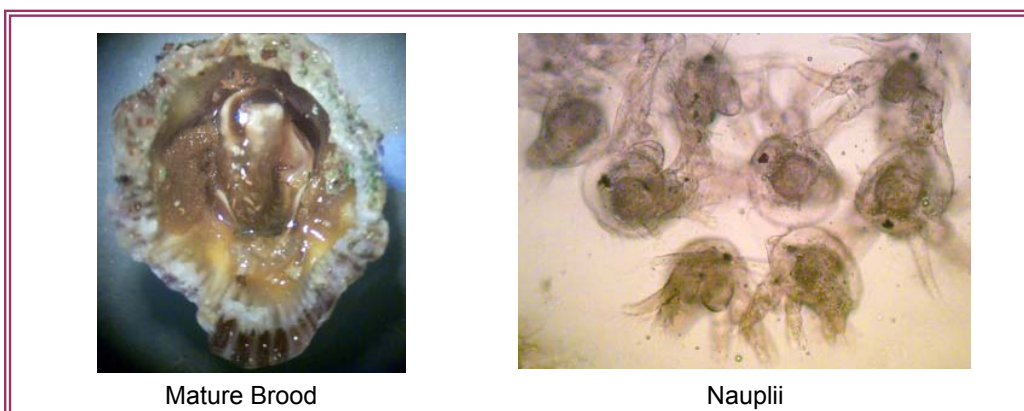


*Chapter 2*  
*Reproduction*

## 2.1 Introduction

Balanomorph barnacles are hermaphrodite (Charnov 1987), fertilization is internal and usually capable of producing eggs and sperms at the same time. Exchange of sperms between adjacent individuals, leading to cross fertilization, appears to be the rule among acorn barnacles. Male barnacles are tiny, and are usually found in close association with much larger females or hermaphrodites. The hermaphroditism of typical acorn barnacle shows a



tendency towards protandry; that is male reproductive function often matures at a smaller size than does female function (Crisp 1983; Newman 1980). Thus some individuals can begin reproducing as males, later to become hermaphrodites. However incidences of self-fertilization are also reported in barnacles (Barnes and Crisp 1956; El-Komi and Kajihara 1991). Self-fertilization seems to be less effective in producing normal embryos than cross-fertilization. Barnacle reproduction falls into two broad patterns, those that produce one brood during the breeding season (e.g. *Balanus balanus* and *Semibalanus balanoides*) and those that produce many broods (e.g. *Balanus amphitrite*). Eggs are retained in the mantle cavity, where they develop into nauplii. Barnacle releases these nauplii to the environment.

Several studies on reproduction and molting in barnacles have been conducted (Crisp and Davies 1955; Crisp and Patel 1958,1961; Patel and Crisp 1960a,b; Hurley 1973; Hines 1978; El-Komi and Kajihara 1991). It was observed for *Balanus balanoides* that the energy available for egg production is distributed among some 4000-10000 small eggs, according to the size of the adult, which hatch into planktotrophic nauplii. Egg size is a compromise between two extremes: the smaller the egg, the greater the fecundity, yet the greater the energy reserves it contains, greater the probability of its survival (Crisp 1986). The greater the concentration of reserves the better and, of those available, lipid is energetically superior to protein or carbohydrate (Crisp 1984). Barnes and Barnes (1975) reviewed the effect of temperature, light and food on population synchrony with particular reference to cirripedes. In general reproductive processes are sensitive to temperature (Barnes 1989). Some cirripedes will breed over a wide range of temperature whereas some will breed until a certain critical temperature is reached. This critical temperature may be above or below that of their natural environment. Thus temperature becomes an ecologically important component in determining the latitudinal distribution of cirripedes. In tropical regions where temperature is more constant or fluctuates slightly around mean values, breeding might be expected to be asynchronous in a population. However in the regions with wide variation or fluctuations in temperature it might synchronize breeding (Barnes 1989).

It is also reported that the food concentration plays an important role in the breeding cycle of the cirripedes. Barnes and Barnes (1967,1975) have shown that feeding in *Balanus balanoides* and *Verruca stroemia* can control the time

at which egg lamellae are produced, also poor nutritional conditions can cease breeding. As far as energy budget is concerned it is shown that in case of *Balanus glandula* the production of eggs takes second place after respiration followed by production of shell and body tissue (Wu and Levings 1978). This indicates the utilization of energy reserves for the growth and reproduction.

In view of the importance of temperature and food available in breeding, experiments were carried out to evaluate their effect on breeding of *Balanus amphitrite* in the laboratory. Experiments were carried out to observe incidences of self-fertilization. It has been reported earlier that the molting cycle is affected by reproduction (Patel and Crisp 1961; Barnes 1962a). Hence observations were also made on molting frequency at different temperatures and food concentrations.

## **2.2 Materials and Methods**

### **2.2.1 Breeding and molting**

Adult barnacles collected from the intertidal area of Dona Paula bay were brought to the laboratory and were cleaned for the epibiotic growth with a nylon brush. The barnacles with rostro carinal basal diameter of 8-12mm were used in the study. These barnacles were mounted on acrylic plates (8x3 cm) as solitary and in pairs (5 replicates) using artificial glue (Super Wiz).

Acrylic plates with barnacles mounted on it were put in filtered seawater (GF/C) in 250ml capacity glass beakers. Seawater (35‰) in beakers was changed everyday. These barnacles were fed with newly hatched *Artemia* nauplii. The number of molts and/or the larvae released were quantified prior

to change of medium and food everyday. The numbers of left out *Artemia* sp. were also counted.

### Experiment 1

Observations on breeding and molting were made for 75 days at different temperatures (20, 25 & 30° C) and food concentrations (50, 100, 150 and 200 *Artemia* ind<sup>-1</sup>).

### Experiment 2

Observations on breeding and molting were carried out for 203 days. This was carried out only at 30° C, at all the food concentrations (50, 100, 150 and 200 *Artemia* ind<sup>-1</sup>). Food and seawater were changed everyday as mentioned above.

### Experiment 3.

The nauplii released by the paired barnacles maintained at 30° C (fed with 200 *Artemia* ind<sup>-1</sup>) were collected and reared till they developed into cyprid instar in the laboratory using *Chaetoceros calcitrans* (unicellular diatom) as food at a cell concentration of  $2 \times 10^5$  cells ml<sup>-1</sup>. These cyprids were put into the polystyrene multiwells (5 ml) for settlement. Subsequently the spats were provided with unicellular algae (*Chaetoceros calcitrans*) and *Artemia* as food. Once these juveniles attained a size of 5mm (rostrum carinal basal diameter) they were separated as solitary and paired individuals and maintained at 30° C at a food concentration of 100 *Artemia* ind<sup>-1</sup>. The number of molts and broods were monitored everyday prior to change of food and seawater.

### 2.2.2 Carbon Quantification

Carbon quantification was done by calculating the amount of food intaken by counting the left out *Artemia* nauplii in each of the beakers. Single *Artemia* nauplius corresponds to  $\sim 0.52\mu\text{g C}$  (Omori and Ikeda 1984). Thus the total number of nauplii fed were multiplied by this factor to get the energy gained in terms of carbon and is expressed as  $\mu\text{g C}$  assimilated.

### 2.2.3 Field Observation of Gonads

Studies on temporal variations in gonad development were carried out for *Balanus amphitrite* population at Dona Paula bay from March 1997 to September 1999, and observations were taken every fortnight. Collection of the individuals was done as solitary (having no other barnacle in the vicinity of 5 to 6 cms) and crowded.

Differentiation of the gonad stages (0-IV) is based on the coloration and developmental condition of the ovary (Anil et al. 1995). At stage 0, the ovary is absent. Stage I is a growing stage, where the ovary is granular and present as a thin cream-colored layer of ovarian tissue on the basal membrane. At Stage II, the ovary is creamy yellow in color and fills about a third of the mantle. At Stage III (mature stage), eggs in the ovary occupy most of the mantle (bright yellow) and at Stage IV nauplii with visible eyespots are present (dark brown).

## 2.3 Results

### 2.3.1 Breeding, Molting and food consumption (Experiment 1)

#### Breeding

In case of paired individuals the frequency of breeding was high when compared to solitary ones (Fig. 2.1 a,b,c). In case of solitary individuals at  $30^\circ\text{C}$ , breeding was observed at all the food concentrations (Fig. 2.1c). However

at lower temperature this was not the case (Fig. 2.1a,b). Breeding interval was comparatively longer for solitary individuals than the paired ones. Breeding interval was longer at 30° C (Fig. 2.1f) and decreased with a decrease in temperature (Fig. 2.1f,d). The maximum breeding interval for solitary individual was 43days and was observed at 30° C (50 *Artemia* ind<sup>-1</sup>) (Fig. 2.1f). The minimum breeding interval observed was 19days at 20° C (150 *Artemia* ind<sup>-1</sup>). In case of paired individuals, an increase in food concentration resulted in an increase in the number of broods almost at all the temperatures (Fig. 2.1 a-c). However only at 100 *Artemia* ind<sup>-1</sup> maximum number of broods were observed at 20° C (Fig. 2.1a).

