International Research Journal of Biological Sciences ________________________________ ISSN 2278-3202
Vol. **7(9),** 6-11, September **(2018)** *Int. Res. J. Biological Sci.*

Comparative study of Dormancy pattern in selected Aquatic and Terrestrial organisms

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Available online at: www.isca.in, www.isca.me
Received 29th May 2018, revised 27th August 2018, accepted 8th September 2018

Abstract

Among the various peculiar characteristics of lower animals the most prominent is avoidance to insensitive conditions as an adaptation to thrive the existing situation which is generally referred as Dormancy. Dormancy is a latent period in the life history of an organism when growth, development, and metabolic activities are provisionally clogged due to prevailing environmental conditions. The minimizations of metabolic activities facilitate an organism to conserve energy as well as life expectancy. This is the period when, an agile creature becomes inactive only to overcome the situation for an extensive period of life. Quiescence is a state of quietness or inactivity due to several reasons such as change in environment, availability of food, predator etc for a very short period. Here the creature sometimes exhibits polymorphism, one of the practices of mimicry only to avoid the available situation. Diapause, on the other hand, is a temporary slowing down of physiological activities in retort to terrible environmental conditions as such found in most of the insects. It is an extrapolative strategy, which is predetermined and inherited by an animal from its ancestors. It is a mode of change in the life style of an organism to suit its metabolic activities according to the conditions to its favor. Indeed the term Dormancy is extensive in its meaning and it includes several types of latent periods such as Quiescence, Diapause, Aestivation, Hibernation and Brumation. Present work was carried out in Wolaita Sodo University about 350 km from the capital city of Ethiopia, Addis Ababa. Aquatic organisms such as Rotifers and insects such as Sorghum chafer were taken for comparing and observing the dormancy patterns; since they were found in two different environments. An attempt was made to understand the dormancy patterns found in the life cycle of Pacnoda interrupta both in the laboratory as well as on field with respect to physical factors such as temperature, moisture, availability of food and predator, length of day light, and factors contributing for their success of overwintering for a long period. Similarly, rotifers were taken from the fresh water bodies in and around the study area for investigating their dormancy pattern in the laboratory. The authors have made an effort to define various terms of dormancy in a novel way for upcoming generations, which of course unwrap for further debate.

Keywords: Crypticism, diapause, dormancy, latency, rotifer, sorghum chaffer, quiescence.

Introduction

Dormancy is a latent period in the life history of animals, when growth, development, and metabolic activities are provisionally clogged due to prevailing environmental conditions. It is very much common among many organisms. In some it is inherited from ancestors while in some others it is a way of adaptation only to thrive from existing situation- may be environmental, accidental or danger of any kind. Many organisms including plants, microorganisms and especially animals undergo this phenomenon. Metabolic activities are either slow or negligible in some cases while completely ceased in some other. All these phenomenon all together cannot be called simultaneously as Dormancy. Reptiles also exhibit this phenomenon but no metabolic activities are stopped but the consumption of water is less and conservation of energy is more specifically during summer months (Brumation). Indeed they do not drink water in most of the cases. Unlike Reptiles, Amphibians exhibit two types of patterns such as aestivation and hibernation are observed differ as per their seasonal activities but simply to avoid the harsh environmental conditions prevailing. In lower organisms the phenomenon of dormancy is exhibited not only for change in the ecology but also for silly reasons such as appearance of multiple predators, unavailability of food, unavailability of partner for mating etc. indeed it is a temporary exhibition of animal only to avoid a situation. It is neither seasonal nor permanent in nature. It also is not long lasting and sometimes subsists for only few hours. Such phenomenon should be called as ‘Quiescence’ or ‘Latency’.

Among multi cellular microscopic aquatic organisms, the Rotifers are well-known for display the phenomenon of Dormancy. Among the rotifers the monogononts, produce dormant eggs capable of latent life after transforming to sexual phase where as bdelloids go through the process of anhydrobiosis at any time during their life history. Monogononts live for short duration but exhibit dormancy for longer periods similar to diapause in Insects. Whereas Bdelloids on the other hand live a long life inhabiting in-conducible environments exhibiting quiescence as a direct response to changing environment mostly for short duration. Both of these dormancy patterns in rotifers can be correlated to the sequential discrepancy of their environments and appear to represent diverse rejoinder at different rates. Both these tactics sometimes are alternative, as no single rotifer species seems to be capable of both diapause and quiescence. Dormancy has immense ecological importance as it can help the species to overcome demanding conditions. In Insects Dormancy is a unique phenomenon to escape from the harsh environmental conditions. It is indeed genetically motivated in nature and exhibited by most of the species for prolonged period sometimes extending up to decades. In some insects, the phenomenon of dormancy is exhibited although there is no major change in the environment can be called as 'Crypticism'.

