails entered heat coma at temperatures 3-9°C above those at which Port Seton snails became comatose. Since there is a latitudinal difference between Port Seton and Cardigan Bay, it is possible that acclimation to temperature such as has been reported in many poikilotherms (Bullock, 1955; Prosser, 1955) may account for the temperature tolerance differences between snails taken from the two locations. Moreover, the snails could be acclimated to when exposed. Both the temperatures of the sea when submerged or the air temperature must therefore be considered.

The median high and low temperature tolerance limits decreased gradually with period of cold acclimation and the minimum and steady temperature values were obtained by about 15 day of acclimation. The median heat and cold tolerance limits increased gradually with period of
warm acclimation and steady temperature were obtained by about 10 days. The changes in tolerance limits were slow and longer on cold acclimation, that is, the adaptation was slower whereas warm adaptation was quicker in *Bellamya begalensis* (Kulkarni et al., 1985).

The physiological adaptation to environmental stress is one of the main problems of an organism (Bullock, 1955; Larry et al., 1972). Shrimp larvae showed a direct relationship between acclimation temperature and survival time, Larry et al., (1972).

*Emerita holthusi* regains its tolerance to high temperature very rapidly after a low temperature history by acclimation in the upper part of the physiological temperature range. A rapid gain in heat tolerance would be of extreme advantage to most intertidal animals. For intertidal animals, the increase in temperature may be great over a period of few hours in the tidal rhythm with the low salinity and low temperature combination, loss in heat tolerance was much greater studied by Kulkarni (1978).

The relation between salinity and temperature was found by Broekhuysen (1940) in *Crangon crangon* and in *Hemigrapsus* by Todd and Dehnel (1960). The salinity optimum for length of life depended on the temperature. Kinne (1956) found that the hydroid *Cordylorrhora caspia*, had slight osmoregulatory abilities and was found to with stand a high temperature better at high salinity than at
lower ones and the upper thermal limit may be modified by decrease in salinity and for many marine animal, the upper lethal temperature decrease as salinity decreases. Nagbhushanam and Sarojini (1969) stated that in *Diogenes bicristimanus* the acclimation to a high temperature generally increased resistance to lethal temperature where as acclimation to low salinity generally decreased it.

Survival time of *Mytilopesis leucophaeta* increased with increasing acclimation temperature and decrease with increasing salinity. In comparison with co-occurring species such as *Mytilus edulis* and *Dreissena polymorpha, M. leucophaeta* appears to be more tolerance to high temperature stress Matthews and Mc Mahon, (1997); Rajagopal et al., (2005); Rajagopal et al., (2005).

Panigrahi, (1998) showed slug exhibited varied rates of mortality and survival days at different temperatures and room temperature. Hundred percent slugs died at different temperature grades at the age of variable days period. Following exposure although 10 and 40% slug survived at 20°C and room temperature (18.5°C to 38°C) respectively they enjoyed most comfortable life at 25°C temperature. Where rate of mortality was 40% at the time of termination (360 days) of the studies.

Studies on warm as well as cold acclimatization have been examined in *Helix pomatia* Mews, (1967) in *Limax flavus* (Segal,
1956) in *Nodilitorina granularis* (Ohsawa and Tsukuda, 1956). David et al., (1995) observed that Zebra mussels were exceptionally tolerant to moderately high temperature. Mussels acclimated to 32°C survived for 35 days without extensive mortality. Mcmahon et al., (1995) estimated a mean tolerance time of 150hr. at 32°C for *D. polymorpha* acclimated at 30°C. Iwanyzki and Mc Cauley (1993) worked on *D. polymorpha* acclimated at 25°C, measured a mean tolerance time of just 95hr. at 30°C.

The survival of fresh water pulmonates and prosobranchs at different temperature ranges was studied. Fresh water snails are rather sensitive to warm temperature above 30°C. Forcart (1948) observed that the snail, *Physa acuta* could with stand up to 46°C for a short period and also they were having resistance to cold and freezing. They can with stand for some time being frozen in ice. Variation in mortality may involve environmental limitations. Environmental temperature has great influence on the survival of snail. The pulmonate snail *Lymnaea peregra* was also said to survive up to 45°C.

