INTRODUCTION

Insect pest management in stored food commodities using chemicals is facing many challenges due to the concerns of human health safety, development of insect resistance against the chemical pesticides and creating environmental hazards (Hagstrum et al., 1999, Phillips et al., 2000, Daglish and Wallbank, 2002, Nayak et al., 2005, Daglish and Nayak, 2006). The loss in quality and quantity of the stored grains and other food materials due to insect attack is a major threat for the future food security of a nation. So, worldwide researches are focused on the search of alternative insect control measures against the stored-insect pests. Among the various alternative options for insect control, the biological control has attracted the interest of the stored entomologists because the bio-control agents are present in the same environment that of the pest insects, reduced risks for beneficial insects, benign for the environment and its biota, and safe for human health.

A number of bio-control agents like parasites, parasitoids and predators have been continually screening against the stored insect pests. The warehouse pirate bug, *Xylocoris flavipes* (Reuter) is a predator and potential in controlling different insect species of the grain (Brower et al., 1996, Scholler et al., 1997, Visarthanonth et al., 1990, 1994; Imamura et al., 2008). This hemipteran predator preys on eggs, larval instars and pupae of different insect species depending on the size of the prey (LeCato and Davids, 1973), and age of prey/host insect (Vinson and Ivantsch 1980). The biological parameters of *X. flavipes* is limited by a number of factors like temperature and humidity of the store, host species, and even on the food on which the host is developed (Press et al, 1976, Russo et al. 2004, Ferdous 2006, Ferdous et al. 2009, Rahman et al., 2009).

The flat grain beetle, *Cryptolestes pusillus* (Schon.), is a member of the family Cucujidae under the order Coleoptera. The beetle is an external feeder and a serious cosmopolitan pest of stored product commodities especially cracked grains (Barker, 1976 and Hole et al., 1976). It multiplies rapidly and subsequently build up into a huge population within very short period of time (Rahman et al., 2008). *C.
*pusillus* is consumed as a prey by the warehouse pirate bug, *X. flavipes*.

This paper intends to report study of the biological parameters of the hemipteran predator *X. flavipes* while preying on different life stages of the host *C. pusillus*.

**MATERIALS AND METHODS**

**Host insect species**

Adults of *C. pusillus* were collected from the stock culture maintained since 15 years in the Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi. The stock culture is maintained on standard food medium (whole wheat flour and powdered Brewer’s yeast in a ratio of 19:1) (Park 1962, Zyromska-Rudzka 1996) at 30±1°C and 70±0.5% RH in a climatic room. Five hundred adult beetles were collected and divided into five groups consisting of 100 adults. Beetles of each group were then kept in 500 ml beaker provided with 25g of sterilized standard food medium. Few pieces of filter papers were placed in the beaker for easy movement of the beetles. Mouth of the beaker was covered with a piece of fine cloth and rubber band to prevent the escape of the beetles. Food present in the beaker was replaced after every three days. After 24h, the adults were removed and the deposited eggs were collected through sieving the food by 125 micrometer aperture sieve. The collected eggs were placed on a piece of black paper and gently cleaned using a fine camel hair brush. Eggs were kept 3-4 days (d) for hatching.

**Collection of larvae, pre-pupae, pupae and adults**

After hatching, 1st, 2nd, 3rd and 4th instars of larvae were obtained from 4-5d, 5-6d, 4-5d and 6-7d respectively. Pre pupae and pupae were observed inside the cocoon at 3-4d and 4-5d respectively. The larvae and pupae were confirmed by random examining through a magnifying glass. Pupae emerged as adults at 4-5d. All the cultures were maintained in the Climatic room at 30±0.5°C temperature and 70±0.5% RH to ensure constant and regular supply of different life stages of *C. pusillus* of known age throughout the study period.

**Predator insect species**

Adult *X. flavipes* were collected from the culture maintained in the Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, rearing since 10 years. The bugs were reared on either eggs or larval instars (1st, 2nd, 3rd and 4th separately) and pupae of *C. pusillus* at 30±1°C and 70±0.5% RH in a Climatic room. For continuous rearing of the predators, 50 g of standard food of the host insect was kept in the culture container. After every three days the host’s food was replaced by a fresh one.

In a 500 ml beaker 200 unsexed adult predators were kept. Either 1st or 2nd instar larvae or pupae of *C. pusillus* were given in the beaker to feed the predators. In such a way separate adult predators of similar number were reared on either one of the food mentioned. Using a fine camel-hair brush the adults were removed after 24h. Eggs laid by those adults were found at the bottom of the beakers. Healthy eggs were examined using microscope, and kept 4-5d for hatching.