Sorghum, *Sorghum bicolor* (L) Moench is among the major cereal crops grown for food in Ethiopia. The production of sorghum is threatened by a wide range of both pre and post harvest pests. Among which the Sorghum chafer plays important role and represents a critical constraint to increased sorghum production because of the level of damage it inflicts due to lack of successful control methods. The adult beetle is the damaging stage. It feeds on the flowers and sucks all the contents of the sorghum, maize and wheat grains at milk stage. *Pacnoda interrupta* feeds towards the end of the rainy season, in late September on milk stage grains of sorghum and pennisetum millet heads.

Materials and methods

To understand the dormancy in aquatic organisms, water samples for hydrographical analysis were collected by dipping a 250ml wide mouthed glass stopper or polythene bottle just below the surface of Lake water in open condition. The water was immediately transported to the laboratory after replacing the stopper, for chemical analysis. Temperature was recorded by an ordinary centigrade thermometer and Transparency by a Secchi disc in the field. Hydrogen-ion concentration was noted by using narrow range pH paper (BDH) and a portable pH meter (Systronics, Type 323). Turbidity was determined by the Hellige Turbid meter with help of standard curves supplied with the instrument. Water samples collected into 125ml capacity bottles were treated in the field for the determination of dissolved oxygen (DO_2) according to Winkler's method but for final corrections by a DO meter (model-JENWAY-9015). Carbonate and bicarbonate alkalinity were determined by titration with standard hydrochloric acid of N/20 (0.05N) using phenolphthalein and methyl orange as indicators. Free Carbon dioxide was determined by titrating the sample against the standard alkali titrant (standard sodium carbonate of 0.0454N) using phenolphthalein indicator. Biochemical oxygen demand (BOD) was estimated by dilution method, after 5 days incubation at 20^oC[1].

Plankton sampling: The collection of plankton samples were taken up by a tow net of No.20 made up of silk bolting cloth (mesh size 70µm), with about 1 foot in diameter. For quantitative analysis of plankton known quantity (100lts) of water was passed through the net. The volume of water filtered through the net varied in different localities depending on the plankton concentration. The inside of the net was thoroughly washed with water to obtain any adhering organisms within the meshes. The organisms were first observed in live condition and then the concentrate was divided generally into three parts. The first part was treated with 5% procaine hydrochloride and then fixed in Schaudinn's fixative. The second part was treated with 5-10% formaldehyde and third part was treated with boiled water and then fixed in formalin. When the organisms treated directly in formaldehyde, the soft parts contracted considerably leaving the clear outline of the Lorica, thus making the identification easy. Hot water treatment gives satisfactory results as well as can fix the organism in its natural position which no other relaxing agent can do. A large number of organisms were placed in a Petri dish and equal amount of boiling water was suddenly poured into the middle of the dish. This method worked well for basically free-swimming organisms and also collects the dormant forms such as desiccating animals, hard and dead structures as well as eggs[2]. During the investigation period, the microscopic features of various rotifer eggs were collected and preserved in separate glass wiles for further studies.

For Insects: Both nervous and hormonal pathways are involved in diapause regulation. Searches for diapausing beetles were made in a number of places although live diapausing adults were found only in a few places, (less than 10%) of the places checked. A field observation was made in north eastern regions of Ethiopia where a total of 58, adult *P.interrupta* beetles out of which 30 females and 28 males were found. The differences in sex of the beetles were not significant at 0.05 level (χ^2 = 0.069, P=0.793). The topography, vegetation and other environmental conditions were noted. The depth of diapauses, soils temperature and other micro-ecological conditions were noted to understand the phenomenon of diapause. The diapausing locations were not exposed to direct sun either because of topography or vegetation cover. *Prosopis juliflora,* plant, was found harboring a considerable number of beetles under its dense shade caused from its very green leaves. The diapausing depth the insects were found in the soils varied from 25-60cm. In all cases the soil temperature was found to be between 25-25.5^{0}C while the atmospheric temperature was varying from 28^{0}C to 35^{0}C at different times of the day.