In cold acclimated *L. saxatilis*, heat coma was also observed at 32°C, although the onset of anaerobiosis occurred at lower temperatures (18°C and 28°C in North Sea and White Sea snails, respectively). The heat coma temperature (HCT) in *Littorina* was within the range of the mean HCTs reported for *L. saxatilis* from some North
Atlantic populations (30.8–31.7°C) (Clarke et al., 2000b; Sokolova and Portner, 2003).

4.3 Neurosecretory cells

During the present study, two type neurosecretory cells are recognized in cerebral ganglion of the slug *Semperula maculata* that is A cell and B cell. A cell type is pyriform in shape measuring about 80 to 130µ in length. The nucleus of the cell is long in comparison to the size of the cell. The nucleus is 60µ in diameter. Normally, the nucleus is spherical but it may be kidney-shaped. Inside the cytoplasm of these type of cells small globules are found. The cytoplasm is weakly stained with Gomori’s and Mollory’s stains. The granules inside the cytoplasm are stained blue-black with Gomori’s stain and deep blue with Mallory’s triple stain. These cells have many features in common with the cell described by Laver (1957) in *Ferrissia* spp. and Nagabhushanam and Swarnamayee (1963) in *Vaginulus* spp. Nagabhushanam (1962) in *Bankia gouldi* and Nagabhushanam and Kulkarni (1971) in *L. alte*. These A cells are found to be equivalent to type II cell of Krause (1960) which are present on the postero-ventral side of cerebral ganglion and in pleural and visceral ganglia.

B cells of *Semperula maculata* are oval in shape ranging from 50 to 65µ in diameter and they are characterized by the intensive staining of the cytoplasm. The granules inside the cytoplasm are stained
red with Gomori’s as well as with Mollory’s stains. The nucleus of this type of cell is 16µ in diameter. These cells are more common than cell type A. They are found throughout the periphery of ganglion. These cells have many features in common with B cells of Vaginulus (Nagabhushanam and Swarnamayee, 1963) Nagabhushanam, (1962) in Bankia gouldi and L. alte (Nagabhushanam and Kulkarni, 1971). These cells are also found to be similar to type I cells of Krause (1960). In both the neurosecretory cell types A and B axonal transport of the neurosecretory material is noticed. Some droplets are found accumulated in the neuropiles of the ganglia as well as inside of some nerves. Similar result observed in Meritrix casta (Nagabhushanam, 1970).

A cell shows resemblance with those designated as grana II by Fahrmann (1961) in Unio, the pyriform-shaped cells of Teredo (Gabe and Rancurel, 1958) and to cell Type I in the oyster (Nagabhushanam, 1962). Grana I described by Fahrmann (1961) in Unio and cell Type II of Crassostrea virginica, Surf calm, Spicula solidislimma (Nagabhushanam, 1962a, b) agree close with B cell of Semperula maculata, Type II neurosecretory cells of Bankia (Nagabhushanam, 1962b), cell types II of Tegulus (Nagabhushanam, 1964) and cell type II of Meretrix casta (Nagabhushanam, 1970).

In Bankia (Nagabhushanam, 1962) concerning the distribution
of the neurosecretory cells Type I cells were found in all the ganglia while cell Type II is observed only in the cerebral and visceral ganglia. In *Unio*, Fahrman (1961) observed granal I and II in the cerebral, pedal and visceral ganglia.

From a study of the histological sections, various authors (Scharrer, 1935; Lever, 1957; Nagabhushanam, 1966) reported that the neurosecretory granules are transported along the axon. The observations in *Semperula maculata* support this view, the secretory material being traced along the axon.