**Collection of nymphs and adults**

The newly hatched nymphs were collected carefully and kept in a 500 ml beaker, using fine camel-hair brush. The nymphs were fed with 1st and 2nd instar larvae of *C. pusillus*. The 2nd, 3rd, 4th and 5th instar nymphs of the predator were obtained from this culture on the 3rd, 5th, 8th and 12th d after hatching. The nymphal instars were determined by counting the exuviae deposited in the beaker. The 5th instar nymph transfer into adults.

**Determination of sex**

Sex of *X. flavipes* was separated at the adult stage. The major character of sex dimorphism is the shape of abdomen. In female the abdomen is bilaterally symmetrical which in male it is notched at left side of the segments 8 and 9.

**Bioassays**

Seventy newly hatched nymphs of 4-6d age were placed in seven Petri dish (9 cm diameter), keeping 10 nymphs per Petri dish. Two hundred fresh eggs or 25 larvae of each instar
(1st - 4th) separately, or 10 pupae of *C. pusillus* were given as food to the nymphs. After every 24 h, consumed or killed life stages of *C. pusillus* by *X. flavipes* were observed and counted. The dead and left over parts of the nymphal food were discarded daily. The daily supply of food for the nymphs were kept constant by adding the similar life stages of the host that consumed by the nymphs. The nymphs were regularly observed for ecdysis. Number of nymphal ecdysis was recorded for each nymph with the duration for each instar. Regular supply of eggs, larvae up to 4th instar and pupae of *C. pusillus* was maintained until the death of *X. flavipes*.

Parameters studied
The following biological parameters of *X. flavipes* were studied:

1) nymphal developmental time, 2) longevity of adult males and females, 3) rate of prey consumption, 4) number of survived nymphs, 5) size of male and female adults (length mm, measured by an ocular micrometer), 6) number of male and female adults and (7) sex-ratio.

All the experiments were replicated three times and conducted in Climatic room at 30 ± 0.5ºC and 70 ± 0.5% RH.

Data analysis
Difference in the effects on the biological parameters of the predator while preying on different life stages of the host insect was compared using the factorial ANOVA. The comparison of mean values of individual parameters was compared by Tukey’s test provided in 1953. Significance difference between the sex-ratio of the predator was tested using χ² test.

RESULTS AND DISCUSSION
Developmental period of *X. flavipes*
*X. flavipes* is able to complete its development on the life stages of the host *C. pussillus*. While this bug only consume the eggs and larvae of the host. The mean total developmental period of the bug was recorded minimum (12 ± 1.15 d) when fed on host’s pupae, and the development was delayed (22 ± 0.58d) when fed on the 2nd instar larvae of the host (Table 1). The developmental period of *X. flavipes* was found differ while feeding on different life-stages of the host, and the P-value was = 0.05.

Adult longevity and total life span of *X. flavipes*
Life span of the predator varied with the different life stages of host. Adult of *X. flavipes* were found to prey very actively on 2nd to 4th instar larvae. In case of males, the maximum longevity was 12±1.15 and the minimum was 6±0.58 while feeding on similar food like the females (Table 1). The highest longevity of the females was 31±1.15 days and the lowest was 14±1.15 days, when fed on 2nd instar larvae and pupae respectively. The effect of different life stages of prey on adult longevity was significant (Table 1). Total life span of *X. flavipes* was found to range from 18-39 days in female and 26-53 days in male (Table 1). The 2nd instar larvae of host extended the adult life span in both cases, and short life spans were obtained when fed on the pupae.

Average prey consumption rate of *X. flavipes*
Average prey consumption rate was found to differ depending on the life stages of *C. pussillus*. The predator preyed maximum number of eggs compared to other stages of the beetle; the pupae of the host was least preferred by all the life stages of the predator (Table 2). The number of eggs preyed varied at different nymphal instars, and the number of prey was increased with the age of the nymphs. The range of numbers of eggs preyed by a single predator was recorded as 7.33±0.33 - 14.33±0.33 per day by the 1st and 5th instar nymphs respectively. A male predator fed on 16.33±0.88 eggs, and a female predator fed on 20.67±0.33 eggs (Table 2). The number of pupae preyed per day by single predator was found to vary as 1.33±0.33 - 2.67±0.33 for the 1st and 5th instar larvae respectively. The male and female predator consumed 4.33±0.33 and 5.67±0.33 pupae of *C. pussillus* per day, and the female predator always consumed more prey than the male. Prey consumption rate was significantly dependent on the life stages of *C. pussillus* (Table 2).
Survivability of X. flavipes
Survival rate of the predator varied with the life stages of the host. Mean survivability of the nymphal instars was higher when fed on 2nd and 1st instar larvae of the prey insect (Table 3). Survivability of male adults was higher when preyed on 1st instar larva, and that of female adults was higher when fed on 4th instar larva and pupae of the prey insect (Table 3). Survivability rate of X. flavipes significantly varied on different life stages of C. pussilus (Table 3).