Most were found diapausing in the open spaces in the soils created by roots of the plant, around and between the roots and sometimes between rocks. These insects were taken to Ambo plant protection research centre (APPRC) kept there and followed up the life cycle. Twenty-six females and twenty-two males survived in a glasshouse for 119 days, after which they were killed for physiological analysis. It was observed that on the last day itself, although they were unable to fly but yet they were moving actively.

In order to study the effect of feeding on diapausing conditions 2.5kg of sun dried, steam sterilized soil which was moistened with distilled water was put in 14 rearing jars where as twenty five pairs (both male and female) of beetles were kept in each jar. The jars were grouped into two and seven experimental groups were set. i. Group 1: fed daily with variety (banana, guava, and flour of acacia flower) food. ii. Group 2: fed daily but only with banana. iii. Group 3: every 4 days with variety of food. iv. Group 4: every 4 days with only banana. v. Group 5: every week with variety of food. vi. Group 6: every week with only banana. vii. Group 7: Without any food.

The impact of feeding on diapause has been given in (Table-1). Variations in number of survival and weight of beetles has been observed in different feeding programs. However, these variations are highly dependent on provision of food early or late, but not on variety, amount or frequency of feeding. As long as the young adults were fed early in their life and able to accumulate food reserve, they are able to live long with small or even without food in the later stages of their life.

Dormant stages of rotifer: The stages of Monogononts are better recognized as *Resting egg* (R.E) by Ricci Claudia[3]. With the fluctuation in temperature, change in sunlight and variation in pH of water the dormancy patterns in rotifers had also undergone spectacular changes. Along with theses parameters when the biotic elements such as predators were recurrently present in the water they undergo change in their body shapes and structures (Cyclomorphosis) failing which they go for temporary dormancy ceasing all metabolic activities. One of the unique phenomenons found in the present investigation was that some predators were selective in the selection of their food and no food is accepted dead. In other words they catch their won selected food live and do not compromise in their choice. The prey itself also tries to save itself from the disaster by undergoing cyclomorphosis, polymorphism or dormancy.

The predator sometimes too in absence of its choice of food undergoes a temporary dormancy (say Quiescence) that may last maximum for a period of few hours. When the predator rotifer *Asplanchna* was found in water the species belonging to *Brachionus, Keratella and Lecane* developed several structures externally to equip themselves to avoid predation but still if killed in large scale they undergo for polymorphism as in *Brachionus*[2] or dormancy as found in *Lecane* and *Keratella.*

Most of the rotifers exhibit parthenogenesis due to either availability of favorable conditions or unavailability of male partners for continuation of sexual cycle. It was observed in monogonont rotifers that the dormant stages of adults were carrying eggs (R.E). When there was change in the environment in its favor these animals underwent sexual phase of life called mictic cycle[3]. In unfavorable conditions they descended down to the underneath and lead a silent life (Latency) for time without undergoing any metabolic as well as reproductive activities. For some rotifers this latent period vary from few hours up to 60 yrs. A sediment sample, estimated to be 13 years old, up to 77% were found to hatch[4]. Commonly, the dormancy of the resting embryo extends longer than the persistence of the harsh condition, is temporally irreversible and is beyond the environmental parameters. Dormancy itself thus a phenomenon not to be restricted to harsh conditions in the environment rather to be taken in wider way as an art of living, as a tool for struggle for existence inherently present in all organisms.

The recommencement of normal life was commonly influenced by a several factors such as temperature, dissolved oxygen and light and of course availability of food. The shell of the egg was having single layer like that of the cysts of protozoans but thicker and resistant to all types of chemicals. In case of Bdelloid rotifers, they lose internal water and enter a particular form of dormancy, called anhydrobiosis.

In preparation for the anhydrobiotic condition, a bdelloid follows a succession of alteration in their morphology and metabolism. The animal contracts itself and condensed shape[5]. This polymorphic variety may help the animal for controlling the rate of water loss during the process of desiccation also to reduce transpiration when the dormant condition was attained. The series of adjustments that allow the rotifer to enter anhydrobiosis properly (preparation phase) require a few hours, during which the rotifer was undergoing desiccation.