Two type of neurosecretory cells were recognized (Nagabhushanam, 1972) in the dorsal and lateral sides of the cerebral, visceral and Pedal ganglia of *Parreysia corrugata*. Cell type I range from 15 to 20µ in length. The cell body is pyriform in shape. The nucleus was small and round or oval measuring about 6-8 µ in diameter. The nucleus may be either central or eccentric in position. The nucleus generally contains one nucleolus, which stains with orange G. The secretory material in the cytoplasm stains red with Mallory’s and blue-black with CHP. Vacuoles were not found in the cytoplasm. These cells were more numerous than the cell type II. Cell type A appear to be identical to the neurosecretory described in *Unio* (Fahrman, 1961), *Anodonta* (Baranyi and Salanki, 1963), *Dreissena* (Antheunisse, 1963) and *P. corrugata* (Nagabhushanam and Lomte, 1981). Cell
type II of *P. corrugata* is larger than cell type I and the cell body is somewhat oval in shape measuring 20-25µ in diameter. It possesses a large nucleus, which may be vesicular or slightly oval with a central or eccentric position. The nucleus measures about 12-16µ in diameter and generally has one or two eccentrically placed nucleoli. The cytoplasm carries fine granules, which stain dark blue with CHP and red with Mallory’s. The vacuolization of these cells was very striking the vacuoles do not possessed a characteristic shape. Occasionally fine granules are observed inside the vacuoles studied in *Calm* (Nagabhushanam, 1972). Cell type B appear to be identical to the neurosecretory described in *Unio* (Fahrmann, 1961), *Anodonta* (Baranyi and Salanki, 1963), *Dreissena* (Antheunisse, 1963) and *P. corrugata* (Nagabhushanam and Lomte, 1981). In both the cell types of *P. corrugata* the neurosecretory materials was seen in the cell body and axons, there by suggesting the axonal transport of the hormones. However, the granules were usually lost when the axon reaches the neuropile of the ganglia.

Nagabhushanam (1970) reported that neurosecretory cells in the bivalve, *Meritrix casta* were found to be distributed along the dorsal surface of the cerebral, pedal and visceral ganglia. On the basis of the difference in size and staining reactions, two kinds of neurosecretory cells were recognized in the cerebral and visceral ganglia and one type
in the pedal ganglia. The cell type I bodies were pyriform in some sections but are often irregular in shape. These cells measured about 25 to 30µ in length and 10 to 15µ across the broadest part. The nucleus was round in shape, with little chromatin, and possessed one large nucleolus; the nucleus may be either central or eccentric in position. The secretory material stains blue-black with Gomori’s stain and red with Mallory’s stain. These cells were present in all the ganglia. This cell type bears a close resemblance to the pyriform shaped cells of *Tegulus* (Nagabhushanam, 1964). Cell types II of *Meritrix casta* restricted to the cerebral and the visceral ganglia, was oval or round and had a diameter of about 20 to 25µ. Their nuclei were similar to those of Type I cells. Some cells contain a few large vacuoles that almost completely filled with the cytoplasm. The secretory material stained red with Gomori’s and Mallory’s stains. These cells appeared to be abundant in the visceral ganglia where they were arranged in some follicles along the dorsal side. They resemble in shape and tinctorial properties with the Type II neurosecretory cells of *Bankia* (Nagabhushanam, 1962b) and the Cell Type II of *Tegulus* (Nagabhushanam, 1964). In both neurosecretory cell types of *Meritrix casta* the secretory material is observed near the proximal end of the axon occasionally.

In Bivalve, *Yoldia limulata* (Nagabhushanam, 1963) the
neurosecretory cells were found to be distributed along the dorsal surfaces of the cerebral, pedal and visceral ganglia. Only one cell type was apparent. The cells were 12µ long in the cerebral and pedal ganglia and 33µ in the visceral ganglia. The cytoplasm showed a dense granular structure and rarely had vacuoles. Minute secretory granules that stained pink with Mallory's and blue black with Gomori's technique appeared in the cytoplasm. The nucleus had one large nucleolus, which stained red with Mallory's stain. In some of the axons of the neurosecretory cells a number of granules were noticed leaving the cell bodies. Apparently the secretory material was transported through the axons but no terminal organ was found. Small granules were also found within the neuropiles of various ganglia. These cells showed resemblance in their shape and tinctorial properties to the cell type I of the Oyster, *Crassostrea virginica* (Nagabhushanam, 1962a) and to the pyriform shaped cells of *Teredo* (Gabe and Rancurel, 1958).