Size of X. flavipes fed on different life stages of C. pussilus
Normally females are larger in size than males. Size of males were greater when they preyed on 2nd and 1st instar larvae, and that of the females was more when they fed on 2nd and 3rd instar larvae, of C. pussilus. The adult size was minimum when they fed on the eggs of the prey insect (Table 4). The largest males and females were measured as 1.80±0.01 and 2.10±0.01 mm respectively feeding on 3rd instar larvae, and shortest sizes were 1.50±0.06 and 1.70±0.06 mm respectively when fed on eggs (Adult size of the predator varied significantly with the different life stages of the host insect (Table 4)).

Adult number and Sex-ratio of X. flavipes
Mean number of male predator was found to range from 30.00±2.89 – 45.00±1.15 feeding on eggs and 1st instar larvae of C. pussilus; the number of females ranged from 70.00±2.89 – 71.67±4.04 feeding on eggs and pupae of the beetle respectively (Table 4). Adult number was significantly varied with the life stage of the host (Table 4).

Table 1: Developmental periods and adult longevity of X. flavipes fed on different life stages of C. pussilus under laboratory condition

| Life stages of C. pussilus | Mean Developmental periods (day) of nymphal instar | Total duration (day) of nymphal stages | Adult longevity (day) of X. flavipes | Total duration (day) |
|---------------------------|---------------------------------------------------|--------------------------------------|-------------------------------------|---------------------|
|                           | 1st      | 2nd      | 3rd      | 4th      | 5th      | Male | Female | Male | Female |
| Eggs                      | 3±0.58   | 2±0.58a  | 3±0.58ab | 3±0.58a  | 4±1.15a  | 15±2.00bed | 8±1.15ab | 20±1.15c d | 23   | 35     |
| 1st larvae                | 4±0.58A  | 3±0.58a  | 5±0.58a  | 4±0.58a  | 4±0.58a  | 20±0.00ab | 10±1.15ab | 25±2.89b c | 30   | 45     |
| 2nd larvae                | 4±0.58A  | 3±0.58a  | 5±0.58a  | 5±0.58a  | 5±0.58a  | 22±0.58a  | 12±1.15a  | 31±1.15a b | 39   | 53     |
| 3rd larvae                | 4±0.58A  | 3±0.58a  | 4±0.58ab | 3±0.58a  | 4±0.58a  | 18±1.00abc| 11±1.15a  | 28±1.15a  | 29   | 56     |
| 4th larvae                | 3±0.58   | 2±0.58a  | 3±0.58ab | 3±0.58a  | 3±0.58a  | 14±1.15de | 9±0.58ab  | 26±0.58b c | 23   | 40     |
| Pupae                     | 3±0.0a   | 2±0.0a   | 2±0.58b  | 3±0.58a  | 2±0.0a   | 12±1.15d  | 6±0.58b   | 14±1.15d  | 18   | 26     |

Note: Means with same letter do not significantly differed from each other Tukey’s Test, P<0.001

ANOVA

| Factors (df) | F-values (significance level) at nymphal instars of X. flavipes | Adult X. flavipes |
|--------------|---------------------------------------------------------------|-------------------|
|              | 1st               | 2nd               | 3rd               | 4th               | 5th               | Male | Female | Male | Female |
| Life stages of host (5) | 11.695 (P=0.054) | 16.111 (P=0.054) | 16.429 (P=0.054) | 10.331 (P=0.054) | 9.650 (P=0.054) | 8.720 (P=0.054) | 7.423 (P=0.054) |
| Replication (2) | 7.476 (P=0.017)  | 11.067 (P=0.054) | 8.513 (P=0.054) | 6.329 (P=0.054) | 5.936 (P=0.054) | 5.388 (P=0.054) | 4.684 (P=0.054) |
| Host* Replication | 2.883 (P=0.528)  | 4.436 (P=0.054) | 4.829 (P=0.054) | 3.469 (P=0.054) | 3.501 (P=0.054) | 3.440 (P=0.054) | 2.869 (P=0.054) |
Sex ratio of the male-female *X. flavipes* was different based on the life stage of the host insect. Sex-ratio was significantly differed from 1:1, when the predator preyed on the eggs, 4th instar larvae and pupae of the prey insect (Table 4).