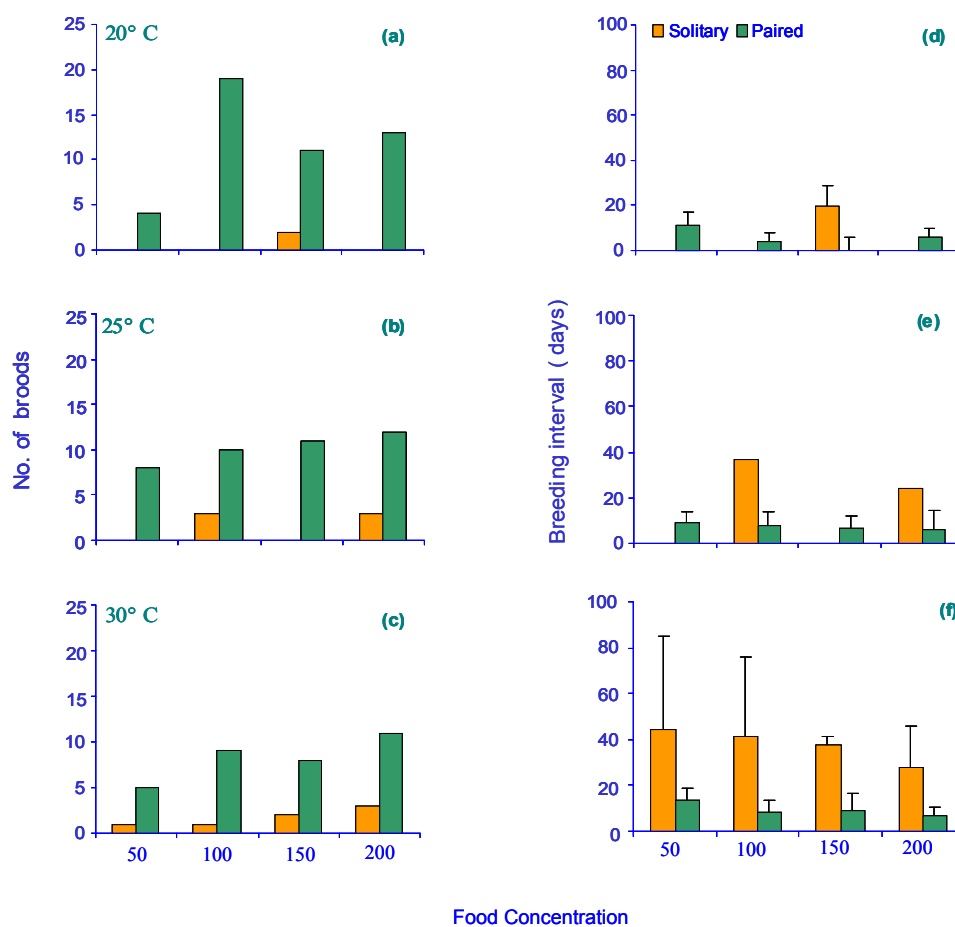


Fig. 2.1 Number of broods and breeding interval for barnacles reared at different temperatures and food concentrations

Regression analysis between the number of larvae released vs. the number of days (experimental schedule) for which the barnacles were raised indicated that the number of larvae released increased with the age of the barnacles. This increase was significant in case of barnacles maintained at 30° C fed with 150 & 200 *Artemia* ind<sup>-1</sup> (Fig. 2.2a,b) and at 25° C (Fig. 2.3a) fed with 200 *Artemia* ind<sup>-1</sup>. At 20° C, such an increase was not evident (Fig. 2.4a-d). The paired individuals (Fig. 2.5a) released maximum number of larvae at 200 *Artemia* ind<sup>-1</sup> at 30° C. However at 20 and 25° C at similar food concentrations, the number of larvae released were less (Figs. 2.6a and 2.7a; Table 2.1).

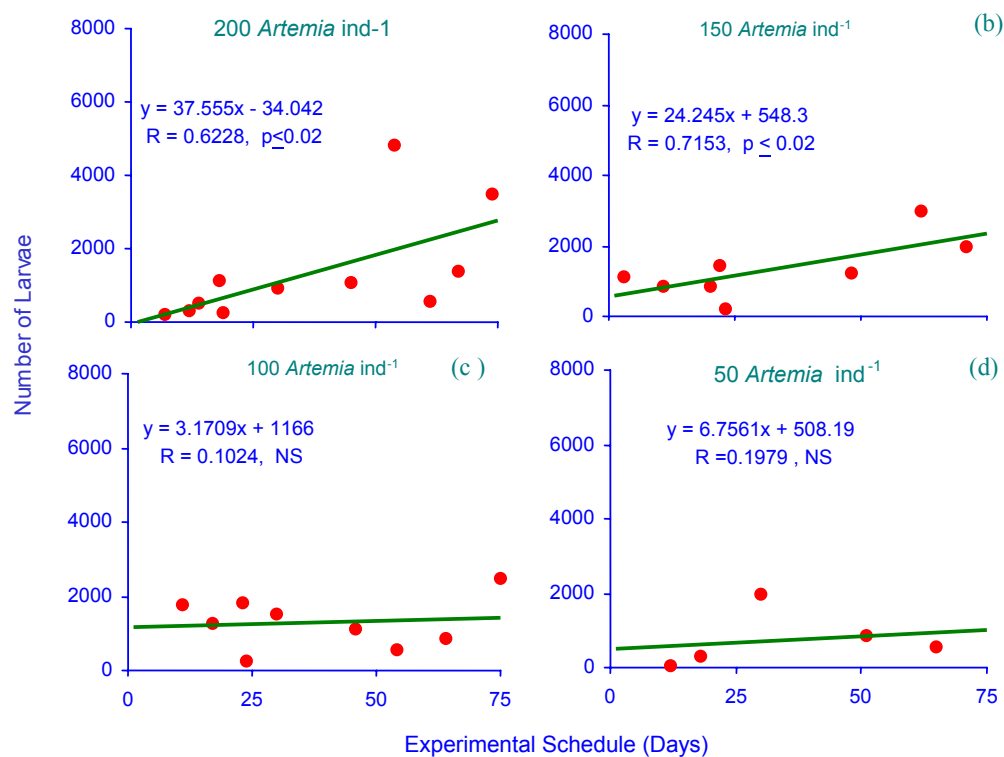


Fig. 2.2 Relationship between the number of larvae released with the experimental schedule (days) in paired individuals at 30° C. Ns – Not Significant



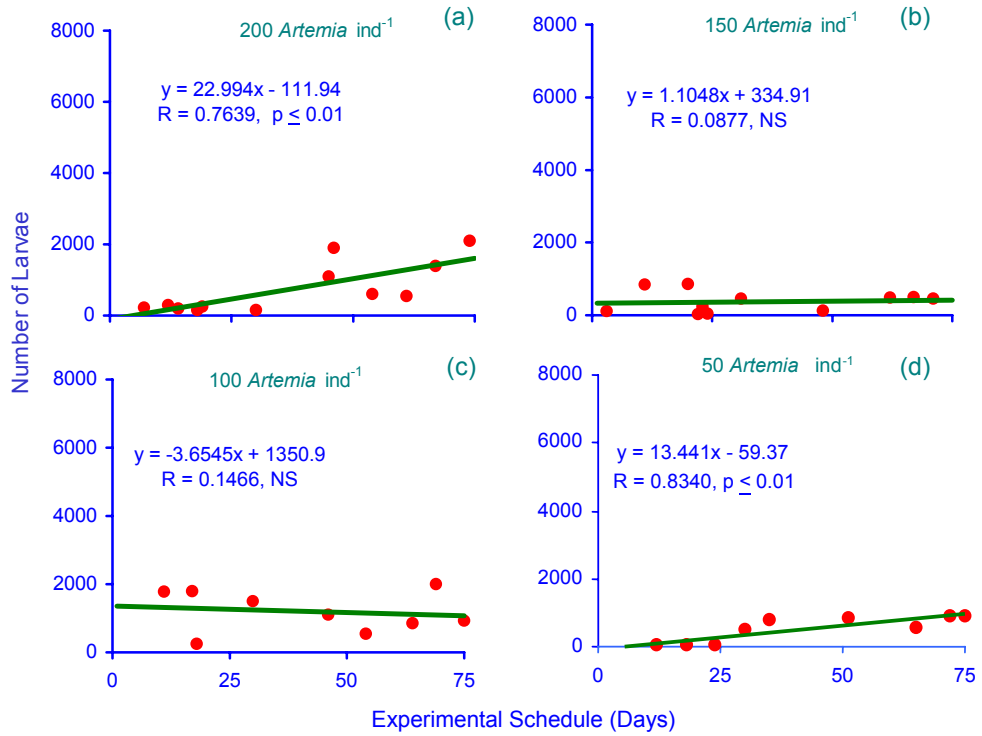


Fig. 2.3 Relationship between the number of larvae released with the experimental schedule (days) in paired individuals at 25° C. Ns – Not Significant

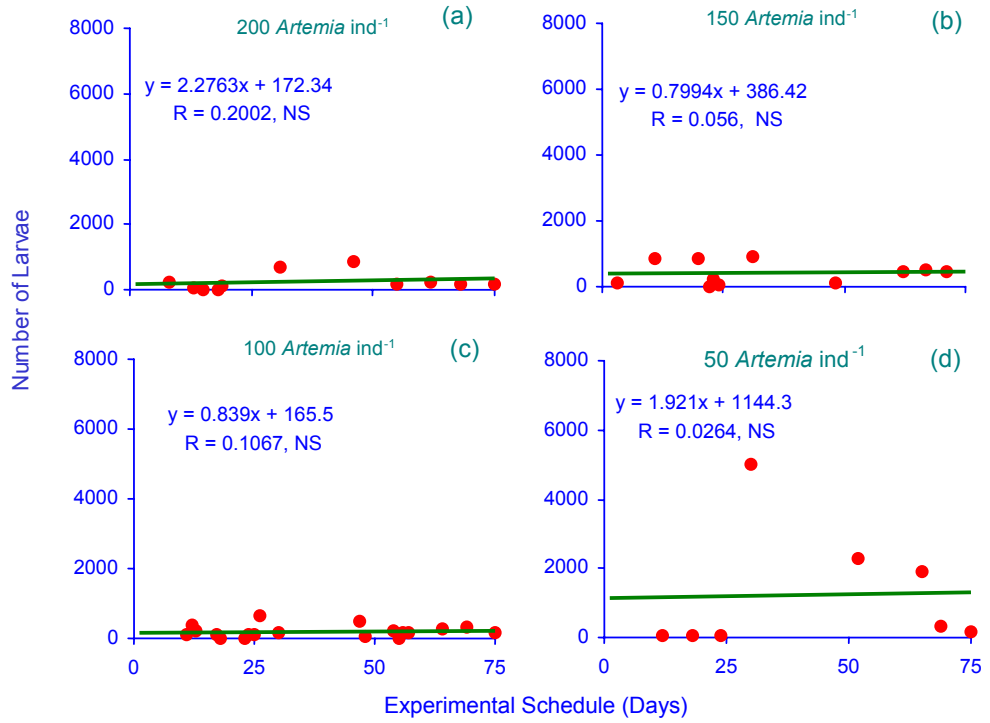


Fig. 2.4 Relationship between the number of larvae released with the experimental schedule (days) in paired individuals at 20° C. Ns – Not Significant