Changes in the neurosecretory fabric in response to thermal acclimation have been evidenced in insects (Janknovic et al., 1969, Ivanovic et al., 1975). In present study the slug *Semperula maculata* during the warm acclimation, super and active A and B cells were encountered in cerebral ganglia as their nuclei displayed enlargement over those of control slugs and scanty neurosecretion was spotted in their perikarya and axons. Hence, it is quite likely that during the warm
acclimation these neurosecretory cells are extremely active and transport and release neurosecretory material probably faster than the synthesis, thereby giving no or very little allowance for the accumulation of the secretory material in the perikarya and axons. Moreover, it is possible, that whatever little quantity that might be present in the perikarya (during the acclimation) is probably beyond the pale of chemical sensors employed in the present investigation. The spectacular pile up of neurosecretory material in A and B cells during cold acclimation may be the resultant of the cessation of the axonal transport and release. Probably, the rate of synthesis in these cells during cold acclimation is not substantially different from that of during warm acclimation as there were no marked differences in their nuclear dimensions, during both warm and cold acclimation.

Neurosecretion may perhaps control the metabolism in pulmonata stylommatophora. Mussels are sensitive to thermal and osmotic stresses, a sudden rise of temperature (10°C) maintained for an hour or a sudden fall of salinity (20%) result in an emptying of the neurosecretory cells of the cerebral ganglion while a fall in temperature or an increase in salinity (45%) are followed by a significant increase in the secretion product in the same cell. No variation takes place in the visceral ganglion (Lubet and Pujol, 1963, 1965). An electrical (20 volts for 30 minutes) or thermal (2 hours at 32°C) shock empties the secretion product (Nagabhushanam, 1949).
Rise of temperature of ganglion cell increases the frequency of action currents; decrease of temperature decreases the frequency. Inactive cells may be activated by cooling, inactivated by warming. (Kerkut and Taylor, 1956; Kerkut and Ridge, 1962).

In *Cerastus moussonianus* (Kulkarni et al., 1987) high temperature (40°C) helped in releasing the NSM whereas the low temperature (20°C) caused the accumulation of NSM in the snail *Cerastus*. But the effect of low temperature was found to be insignificant.

### 4.4 Biochemical profiles

#### 4.4.1 Glycogen

Glycogen is the major complex organic constituents of the living organisms. Glycogen is distributed throughout the tissues, which constitutes main food reserves of pulmonates. It is accumulated during summer from May to November. Carbohydrate that is glycogen is the major foodstuff of the animal. This food gets oxidized to meet most of the energy requirement of the body.

In *Helix*, it reaches a maximum value of 13.2 percent of the dry weight and is utilized as an energy source of hibernation, falling to 4.8 percent of the dry weight by spring (Thiele, 1959). Terrestrial pulmonate are able to regulate their body metabolism with reference to temperature, their metabolic rate lower than would be expected at that
temperature, when kept at a low temperature, the rate is higher than expected (Vaughn, 1953). Glycogen occurs in the foot (Fretter, 1968) and in the wall of the stomach and intestine (Gabe and Prenant, 1949 C).

During present study, glycogen content was found to be lower in warm acclimated animals in both the tissues that are in hepatopancreas and foot. However, the high depletion of glycogen in hepatopancreas followed by foot muscles. As compared to total protein and total lipid, glycogen showed more decrease it might be due to the stress condition during exposure. These results are similar to that of Das and Prosser (1967); Das 1967 and Rao (1967). While glycogen content in cold acclimated animals increased in both the tissues but that change was not remarkable in both the tissues.

In case of cold acclimation, as there is no activity of the animal, the constituents are not used, so there is an anabolic activity; thus, there is increase in the constituents. When the animals were acclimatized for high temperature the much of the energy must have been used to compensate the stress. So there is depletion in the organic constituents.

The slugs stores excess food in the form of glycogen in different body tissues, anticipating the adverse conditions of the nature. When any stress condition occurs these stores are utilized to obtained energy
for counteracting this stress. The artificial stress by temperature is also
tackled in the same manner. The effect of temperature on the alterations
in the biochemical substances of the body has been profusely studied
in slugs by (Martin, 1961).