From the results it is revealed that developmental period, longevity, prey consumption, survival, size, adult number and sex-ratio of the predator *X. flavipes* differs depending on the life stages of the prey insect, *C. pussilus*. Growth and development of an organism fully depends on their diet. The predator *X. flavipes* preys on a number of insect species of the stored food commodities. The total developmental time of the predator was reported as 16.53±0.13 days when preyed on *Tribolium castaneum* at 30±1°C and 70% RH (Saha et al. 2012), whereas, in the present experiment the total developmental time of the predator was recorded minimum as 12±1.15 days when preyed on pupae of *C. pussilus* at similar temperature and relative humidity. So, development of the predator is faster when it feeds on young stages of *C. pussilus* than when fed on larvae of *T. castaneum*. According to Brower and Press (1992) and Abdel Rahman *et al.* (1978-79), *C. pussilus* and *Rhizopertha dominica* were the most suitable prey of *X. flavipes*. The predator developed faster, lived longer as an adult, survived better in the immature stage and laid more eggs when fed on coleopteran larvae rather than lepidopteran larvae (Abdel Rahman *et al.* 1978-79). As intrinsic factors both temperature and relative humidity considerably affect the duration of nymphal and adult stages of *X. flavipes* (Abdel Rahman *et al.* 1977 and Arboagast 1978), however, in the present experiment both these factors were kept constant throughout. In the present study mean developmental periods of nymphal and adult stages of *X. flavipes* (Abdel Rahman *et al.* 1977 and Arboagast 1978), however, in the present study both these factors were kept constant throughout. 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In the present study mean developmental periods of nymphal and adult stages of *X. flavipes* (Abdel Rahman *et al.* 1977 and Arboagast 1978), however, in the present study both these factors were kept constant during the egg, 1st, 2nd and 3rd instar larvae and pupae of the prey insect (Table 4).
ever, the present study revealed that the adult males lived for 8±1.15-12±1.15 days and the adult females lived for 20±1.15-31±1.15 days feeding on eggs and 2nd larvae of C. pussilus, respectively at 30°C. Whereas, when temperature is 35°C development of the eggs and the nymphal stages were decreased and shortened the life span of the adults of X. flavipes on T. castaneum (Abdel Rahman et al. 1977). Daily consumption rate of adults varies with the size and life stage of the prey, and gut capacity of the predator. X. flavipes killed significantly more ‘stimulating’ larval prey than ‘easy’ egg prey (Lecato and Arbogast, 1979; Russo and Vasta, 2004). Lecato and Collins (1976) mentioned that X. flavipes destroys large quantities of prey when prey is abundant. In the present study it was observed that when an excess of eggs, 1st, 2nd, 3rd, 4th instar larvae and pupae of C. pussilus were provided, each predator killed an average of 300 eggs, 49 larvae and 25 pupae of C. pussilus, but when different life stages of the host were provided separately, each predator destroyed an average of 400 eggs, 60 larvae and 28 pupae. The predator when preyed on the larvae of different pest insects separately, it fed on 105 larvae of Cocyra cephalonica, 112 larvae of T. confusum, 30 larvae of Stegobium panicerum, 148 larvae of Lasioderma serricorii during 43 days of life span (Awadallah et al., 1986).

**CONCLUSION**
The present investigation revealed nymph up to 5th instar and adult X. flavipes can kill and

Table 3: Average number (±SE) of survivability of different life stages of X. flavipes on different life stages of C. pusillus

| Life stages of C. pusillus | Nymphs | Adults |
|----------------------------|--------|--------|
|                            | 1st    | 2nd    | 3rd    | 4th    | 5th    | Male | Female |
| Eggs                       | 9.00±0.58a | 8.33±0.33abc | 7.33±0.33ab | 6.33±0.33a | 5.33±0.33a | 3.67±0.67a | 6.33±0.67a |
| 1st larvae                 | 10.00±0.00a | 9.67±0.33ab | 8.00±1.53ab | 8.00±1.53a | 7.00±1.15a | 4.67±0.88a | 5.33±0.88a |
| 2nd larvae                 | 10.00±0.00a | 10.00±0.00a | 10.00±0.00a | 8.67±0.33a | 6.67±0.88a | 3.33±0.88a | 6.67±0.88a |
| 3rd larvae                 | 9.67±0.58a | 6.67±1.20c | 5.00±1.15b | 5.00±1.15a | 4.67±1.45a | 3.67±1.45a | 5.67±0.88a |
| 4th larvae                 | 8.33±0.58a | 6.00±0.58c | 5.67±0.33b | 5.00±0.58a | 4.67±0.33a | 2.33±0.33a | 7.67±0.33a |
| Pupae                      | 8.00±0.58a | 7.00±0.58bc | 6.33±0.33ab | 5.33±0.67a | 4.33±0.33a | 2.67±0.33a | 7.33±0.33a |