Results and discussion

The term 'Dormancy' includes all types of cryptic phenomenon exhibited by animals to lead a latent life that includes both Diapause and Quiescence. The two phenomena are distinguished by being under intrinsic and extrinsic conditions respectively. Diapause was commenced in response to a variety of stimuli such as availability of food, population density, temperature and photo-periodism. Diapause got terminated by the return of favorable conditions. Quiescence refers to states such as hibernation, aestivation and all forms of Cryptobiosis (such as cryobiosis, anhydrobiosis, osmobiosis). Quiescence is directly induced and maintained by the occurrence of adverse environmental conditions and promptly broken once the condition was removed; it lasts as long as the unfavorable situation. Animals can enter quiescence at any age, and do not need to produce specific dormant stages. Both dormancy forms occur in rotifers.

The pattern of Dormancy in terrestrial conditions is quite different than that of aquatic organisms since the ecological conditions are entirely different. Moreover the food and feeding, metabolic activities, presence or absence of exoskeleton, osmo-regulation play vital role in exhibiting this phenomena. In most of the aquatic organisms there is no need for conservation of water and minerals consistently where as in terrestrial conditions it's obligatory concern- one of the primary cause of dormancy in Insects. In most aquatic organisms, the body outer covering is soft but thin to enable permeability (as Lorica in rotifers) but chitinous and tough in several species belonging to Cladocera, Celanoids, cyprinoids and other crustaceans. More the thickness less is the permeability and more chances for desiccation in warmer climates (anhydrobiosis), one of the causes again for Latency or Dormancy. The present work provided solid evidences to prove such idea. Indeed in rotifers also the species, in which the lorica was thin and more permeable such as *Brachionus and Lecane,* undergo quickly for quiescence than the one with thicker cuticle or lorica such as *Asplanchna*. In most of the cases food also play essential role before and after the commencement of dormancy. Insects feed voraciously before going for diapause, stop feeding and again began feeding as soon as relapsed from diapause. Whereas the aquatic organisms neither so hurry nor slow in their feeding behavior, indeed they go for dormancy if the food either of their choice or in plenty is unavailable.

The genera of *Brachiounus, Lecane* and *Rotatoria* do have good varieties of egg carrying capabilities. Many animals live in momentary habitats, and when the conditions become unsuitable for their life, they may either move to a better habitat, or if unable to actively migrate, they may persist in their environment by inflowing to dormancy. Dormancy is a temporally suspended life, characterized by reduced metabolism and arrested development. Dormant forms often present enhanced resistance to environmental stress, such as drought or extreme temperature.

The commencement and termination of dormancy were basically under the environmental conditions, although some secondary factors also apparent. The temporally suspended life may be achieved through a number of diverse physiological states, and form and duration vary consistently among animals[6]. The Monogononta and Bdelloidea rotifers consist mostly of freshwater animals those reproduced parthenogenetically and commonly found in unstable habitats. To resist uninhabitable conditions rotifers of these classes undergo dormancy.

Dormancy pattern in rotifers varies also from temperate zone to that of tropical since their ecology change according to these zones. Most rotifers such as *Brachionus, Keratella, Lecane, Fillinia and Hexarthra* were short-lived and complete their life cycle within a week in optimum temperature range of 15–25°C. The ecological conditions in freshwater environments differ from tropical to temperate zones which are likely to change steadily between cold to hot.

Thus, their life cycle either includes or does not include prolonged seasons and hence sometimes the role of environment also remains limited. In the laboratory conditions although there was no alarming change in physic-chemical conditions as well as availability of food yet the rotifers went for dormancy.

The need to understand secondary reasons for dormancy came to picture in this context thenceforth it is not only the environment, food and partner alone but also the inherent acquired traits from generations that drove them to such action.

As far as availability and choice of food was concerned, they produced cysts like encapsulated structures to escape adverse environmental conditions. It was found that when populations of *Brachionus sp.* were deprived of food in the lab for long periods of time able to produce cysts more frequently. *Keratella cochlearis* could live long for about 47–55 days in lower temperatures in contrast to *Brachionus* that lived for up to 26 days but the reverse was true if the temperature was altered. Interestingly both these forms exhibit a higher order of cyclomorphosis which also connotes with the ecological parameters especially to dormancy[2].

Insects: Grunshaw[7], used the term quiescent to express, overwintering in *P. interrupta.* In the present study however diapause was preferred, following the definitions given by Leather *et al*[8] who explained diapause as most highly evolved system of dormancy for overwintering cyclic, long-term, extremes in environmental conditions.