The O:N ratios of the mussels in acclimation groups (20, 24, 28,
32°C) was 64, 68, 35, 35. This indicated that at 20°C and 24°C the mussels
were primarily catabolizing carbohydrates and lipids (O:N > 50) (Bayne
and Widdows, 1978). The higher weight specific metabolic rates of
smaller animals than larger ones, it might be expected that smaller D.
polymorpha might be more adversely affected by higher temperatures
than larger individuals, but McMahon et al., (1994) have shown that
smaller D. polymorpha tolerate chronic exposure to high temperature
better than larger individuals. Likewise, Bayne and Newell (1983) have
shown that on the basis of the comparison of actual biomass changes
with those predicted from measured rate of oxygen consumption in
Cerastoderma edule (L.) and Mytilus edulis L., smaller individuals
are better at reducing energetic losses during periods of food shortage
than larger individuals. Hence, from the evidences it was suggested that
high temperatures may negatively affect the physiological energetic of
large mussels more than smaller ones (David et al., 1995).

Glycogen content of Cryptozona acclimated to different
temperature indicated that the glycogen content in foot and digestive
diverticula was lowered at the higher temperature, Mantale (1970); Kulkarni and Baramatiwala (1987) in Bellamya bengalensis.

Newell and Northcraft (1965, 1967); Newell and Branch (1980) have shown that in a number of littoral marine invertebrates representing several different phyla the basal metabolic rate of quiescent animals remains remarkably constant over the approximate temperature range 6.5°C - 22.5°C. Yet, at temperature between about 20°C and 25°C, the latter being the highest experimental temperature employed, they found that the basal metabolic rate of L. littorea and other species either increased or became irregular. Davies (1966) worked on Patella aspersa and P. vulgata and Micallef and Bannister (1967) on Monodonta turbinata also noted abnormally irregular respiratory rates between 25°C and 35°C.

Vedpathak et al., (1980) reported that the rate of respiration increased with rise in temperature in fresh water bivalve Indonaia caerulcus and this was more marked after 7 days than 15 days exposure to temperature. After 7 days of exposure, the glycogen content in foot and hepatopancreas of bivalve was decreased and similar change seen in foot and hepatopancreas after 15 days of exposure.

At 28°C and 32°C, the mussels used more protein than 20°C and 24°C in catabolism (O:N < 40). Among mollusks such reduction in
Matveeva (1974), Galaktionov (1993) did not demonstrated a compensatory increase in respiration during cold acclimation in white sea Littorina. A depression of metabolic rates, which correlates with reduced levels of feeding and general activity, was observed in many marine and freshwater molluscs in winter (Innes and Houlihan, 1985) and was consistent with an important role of hypo metabolism as a survival strategy during prolonged periods of cold exposure or resource limitation. This strategy was found across a wide variety of phyla, from resting bacterial spores through aestivating and dormant invertebrates to hibernating mammals (Hochachka and Guppy, 1987; Sokolova and Portner, 2003).

4.4.2 Total Protein

Total protein is also the major complex organic constituent of the living organisms. Proteins get oxidized to meet energy. It is main constituent of cell membrane, plays an important role in the process of interaction between intra and extracellular media. As enzyme it take parts in many physiological activities. The observations on Periplaneta americana (Singh and Das, 1978) revealed that this insect is capable of demonstrating respiration compensation against thermal alteration at the organismal as well as the cellular level. Besides, the thermal metabolic adaptation of cockroach also cause appreciable alteration in
the levels of accumulation of macromolecules like protein, RNA and lipid in different tissues. Similar changes in biochemical composition of tissues have been reported on other poikilotherms during thermal acclimation (Das, and Prosser, 1967; Rao, 1967; Das, 1967).

During present study, total protein content was recorded in warm acclimated (32°C and 36°C) slug *Semperula maculata* was found to be decreased in tissue like hepatopancreas and foot. This clearly suggests that the warm acclimated slugs were subjected to stress condition. Hence the depletion in protein content was observed. These results are similar to Das and Prosser (1967); Das (1967) and Rao (1967).

The slug stores excess food in the form of protein in the body tissues to counteract unfavorable condition. Hence, during stress condition such contents utilized and quantity of protein decreased (warm acclimation). In cold acclimated slug protein contents was hardly changed. Same trend was seen in the hepatopancreas and foot. As the physiological activities were slowed down in cold acclimation (10°C and 15°C), the proteins were less used and this resulted in anabolic phase that is increase in the protein content.

Kulkarni *et al.*, (1984) showed that degradation of protein band and concentration of free amino acids increased on high temperature acclimation and decreased on low temperature acclimation with slight variation of total number of amino acids in the slug *Semperula maculata*. 