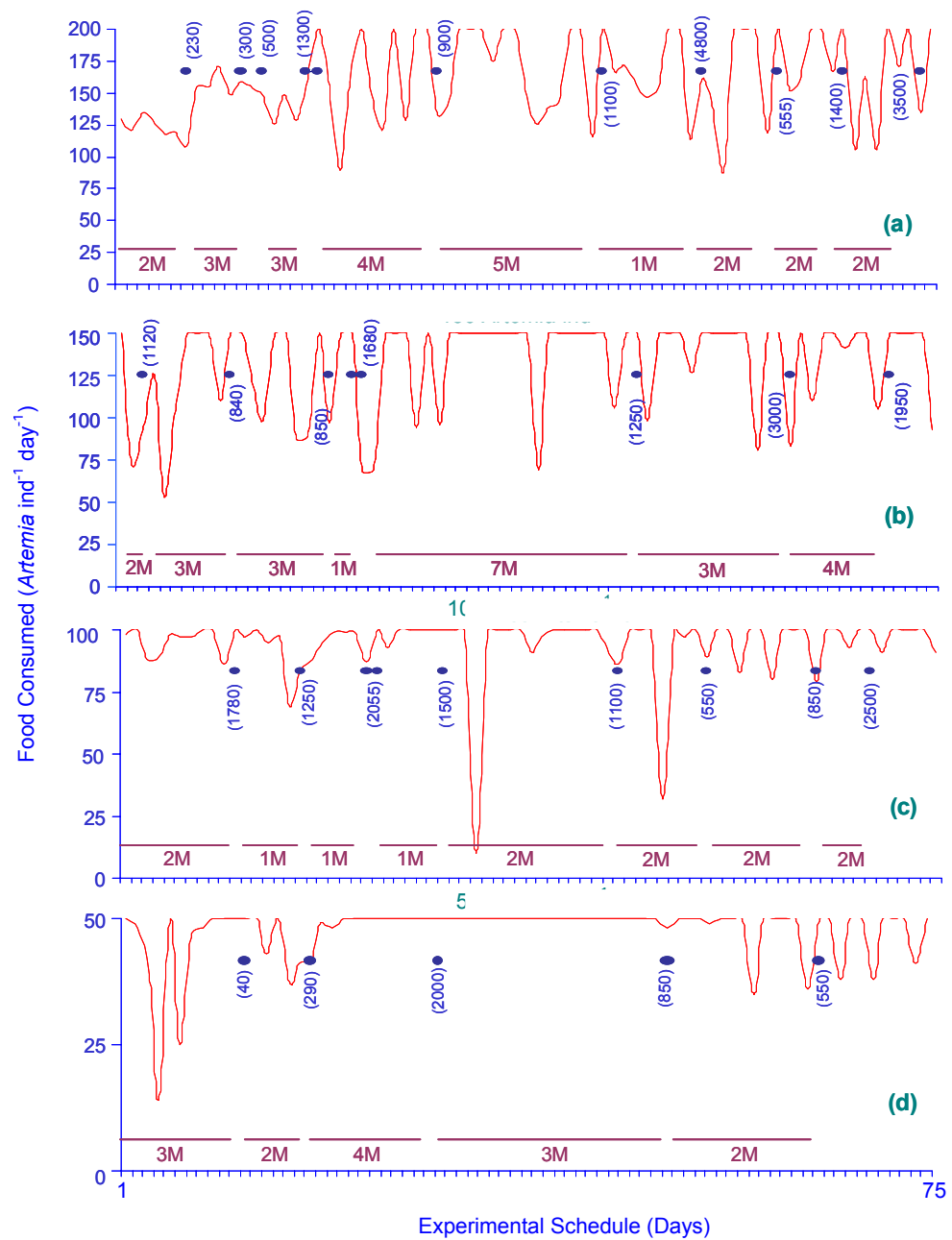


Fig. 2.5 Food consumed at different food concentrations by paired individuals at 30°C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods

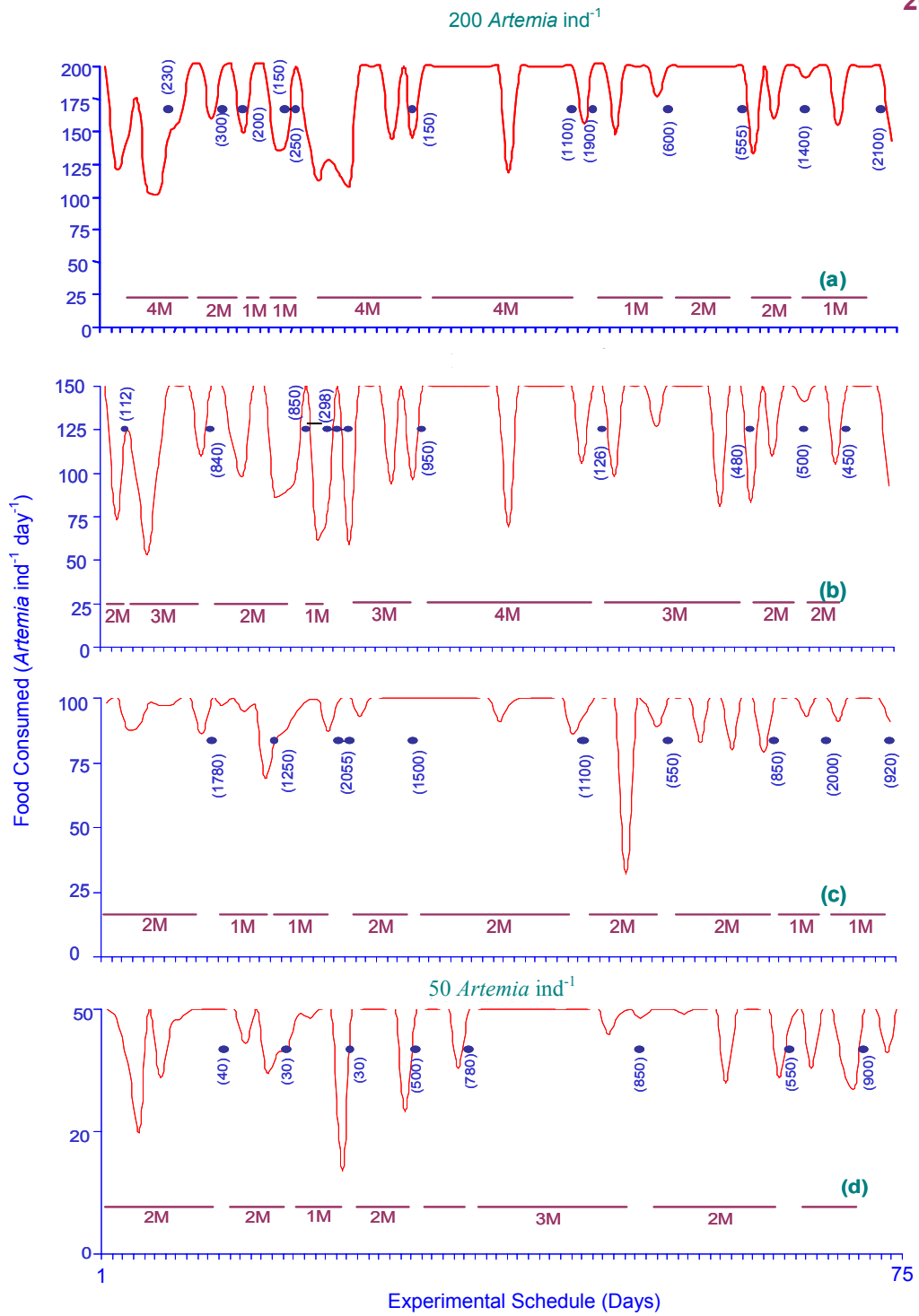


Fig. 2.6 Food consumed at different food concentrations by paired individuals at 25°C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods

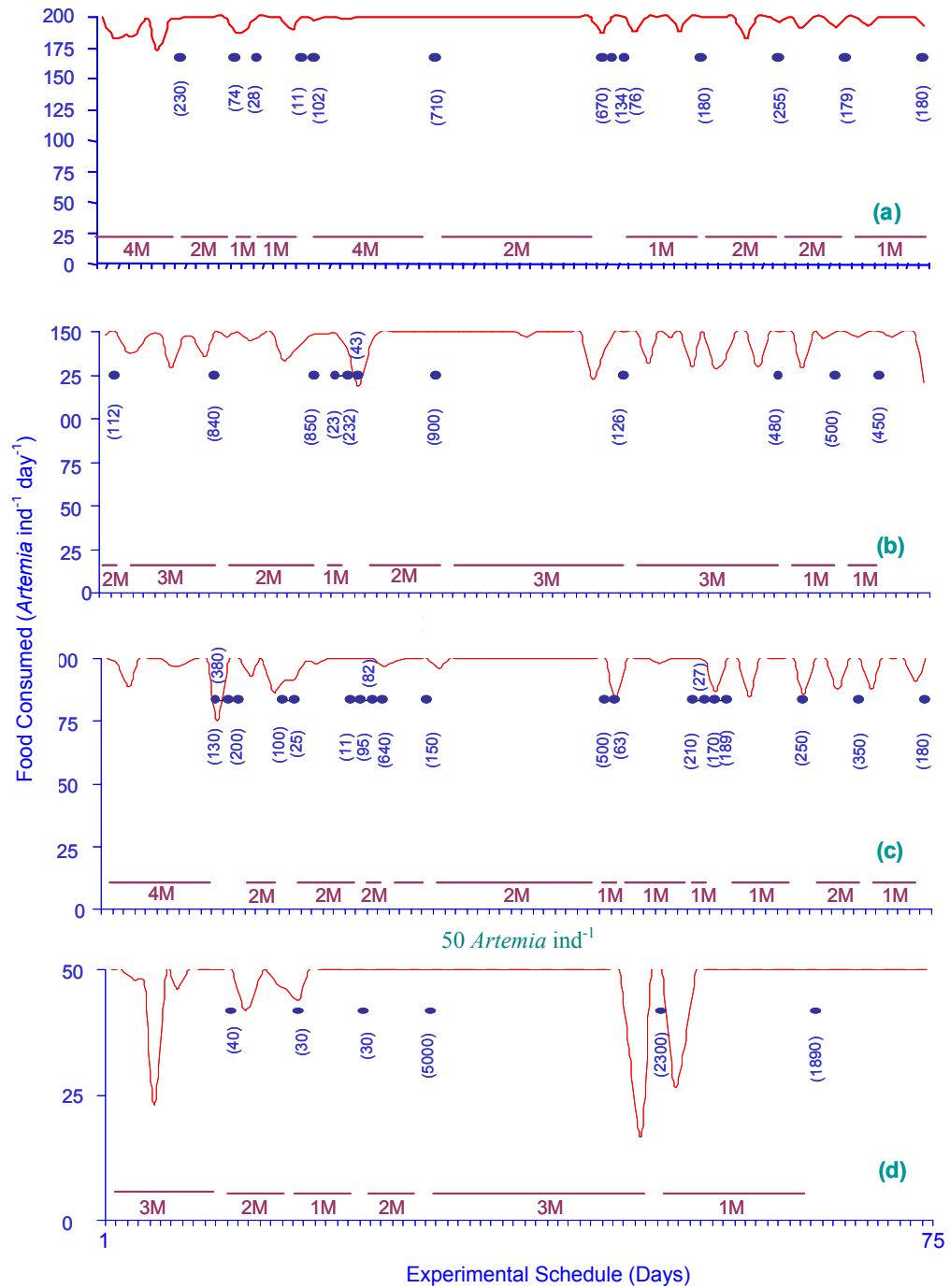


Fig. 2.7 Food consumed at different food concentrations by paired individuals at 20°C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods

Similar is the case at other food concentrations (150, 100 and 50 *Artemia* ind<sup>-1</sup>) at all the temperatures (Figs. 2.5 b-d, 2.6 b-d and 2.7 b-d) except at 50 *Artemia* ind<sup>-1</sup> at 20° C where maximum larvae were observed among all the food concentration (Table 2.1).