It was also believed that, unlike most other insects, diapause in *P. interrupta* was not initiated by abiotic factors, but by biotic factors such as nutrition. Both environmental and laboratory observations were the bases for this conclusion. In the environment, the beetles disappeared when their host (mainly sorghum) was maturing or scarce. In the laboratory, all the experimental beetles died when denied food for 15 days and continued to survive for more than 3 months without diapausing when they were provided with food every day, at intervals of every 4 days or every week (Table-1). This agrees with that of Blossey and Hunt[9] that newly emerged adults require a feeding period to accumulate fat reserves to overwinter successfully.

The new generation, emerging directly from pupae, fed actively (so become worst pest), store sufficient amounts of nutrient and enters in to the soil for diapausing. In Ethiopia the natural environment for breeding as well as diapausing areas are very hot because of the latitude (around 9^0N) and mainly because of low altitudes usually below 1800msl., particularly the diapausing season from November to end of May is known as dry season and months from February to May are especially very hot. Concerning the type of soil as physical factors, *P. interrupta* adults were found diapausing in the depth of soils ranging from 10-30cm. and mean temperature of 23^0C around Shewa-Robit and 50-60cms depth and temperature of 25-25.5^0C around Melka-worer, while average environmental temperatures were 30 and 35^0C, respectively.

This was possible because according to Leather[8] *et al.*, by locating suitable overwintering site before on set of harsh conditions, insects can mediate the adverse effects. Conditions can be modified by local effects such as inclinations and aspects of slopes, vegetation, the nature of ground surface etc.[10]. Solid ground surfaces absorb all the energy in their top few centimeters. Heat transfer in the soil is by conduction and soil is poor conductor of heat. The range of daily temperature variation decreases with increasing depth, thus providing more stable temperature environment[8].

Table-1: Mean number of survivals of *P.interrupta* beetles and their mean weight after fed for 45 days on different food and different time interval.

Number feeding	Female Mean± SE	Male Mean± SE
Fed daily with- variety of food	21.5±1.5b	23.0±0.0b
Fed daily with - banana	23.5±0.5ab	24.5±0.5a
Fed every4days-variety food	23.5±0.5ab	25.0±0.0a
Fed every 4days-onlybanana	24.0±0.0a	25.0±0.0a
Fed every week-variety of food	24.5±0.5a	25.0±0.1a
Fed every week-only banana	24.0±0.0a	24.0±0.5a
Not fed	0.0±0.0c	0.00±0.0c
Weight-Feeding		
Fed daily with- variety of food	6.67±0.63b	7.42±b
Fed daily with - banana	7.71±0.06a	7.72±b
Fed every4days-variety food	7.34±0.07ab	8.22±a
Fed every 4days-onlybanana	7.64±0.28a	7.72±b
Fed every week-variety of food	7.56±0.1ab	7.25±b
Fed every week-only banana	7.07±0.0ab	7.9±a
Not fed	0.0±0.0c	0.00±c

Means within columns followed by the same letter are not significantly different by P<0.05; DMRT.

The depths the beetles were found in the soil around Melka-Worer and Shewa-Robit were different. This is in agreement with that by Danks[11], in which he explained that the increased costs of burrowing, the deeper they go down and the fact that improved conditions effectively come latter, to deeper layers, insects cannot simply burrow as deeply as they need, to escape, the cold /hot. Instead they have to balance the advantages of escaping the harsh winter conditions with the disadvantages of burrowing well in the soil, so migrate down wards to keep just ahead the advance of the adverse conditions[12].

As far as use of vegetation as an ideal location for dormancy is concerned, in the present work all the diapausing adults were found under plant shades which is in agreement with the elucidatation that the thermal environment above the ground is further modified by vegetation. In forests, shelter from the sun, heat loss by evaporation blanketing at night, reduction of wind speed and the impeding of vertical air movement, all influence the temperature effects.

Before a successful overwintering can occur, insects must prepare themselves for the potentially dangerous conditions that lie ahead; these biochemical changes associated with cold hardness are often substantial e.g 25 percent of the fresh weight of over-wintering *Bracon cephi* (Hymenoptera, Braconidae) is glycerol. Cryoprotectants are usually manufactured only as winter approaches[13]. Abdominal lipid contents are vital to the overwintering survival of adult Lepidoptera[14]. The death of all insects in the group which were not fed for 15 days in the present work (Table-1) agrees with statements mentioned above. That is unless they are pre-fed and prepared for the coming harsh conditions, the beetles could not undergo diapause successfully. At the same time the persistently increasing of lean dry weight, or size of the beetle and fat weight both in females and males obtained for prediapausing adults (days 1-3) (Figures-1 and 2) of the present work indicates that they were preparing themselves for diapausing successfully. This is in agreement with Pullins[14], who demonstrated that the feeding time allowed to the adult butterflies prior to diapause was significantly correlated with the weight of lipids present in the abdomen. The longer the adult butterflies fed before diapause, the longer they were able to survive over the winter. These could serve as an indications that the short pre-diapause period was used for fast growing and accumulation of food reserves, the absence of which led to death of all the experimental insects (Table-1).