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O:N ratios are usually associated with tropic stress (Bayne 1973; Widdows 1978; Bayne et al. 1979, 1981; Bayne and Newell, 1983; Aldridge et al., 1987; David et al., 1995).

The potassium was known to stimulate and sodium to inhibit some of the enzyme systems involving ATP (Boyer, 1953). Walser (1960) stated that the sodium might influence the protein building capacity of calcium. Calcium was also known to alter protein building in relation to temperature (Halsbad, 1953). Variations in protein synthetic activity on thermal acclimatization were reported in a few poikilotherm (Iwanyzki, 1993; Saroja and Rao, 1965). Dean and Vernberg (1965) observed higher level of protein in the high temperature acclimatized Crab *Uca pugilator* and Mantale (1970) in snail *Cryptozona* in the whole body. (Kulkarni and Baramatiwala, 1987) in *Bellamya bengalensis*.

Vedpathak et al., (1980) reported that after 7 days of exposure to temperature, the protein content in fresh water bivalve *Indonaia caeruleus* was increased in hepatopancreas and foot and after 15 days of exposure, protein content of hepatopancreas and foot was decreased.

**4.4.3 Total lipids**

Total lipid is the major complex organic constituent of the living organism. Lipid is also one of the major foodstuffs of the animal. Lipid provides rich source of energy to the body. Cockroach, *Periplaneta americana* caused appreciable alteration in the levels of accumulation
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of macromolecules like lipid in different tissues. Similar changes in biochemical composition of tissues have been reported on other poikilotherms during thermal acclimation (Das and Prosser 1967; Rao, 1967; Das 1967).

The present investigation showed that decreased in the level of total lipid in hepatopancreas and in foot on warm acclimation (32°C and 36°C) in the slug *Semperula maculata*. The change was due to stress condition. More and more lipid was used to counteract the stress. But in cold acclimation (10°C and 15°C) the total lipid content increase was less because of slow activity of animal. In any animal lipids form the main store of reserve food supply being derived from the fat and carbohydrates diet. It means that under stress conditions lipids are utilized for the energy production as an alternative source of carbohydrate. During present study, decline in glycogen level was noticed and it appeared that lipids are utilized for the production as alternate source of carbohydrate to minimize stress caused by temperature.

At 20°C and 24°C, the mussels were primarily catabolizing lipids (Bayne and Widdows, 1978). Decrease in fat content with rise in temperature was probably due to an elevation in the rate of metabolism Rao et al., (1887) in freshwater bivalve *Indonaia caeruleus*; Kulkarni and Baramatiwala (1987) in *Bellamya bengalensis*. Mayer and Schaeffer
(1914) found the ratios of cholesterol to fatty acid and cholesterol to phospholipid to vary directly as the water content of the tissues. Mantale (1970) studied that water content varied inversely as the fat content. Vedpathak et al., (1980) showed that, after 7 days and 15 days exposure to temperature, lipid contents of hepatopancreas and foot was decreased in fresh water bivalve Indonaia caeruleus.

In molluscs, high thermal sensitivities of metabolism within the environmental range were associated with increased long-term metabolic costs and with a lower tolerance to extreme temperatures. Hawkins (1995), suggested that a reduced temperature dependence of metabolic rate can be selectively advantageous in thermally unstable intertidal environments. L. saxatilis adopted an 'exploitative strategy' that allowed them to utilize resources at a high rate in order to maximize growth, and their metabolic rates increase with increasing temperature (Branch et al., 1988). Acute temperature increase resulted in a considerable metabolic disturbance in L. saxatilis at critically high temperatures indicated by the onset of anaerobic metabolism and the adverse changes in the cellular energy status. In warm acclimated L. saxatilis from the White Sea and the North Sea, the onset of anaerobiosis between 28°C and 32°C coincided with entering heat coma, characterized by the loss of nervous integration and muscle relaxation (Clarke et al., 2000a; Sokolova and Portner, 2003).
4.4.4 Uric acid

In the present investigation, the content of the uric acid during warm acclimation (32°C and 36°C) was found to increase in the slug *Semperula maculata*. This probably indicates the higher metabolic activity of the slug. During warm acclimation, the catabolism of proteins must be at a higher degree than the cold acclimation (10°C and 15°C). Hence the content of uric acid during cold acclimation was decreased.