Table 2.1 Average carbon assimilated between successive breeding at different food concentration, total number of broods, number of larvae produced, number of molts between two successive broods and total number of broods at all the temperatures

|              | Food Conc. | C Assimilated (µg C) | No. of Larvae | No. of Molts | Total No. of broods | Total No. of Larvae | Total No. of Molts |
|--------------|------------|----------------------|---------------|--------------|---------------------|---------------------|--------------------|
| <b>20° C</b> | 200        | 688 (±356)           | 300 (±250)    | 2 (±1)       | 13                  | 2829                | 21                 |
|              | 150        | 479 (±350)           | 500 (±290)    | 2 (±1)       | 11                  | 4743                | 17                 |
|              | 100        | 272 (±200)           | 300 (±225)    | 2 (±1)       | 19                  | 3643                | 15                 |
|              | 50         | 255 (±150)           | 1500 (±1900)  | 2 (±1)       | 6                   | 9290                | 13                 |
| <b>25° C</b> | 200        | 603 (±370)           | 800 (±700)    | 2 (±1)       | 12                  | 8935                | 23                 |
|              | 150        | 513 (±380)           | 460 (±260)    | 2 (±1)       | 11                  | 4108                | 17                 |
|              | 100        | 392 (±178)           | 1300 (±500)   | 2 (±1)       | 10                  | 12005               | 17                 |
|              | 50         | 210 (±108)           | 460 (±350)    | 2 (±1)       | 8                   | 3680                | 12                 |
| <b>30° C</b> | 200        | 576 (±335)           | 1500 (±1500)  | 3 (±1)       | 11                  | 14585               | 28                 |
|              | 150        | 660 (±556)           | 1500 (±750)   | 3.5 (±2)     | 8                   | 10690               | 20                 |
|              | 100        | 400 (±172)           | 1400 (±600)   | 2 (±1)       | 9                   | 11585               | 22                 |
|              | 50         | 303 (±147)           | 750 (±760)    | 3 (±1)       | 5                   | 3730                | 13                 |

(Number in the parenthesis indicate the standard deviation from the average)

Two-way ANOVA results indicated that breeding interval was significantly influenced by the temperature and the occurrence of barnacles as solitary or paired and the interaction of both at all the food concentrations (Table 2.2).

Table 2.2 Two-way ANOVA of the influence of temperature and mode of occurrence (solitary/paired individuals) on breeding at different food concentrations

(df. Degree of freedom; SS. Sum of squares; MS. Mean of squares; Fs. Fischer constant)

| Factor                 | 50 <i>Artemia</i> ind <sup>-1</sup> |      |      |         | 100 <i>Artemia</i> ind <sup>-1</sup> |      |          |
|------------------------|-------------------------------------|------|------|---------|--------------------------------------|------|----------|
|                        | df                                  | SS   | MS   | Fs      | SS                                   | MS   | Fs       |
| A. (Solitary / Paired) | 1                                   | 57   | 57   | 4.1 ns  | 1582                                 | 1582 | 101***** |
| B. Temperature         | 2                                   | 2252 | 1126 | 82***** | 1744                                 | 872  | 56*****  |
| A*B                    | 2                                   | 1646 | 823  | 60***** | 1336                                 | 668  | 43*****  |
| Within sub. Gr.err.    | 12                                  | 165  | 14   |         | 187                                  | 16   |          |
| Total                  | 17                                  | 4120 |      |         | 4849                                 |      |          |

| Factor                 | 150 <i>Artemia</i> ind <sup>-1</sup> |      |     |          | 200 <i>Artemia</i> ind <sup>-1</sup> |     |           |
|------------------------|--------------------------------------|------|-----|----------|--------------------------------------|-----|-----------|
|                        | df                                   | SS   | MS  | Fs       | SS                                   | MS  | Fs        |
| A. (Solitary / Paired) | 1                                    | 586  | 586 | 273***** | 512                                  | 512 | 1341***** |
| B. Temperature         | 2                                    | 1172 | 586 | 273***** | 701                                  | 351 | 919*****  |
| A*B                    | 2                                    | 896  | 448 | 209***** | 614                                  | 307 | 805*****  |
| Within sub. Gr.err.    | 12                                   | 26   | 2.1 |          | 5                                    | 0.4 |           |
| Total                  | 17                                   | 2680 |     |          | 1832                                 |     |           |

(\*\*\*\*\*p≤0.001, ns – not significant)

## Molting

The maximum number of molts were found at 30° C (Fig. 2.8c) and decreased with the decrease in the temperature at which the barnacles were raised (Fig. 2.8a,b). An increase in the number of molts was observed with an increase in food concentration (from 50 to 200 *Artemia* ind<sup>-1</sup>) at all the temperatures (Fig. 2.8a-c).

In general, solitary individuals had less number of molts compared to paired individuals (Fig. 2.8a-c). At 20° C the molting interval was longer (Fig. 2.8d). However at 25 and 30° C this interval was almost similar (Fig. 2.8e,f).

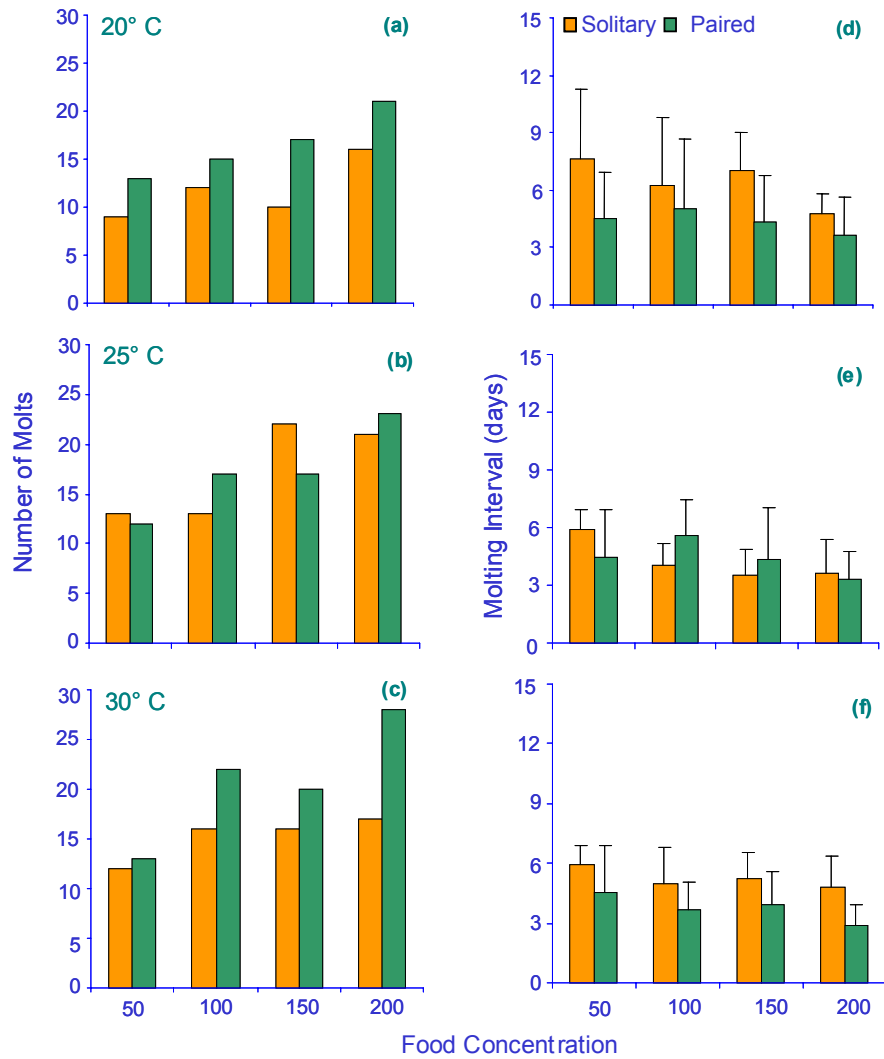


Fig. 2.8 Number of molts and molting interval at different temperatures and food concentrations

The results of the two-way ANOVA indicated significant influence of temperature and mode of occurrence (solitary/paired) on the number of molts at all the food concentrations (Table 2.3).