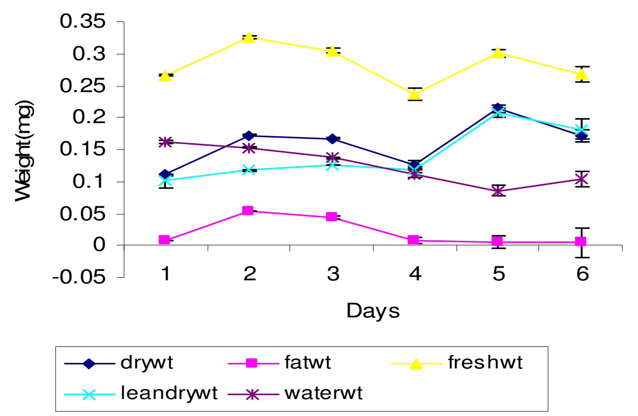

Figure-1: Physiological factors measured for females *P. interrupta* at different days, 1-3 (pre-diapause) and 4-6 days (during diapause).

According to Wolda and Delinger[15], the major obstacle to be surmounted by insects in diapause is obtaining sufficient energy. This problem is especially acute on adults, as these require more energy than larvae, pupae or eggs. During diapause, the metabolic activity is lower than when the insect is active but some energy is still needed for maintenance and this is drawn from fat body reserves. As indicated in (Figure-1 and 2), the amount of fat continued decreasing particularly by day 5 and finally the least was recorded by day 6. This extremely least amount of fat was besides its utilization to maintain metabolic activity; more fat was also needed to support energy for newly regenerating muscle tissues that occur at the end of diapause that agrees with the findings by Wolda and Delinger[15], in which they observed samples taken for one full season from diapausing tropical beetle known as *Stenotarsus rotrundus.* Variations were observed, in phase I and phase III (last phase) where amount of lean dry weight and fat weight were seen decreasing and this was associated to flight muscles which were not degenerated in phase I and in phase III (last phase), when flight muscles were developing. There was no clear general decrease in lean dry weight and this was an indication that non-lipid reserve was not used. During phase I, where degeneration and during phase III, when the flight muscles and ovaries were developing the decrease in fat content, that is fat utilization was larger than phase II (long period of diapause).

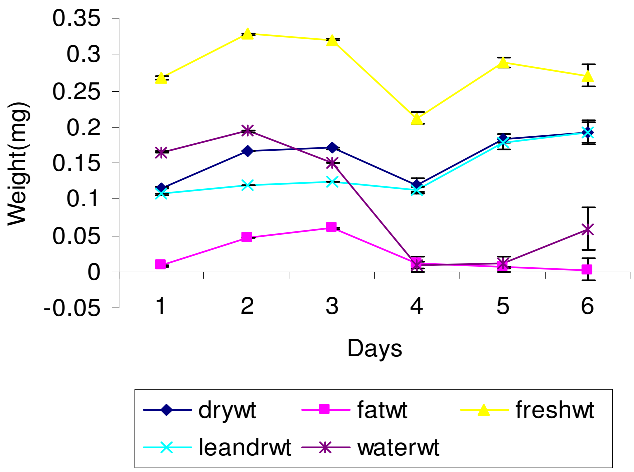

Figure-2: Physiological factors measured for males *P. interrupta* at different days, 1-3 (pre-diapause) and 4-6 days (during diapause)].

The phenomenon of dormancy in all animals is hence a natural process to avoid a particular cause for certain period which may last for few hours up to decades in some animals. In higher animals the behavior may not be visible directly as found in case of the lower but more or less they follow the same principle. The presence of highly developed integumentary system, presence of metanephric kidneys and rich osmo-regulation made the higher animals more suitable to avoid dormancy which was also the drawback for the lower animals. The phenomenon of dormancy is very much wide and wild that needs more understanding and further study. For the time being it can be assumed only as a tool of adaptation to make the animal sustain its life to avoid unnecessary extinction.

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