Meenakshi (1956) showed accumulation of uric acid in vagina, uterus, penis and foot of *Pila globosa* during aestivation. It is because of cessation of reproductive activity and locomotive activity during aestivation. The aestivating animals might perhaps be finding these organs as convenient site to accumulate uric acid formed in the whole body.

The catabolism of amino acid and protein can serve as a significant source of metabolic energy, since these substances are the major constituents of molluscan tissues (Claybrook, 1983). Ammonia is the major end product of nitrogen metabolism in molluscs and they are predominantly ammonotelic. Values for blood ammonia in molluscs (mg/100ml) had been 0.05 for *Anodont*; 1.4 for *Unio*; 2.8 to 4.8 for *Sepia*; 1.2 for *Helix*; 0.2 to 0.7 moles for *Octopus* and 0.7 to 2.0 for aquatic snails. The blood ammonia concentration in *Mytilus edulis*...
varies from 0.31 to 1.79 mg/100ml seasonally as evident from the seasonal data for animals acclimated to 15°C.

Nitrogen excretion in Lamellibranch molluscs has been very little studied. Spitzer (1937) found some uric acid in the excreta of *Mytilus edulis* and *Unio* but the results of the most complete analysis of nitrogen excretion of the oyster, *Crassostrea virginica* made by Hammel et al. (1964) and in cephalopod *Octopus dofleini* (Harrison and Martin, 1965) allowed to conclude, the ammonotelism of these two species. In *Crassostrea*, about 65% of total nitrogen is excreted in the form of ammonia, 13% in the form of urea and 17% is unidentified. It was also found that 72% of nitrogen excreted in the form of ammonia and 28% as urea. Some nitrogen is also excreted in the form of amino nitrogen, especially in *Modiolus* where it was about three times less important than ammonia (Lum and Hammel, 1964).

Temperature is an important factor that influences biological function and nitrogen excretion rates are influenced differently. The increase in temperature increases the ammonia and urea in different tissues but the percentage increase is found to be inconsistent. The increased level of the nitrogenous wastes in the tissues may be due to the increased catabolism of nitrogen compounds, since temperature is known to accelerate the metabolic processes in animals. The simultaneous are, relatively greater increase in the nontoxic compounds
probably suggest that ammonia is detoxified at higher temperatures. The rates of ammonia and urea excretion are also shown to increase with temperature and this may be to clear the larger quantities of these compounds formed at higher temperatures. These results are comparable with those of crustaceans.

According to Hesse (1910), *H. Pomatia* excretes 3.85 mg of uric acid per kilogram Per hour. Marchal (1889) had isolated the uric acid contained in the kidneys of one hundred fifty snails and after its purification had concluded that a kidney contains more than 7 mg uric acid. In fact, at the end of hibernation as Claybrook (1983) have shown a snail kidney contains a mean of the weight of 32 mg of uric acid, that is, about three quarters of the dry weight of the organ. Uric acid is mostly excreted in its acid form, a small portion being in the form of urates (Heidermans, 1937).

At the end of hibernation the nephridium of *Helix* contained 32 mg of uric acid. The end product of nitrogen metabolism of marine gastropods is uric acid (Needham, 1935, 1938), wanting in *Onchidella*. The synthesis of uric acid in *Aplysia* takes place in the midgut gland (Meenakshi, 1956), which has a uric acid content of 0.06 to 0.3 percent. A brief of the midgut gland of *Aplysia* incubated at 39°C produces uric acid in about 3 hours.
4.5 Enzymalogical profiles

Majority of enzymes catalyzed reactions, takes place within the cells. An energy imbalance occurs in the cells due to exposure to temperature. The measurement of enzyme levels in tissues shows the degree of effect on tissues. The bioassay of acid phosphatase and alkaline phosphatase can provide diagnostic means to found effect caused to organism due to exposure temperature.

Any change to overcome the stress needs energy, normally various sources of energy metabolism are acquainted by the metabolic cycles involved in the interchange of organic constituents, that are responsible for the production of energy, undergo a drastic change.