Table 2.3 Two-way ANOVA of the influence of temperature and mode of occurrence (solitary/paired individuals) on molting at different food concentrations

(df. Degree of freedom; SS. Sum of squares; MS. Mean of squares; Fs. Fischer constant)

| Factor                 | 50 <i>Artemia</i> ind <sup>-1</sup> |    |    |        | 100 <i>Artemia</i> ind <sup>-1</sup> |    |        |
|------------------------|-------------------------------------|----|----|--------|--------------------------------------|----|--------|
|                        | df                                  | SS | MS | Fs     | SS                                   | MS | Fs     |
| A. (Solitary / Paired) | 1                                   | 24 | 24 | 24**** | 8                                    | 8  | 8**    |
| B. Temperature         | 2                                   | 13 | 6  | 6**    | 79                                   | 39 | 39**** |
| A*B                    | 2                                   | 7  | 3  | 3 ns   | 67                                   | 33 | 33**** |
| Within sub. Gr.err.    | 12                                  | 12 | 1  |        | 12                                   | 1  |        |
| Total                  | 17                                  | 56 |    |        | 166                                  |    |        |

| Factor                | 150 <i>Artemia</i> ind <sup>-1</sup> |     |    |        | 200 <i>Artemia</i> ind <sup>-1</sup> |     |         |
|-----------------------|--------------------------------------|-----|----|--------|--------------------------------------|-----|---------|
|                       | df                                   | SS  | MS | Fs     | SS                                   | MS  | Fs      |
| A.(Solitary / Paired) | 1                                    | 20  | 20 | 23**** | 168                                  | 168 | 189**** |
| B. Temperature        | 2                                    | 119 | 60 | 67**** | 55                                   | 27  | 31****  |
| A*B                   | 2                                    | 119 | 60 | 67**** | 59                                   | 29  | 33****  |
| Within sub. Gr.err.   | 12                                   | 11  | 1  |        | 11                                   | 1   |         |
| Total                 | 17                                   | 269 |    |        | 293                                  |     |         |

(\*\*\*\*p≤0.001, \*\*p≤0.025, ns – not significant)

## Food Consumption

The food consumption rate was almost similar for both solitary and paired individuals at all the food concentrations. The solitary individuals, which released larvae once or twice did not indicate any change in the feeding rate (Figs. 2.9a-c, 2.10a-c and 2.11a-c) except at 50 *Artemia* ind<sup>-1</sup> at all the temperatures (Figs. 2.9d, 2.10d and 2.11d) which was the lowest food concentration. In case of solitary individuals maximum number of larvae (2700 brood<sup>-1</sup>) were released at 150 *Artemia* ind<sup>-1</sup> at 20° C (Fig. 2.9b). At this temperature the number of molts between the two broods were less (Fig. 2.9b) compared to 25 and 30° C (Figs. 2.10a,c and 2.11a-d).



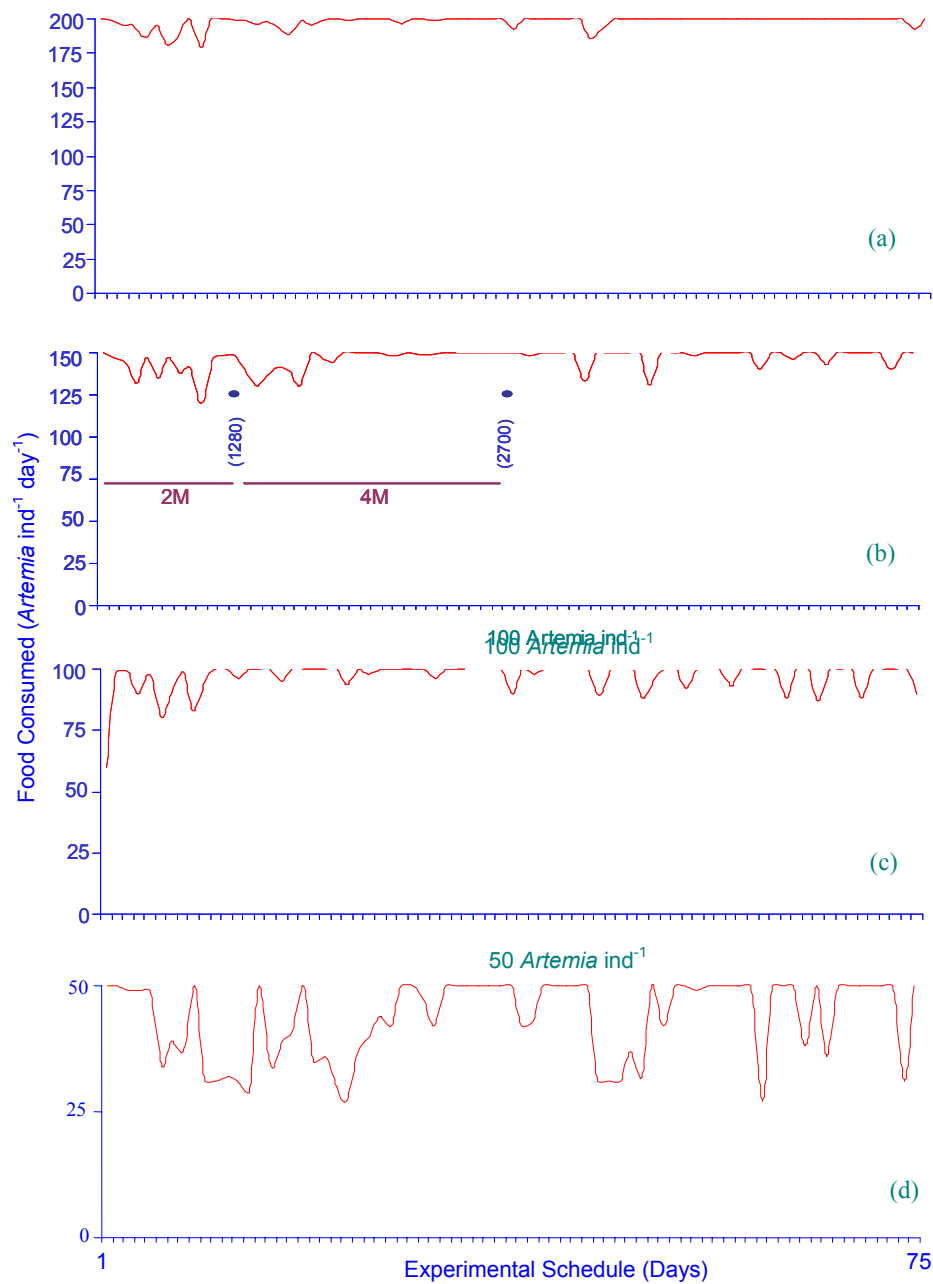


Fig. 2.9 Food consumed at different food concentrations by solitary individuals at 20°C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods

In case of paired individuals, the feeding rate was not uniform throughout. In general prior to/or during larval release there was a decrease in the food consumption rate and was more prominent at higher temperature. Food consumption was high at 20° C (Fig. 2.7a-d) compared to 25 and 30° C (Figs.

2.5 and 2.6). The number of larvae released were less compared to higher temperature (Figs. 2.5 and 2.6) except at 50 *Artemia* ind<sup>-1</sup> (Fig. 2.7d) where the number of larvae released were 5000 brood<sup>-1</sup>.

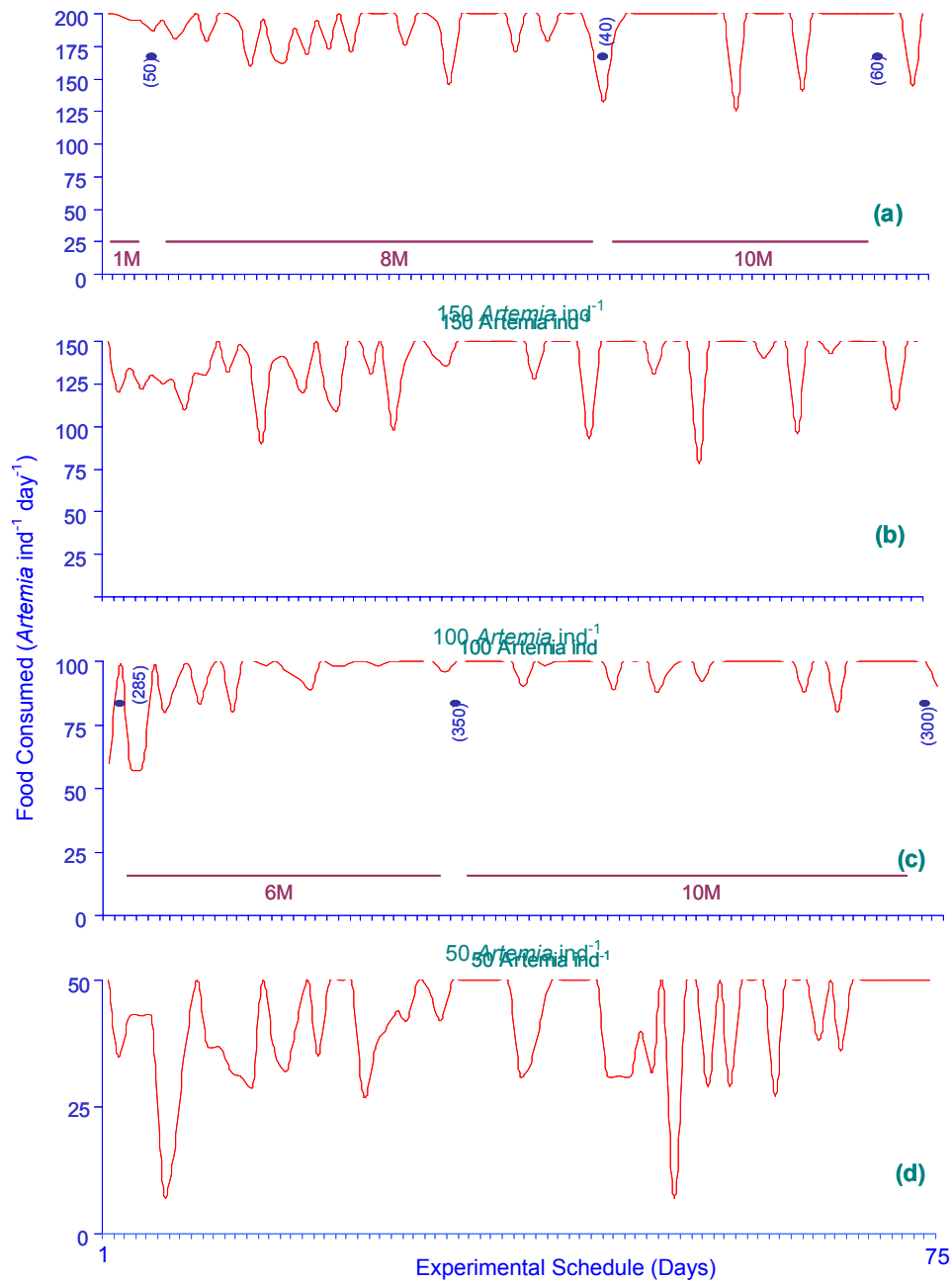


Fig. 2.10 Food consumed at different food concentrations by solitary individuals at 25°C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods

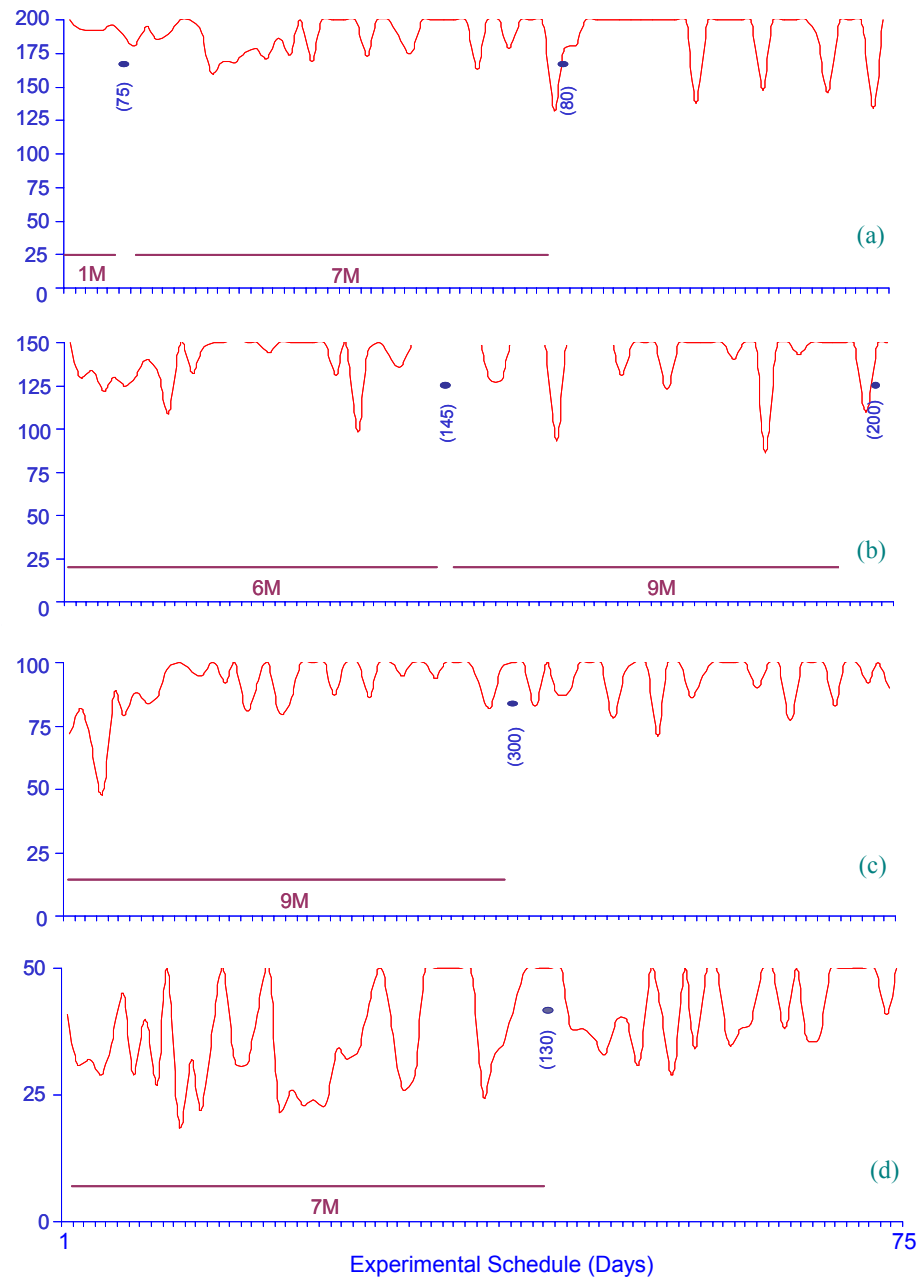


Fig. 2.11 Food consumed at different food concentrations by solitary individuals at 30°C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods

The number of larvae released and molts between successive breeding varied with the assimilation of energy in terms of carbon (Table 2.1). The amount of carbon assimilated between the successive broods was maximum at 200 *Artemia* ind<sup>-1</sup> at 20° C (Table 2.1). This decreased with decrease in food concentration. Similar was the case with the other two temperatures. At 20° C maximum number of larvae were released at 50 *Artemia* ind<sup>-1</sup> even though the carbon assimilated was more at 200 *Artemia* ind<sup>-1</sup> (Table 2.1). It was observed that at 50 *Artemia* ind<sup>-1</sup> breeding was 6 times, however at 200 *Artemia* ind<sup>-1</sup> it was 13 times (Table 2.1). The total number of molts also decreased with a decrease in the food concentration and carbon assimilation.

Regression analysis between the number of larvae released vs. carbon assimilated during interbreeding interval indicated significant increase in the number of larvae with an increase in the carbon assimilation only at 20° C (200 and 150 *Artemia* ind<sup>-1</sup>) (Fig. 2.12a,b). Molting rate between two successive breeding increased with the assimilated carbon at 200 and 150 *Artemia* ind<sup>-1</sup> (Fig. 2.12e,f) at 20° C, 150 and 100 *Artemia* ind<sup>-1</sup> at 25° C (Fig. 2.13f,g) and at 150 *Artemia* ind<sup>-1</sup> at 30° C (Fig. 2.14f) respectively.

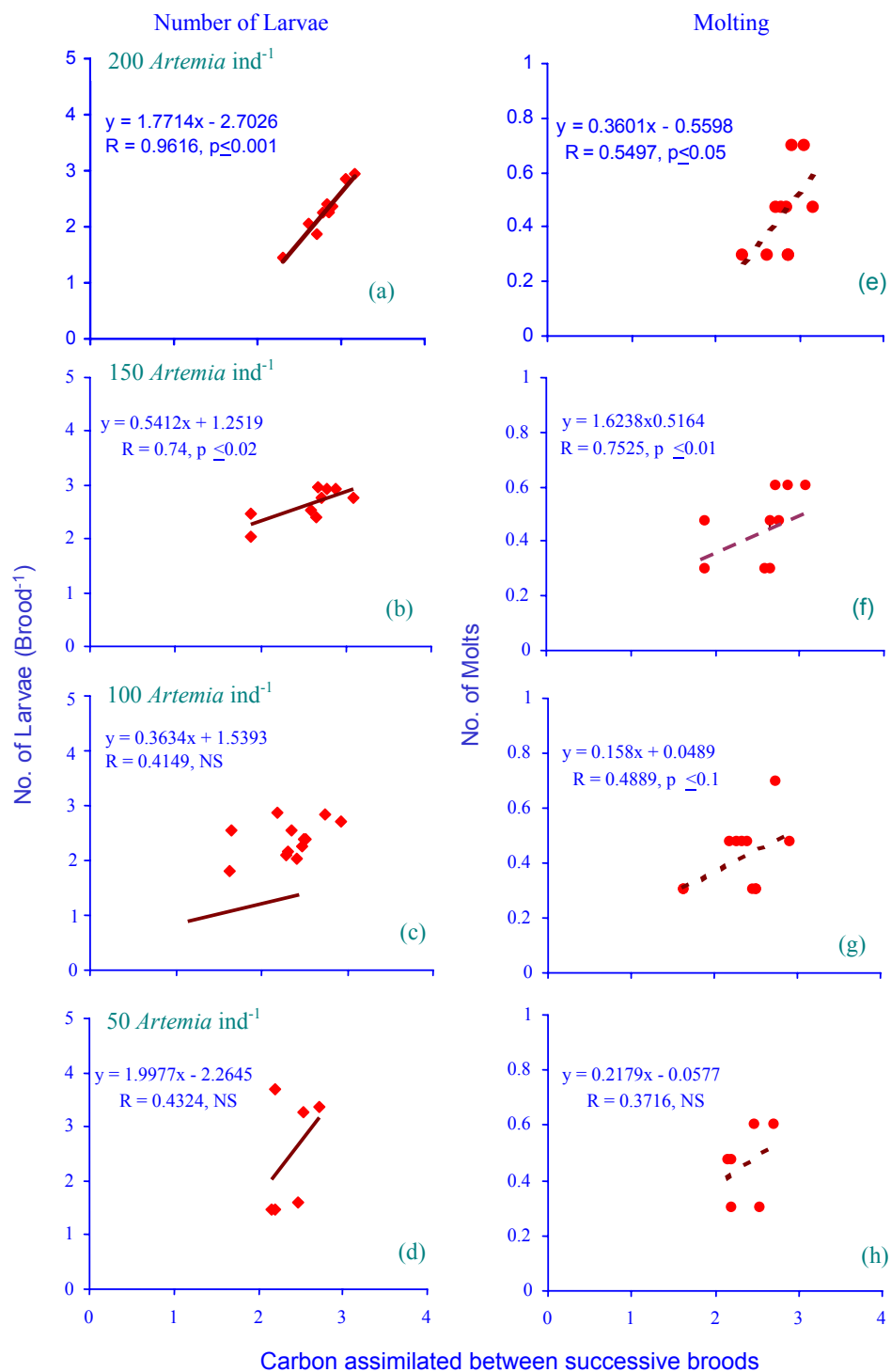


Fig. 2.12 Regression analysis between number of larvae released and the number of molts between successive breeding at different food concentrations at 20°C. Ns – Not significant