Note: Means with same letter do not significantly differed from each other Tukey’s Test, P<0.001

ANOVA

| Factors (df) | F-values (significance level) at nymphal instars of X. flavipes | Adult X. flavipes |
|--------------|-----------------------------------------------------------------|-------------------|
|              | 1st                 | 2nd               | 3rd               | 4th               | 5th       | Male       | Female       |
| Life stages of host (5) | 30.09 (P=0.000) | 36.245 (P=0.001) | 38.471 (P=0.005) | 36.061 (P=0.005) | 27.077 (P=0.005) | 28.106 (P=0.005) | 29.282 (P=0.006) |
| Replication (2) | 10.85 (P=0.922) | 15.208 (P=0.922) | 12.736 (P=0.985) | 5.422 (P=0.995) | 3.559 (P=0.725) | 4.191 (P=0.814) | 5.545 (P=0.858) |
| Host* | 4.618 (P=0.33) | 4.215 (P=0.623) | 5.307 (P=0.521) | 4.620 (P=0.936) | 3.540 (P=0.859) | 4.160 (P=0.693) | 5.698 (P=0.574) |
| Replication | (P=0.33) | (P=0.623) | (P=0.521) | (P=0.936) | (P=0.859) | (P=0.693) | (P=0.574) |
consume eggs, larvae up to 4th instar and pupae of *C. pusillus*. Eggs, larvae up to 4th instar and pupae of host fluctuated the duration of developmental periods of each nymph, adult longevity, consumption rate, survivability rate, size and sex ratio of the predator. *X. flavipes* preferred 1st, 2nd and 3rd instar larvae followed by the 4th instar larvae and pupae. The female predator always consumed more prey than the male.

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**Table 4:** Average (±SE) adult size (mm in length) and number of male and female *X. flavipes* preyed on different life stages of *C. pusillus*

| Parameters          | Sex          | Eggs       | 1st | 2nd | 3rd | 4th | Pupae |
|---------------------|--------------|------------|-----|-----|-----|-----|-------|
| Adult size (± mm in length) | Male         | 1.50±0.06c | 1.60±0.03ab | 1.70±0.03ab | 1.80±0.01a | 1.65±0.03abc | 1.55±0.04bc |
|                     | Female       | 1.70±0.06c | 1.90±0.03b  | 2.00±0.03ab  | 2.10±0.01a  | 1.95±0.03ab  | 1.85±0.04bc |
| Number of Adults    | Male         | 30.00±2.89b | 45.00±1.15a | 38.33±2.73ab | 38.00±2.31ab | 35.00±3.46ab | 28.33±2.03b |
|                     | Female       | 70.00±2.89ab | 55.00±1.15b | 61.67±2.73b  | 62.00±2.31ab | 65.00±3.46ab | 71.67±4.04a |
| Sex-ratio           | Male:Female  | 1:2.33     | 1:1.22      | 1:1.61       | 1:1.63       | 1:1.86       | 1:2.53     |
| X2- value (df=1)    |              | 16.00      | 1.00 (NS)   | 5.44 (P=0.02) | 5.76 (P=0.02) | 9.00 (P=0.01) | 18.78 (P=0.001) |

Note: Means with same letter do not significantly differed from each other Tukey’s Test, P<0.001

**ANOVA**

| Factors (df) | Size of Adult *X. flavipes* | Number of Adult *X. flavipes* |
|--------------|----------------------------|-------------------------------|
|              | Male | Female | Male | Female | Male | Female |
| Life stages of host (5) | 8.52 | 0.001 | 9.217 | 0.002 | 9.611 | 0.005 | 9.332 | 0.007 |
| Replication (2) | 1.484 | 0.271 | 2.141 | 0.262 | 4.122 | 0.025 | 4.004 | 0.026 |
| Host* Replication | 4.752 | 0.006 | 5.182 | 0.007 | 6.769 | 0.006 | 6.570 | 0.009 |
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