Acid and alkaline phosphatase activities are known to be involved in a number of cellular functions like synthesis, transport and metabolic regulations etc. under such conditions acid and alkaline phosphatase activities may also be effected.

Acid and alkaline phosphatases area group of enzymes, having pH specificities, which hydrolyze various phosphate esters, hence also termed as nonspecific phosphomonoesterases. Temperature can also produce metabolic disorders at cellular levels in case of processes like synthesis, transport, metabolic regulations etc. under such circumstances acid and alkaline phosphatase activities may also be affected.

The active role of acid and alkaline phosphatase in different
tissues shows the dominance and important role in the activity of the animal. At warm acclimated temperatures the activity of acid and alkaline phosphatase decreased. This would be because of the reduced activity of the animal, that is digestion and locomotion. The result of this is the minimum requirement of energy and which is reflected by reducing the acid and alkaline phosphatase activity (Reddy, 1967; Srinivasa and Swami, 1976). In *Pila globosa* there was nearly 12% increase in alkaline phosphatase activity in the mantle tissue of aestivated snail (Srinivasa et al., 1976). Studies on the alkaline phosphatase activity in *pila globosa* had shown the enzyme to be present in different regions of alimentary canal, nephridium and the activity of the enzyme was high in regions concerned mainly with reaction (Shyalaja and Alexander, 1974). The enzyme is known to have significant role in calcium and phosphate metabolism. They play an important role in general metabolism (Meenakshi, 1956).

During present investigation, the activity of acid phosphatase and alkaline phosphatase at warm acclimated (32°C and 36°C) was found to be reduced in the slug *Semperula maculata*. These animals tolerate the low temperature more than the higher temperature. Hence at cold acclimated (10°C and 15°C) the activity of both phosphatase was raised.

In the previous chapter, change in the neurosecretory fabric in response to thermal acclimation has been reported in animal under
present study. During the warm acclimation there was scanty neurosecretion in the neurosecretory cells of cerebral ganglia while in cold acclimation there was blooming in neurosecretory material. This has been interpreted in the present studies. During cold acclimation, there is synthesis of neurosecretory material, which is supported by higher activities of acid and alkaline phosphatase. In warm acclimated conditions there was depletion of neurosecretory material, which is completely strengthened by the acid alkaline phosphatase role. Because of higher temperature there is no more synthesis of secretory granules, which in turn is because of reduced synthetic activity of acid alkaline phosphatase.

Changes in neurosecretory cells in response to thermal acclimation have been evidenced in insects (Jankovic et al., 1969; Ivonovic et al., 1975). Shukla and Tripathi (1979), have already observed gradual reduction in activity of protocerebral neurosecretion of *corynidas perigrinas* with fall in temperature. It is an established fact that every enzyme has optimum and minimum temperature and their activities cease at 0°C (Anderson, 1953; Jadhav and Lomte, 1983). It may be true in case of phosphatase also. It has been found that the activity of these enzymes increases in 25°C - 26°C temperature and there was fall in the activity at 30°C and above temperatures. The activity was seen increasing during cold temperatures 20°C and 25°C, while at
10°C it was lowered. This animal tolerates lower temperature. The activities were also maintained during the cold temperature (Kulkarni et al., 1982). At higher temperature animals are almost inactive. These enzymes thus play an important role in elaboration of neurosecretory material in the cerebral ganglion of the slug, *Semperula maculata*. Thus variations in the neurosecretory activity of *Semperula maculata* at different temperatures (Kulkarni et al., 1982) are due to variation in the enzymes activity at that temperature.

Kulkarni et al., (1984) presented that in cold acclimation, the acid and alkaline phosphatase activity in the cerebral ganglion of the slug *Semperula maculata* was higher while in warm acclimation acid and alkaline phosphatase activity was lowered.

In the roach *Rutilus rutilus*, seasonal cold acclimatization and cold acclimation led to a compensatory increase in the activity of several key metabolic enzymes and of Na⁺ / K⁺ ATPase but did not result in the overall increase of the metabolic rate (Koch et al., 1992). This showed that increased enzymatic capacity is not always directly translated into increased metabolic flux and emphasizes the complex relationships between enzyme capacity and the regulation of enzyme in the whole animal (Hoffmann, K. H., 1983).