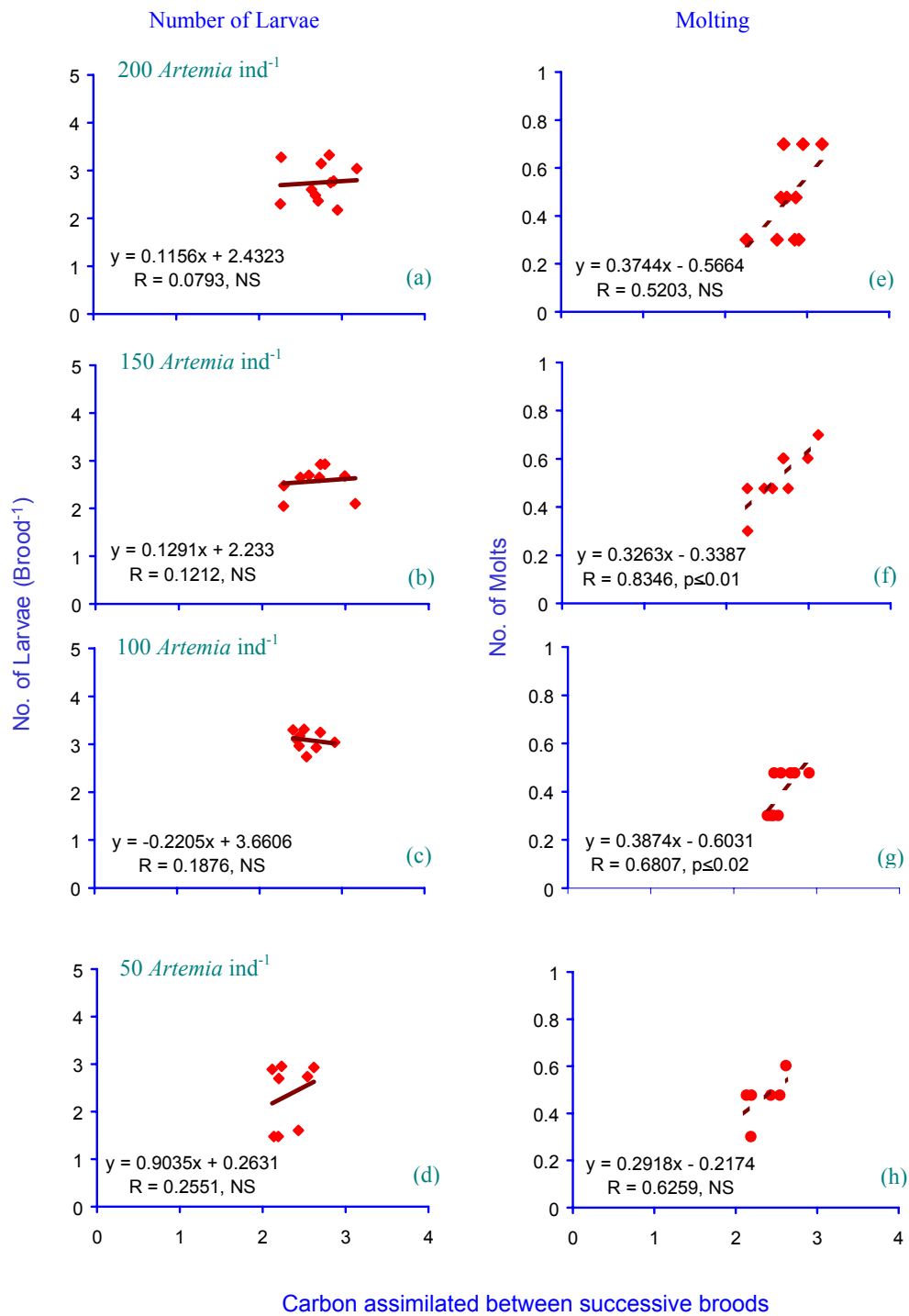
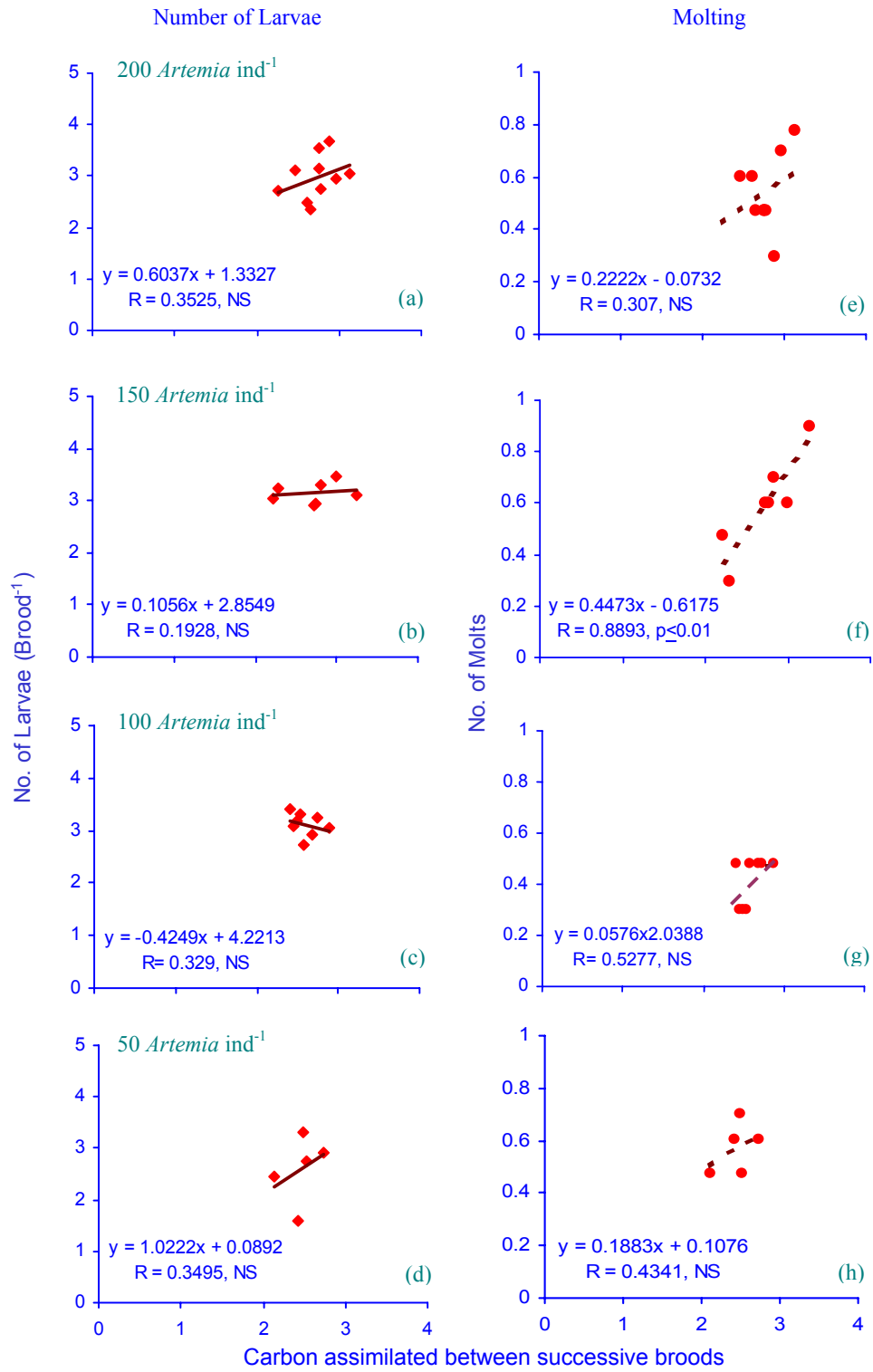


Fig. 2.13 Regression analysis between number of larvae released and the number of molts between successive breeding at different food concentrations at 25° C. NS – Not significant



### 2.3.2 Breeding, Molting and food consumption (Experiment 2)

#### Breeding

The observations on breeding for a longer duration (203 days) at 30° C at all the food concentrations did not indicate any difference in their breeding interval (Fig. 2.15a,b) when compared to that in experiment I (Fig. 2.1c,f). The breeding interval decreased with an increase in food concentration.

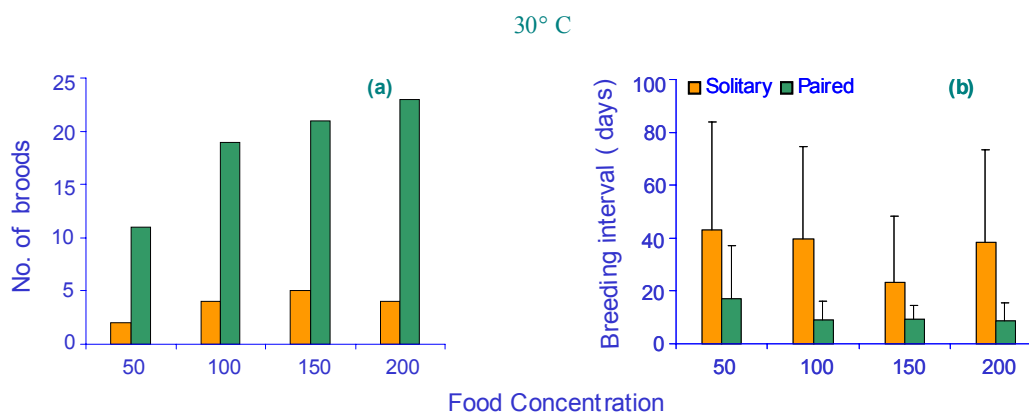


Fig. 2.15 Number of broods and breeding interval of the barnacles reared for 203 days at 30° C at different food concentrations

Regression analysis indicated an increase in the number of larvae with the age of barnacle (day 1 to day 203) only at 200 *Artemia* ind<sup>-1</sup> (Fig. 2.16a). The maximum numbers of larvae released were 7000 brood<sup>-1</sup> at 150 *Artemia* ind<sup>-1</sup>. However in general, number of larvae released were more at 200 *Artemia* ind<sup>-1</sup>.



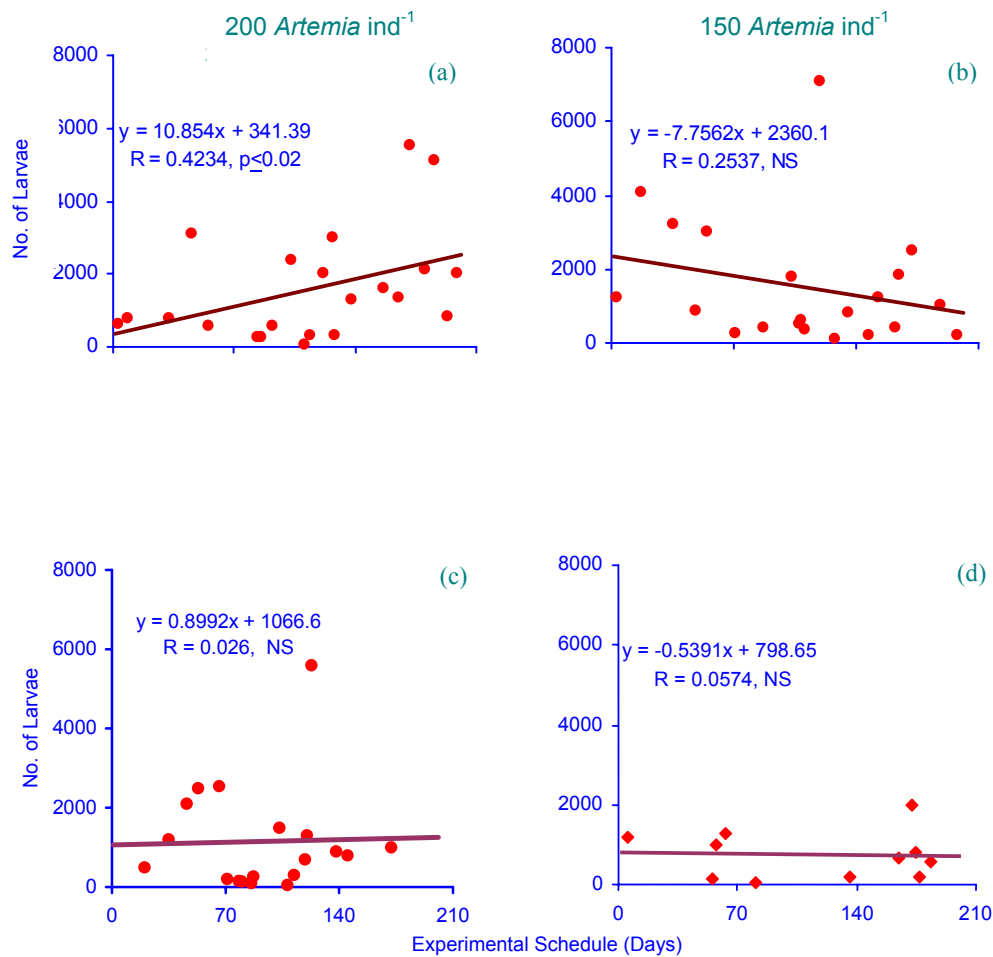


Fig. 2.16 Relationship between the number of larvae released with the experimental schedule (days) in paired individuals at 30° C. Ns – Not significant

The liberation of unfertilized egg masses from solitary individuals at higher food concentrations was observed (Fig. 2.17a,b). In case of paired individuals at higher food concentration (200 and 150 *Artemia* ind<sup>-1</sup>) the liberation of broods was faster in the aged barnacle (Fig. 2.18a,b). This was not the case at 100 and 50 *Artemia* ind<sup>-1</sup>.

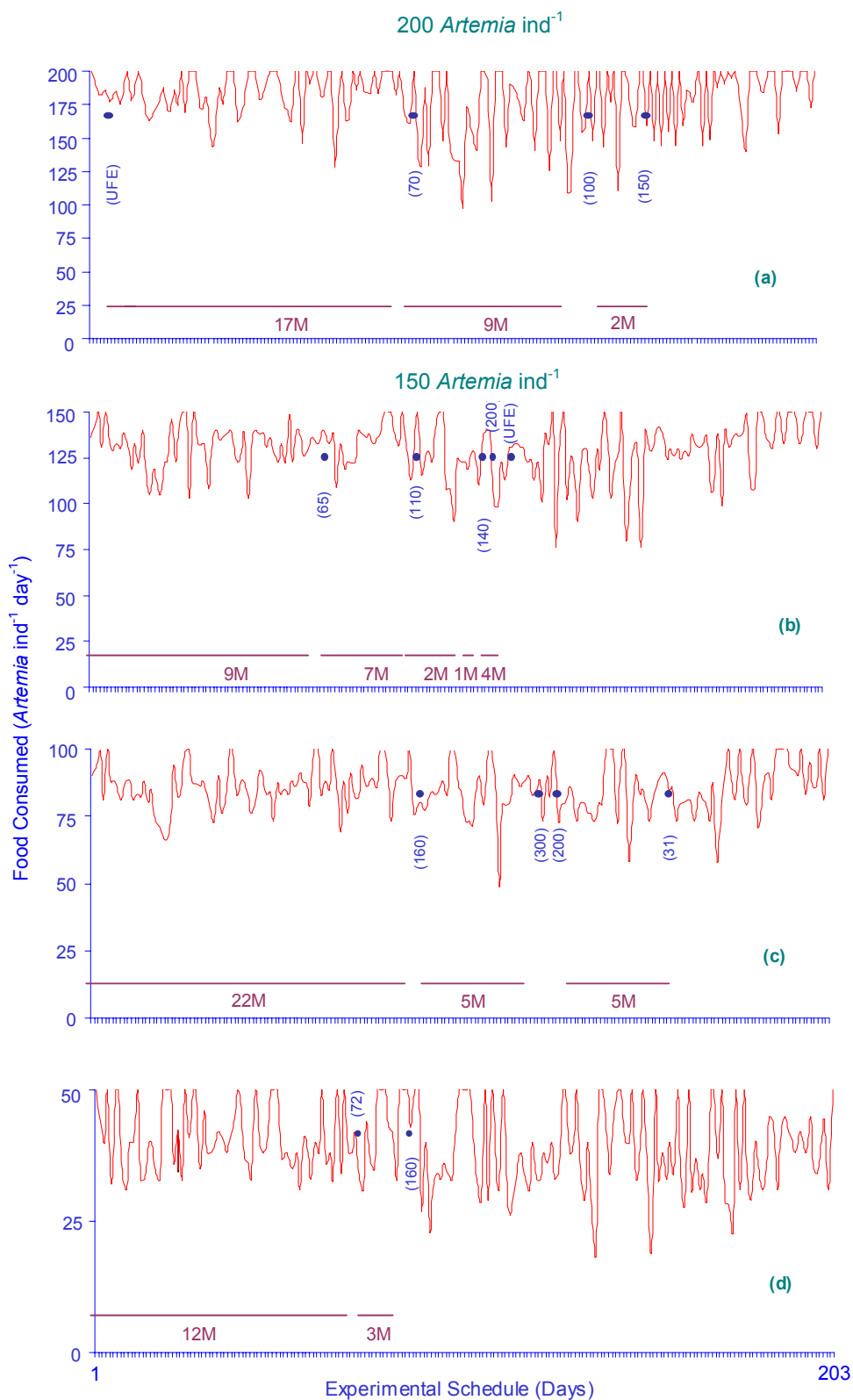


Fig. 2.17 Food consumed at different food concentrations by solitary individuals at 30° C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods. UFE – Unfertilized eggs

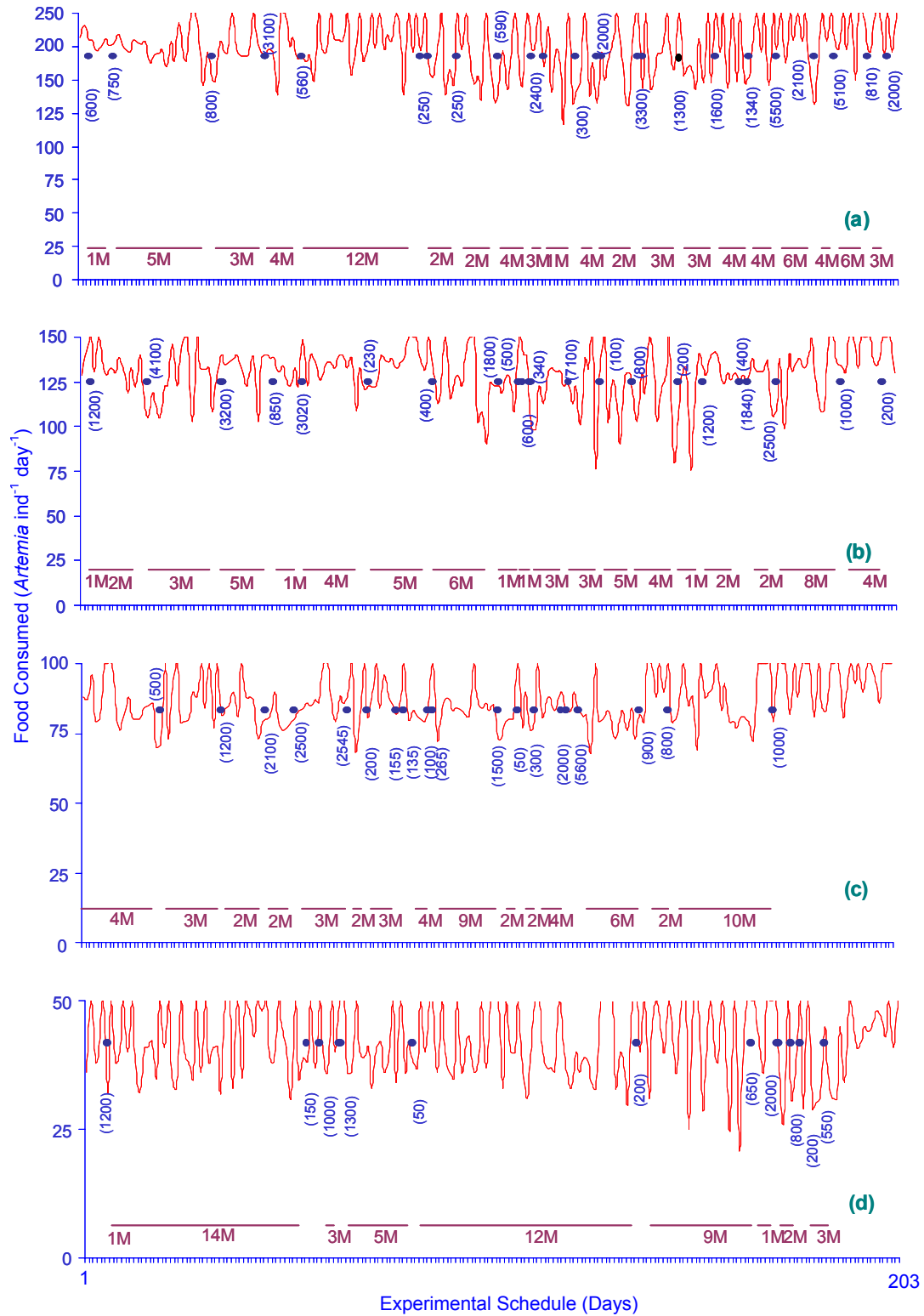


Fig. 2.18 Food consumed at different food concentrations by paired individuals at 30° C. Numbers in the parenthesis indicate the number of larvae released per brood and the horizontal lines indicate the breeding interval. The numbers below the line indicate the number of molts between successive broods

## Molting

The barnacles maintained for a longer duration (203 days) did not show any marked difference in the number of molts and the molting interval with reference to food concentration (Fig. 2.19a,b), and the interval was similar to that observed in experiment 1; 30° C (75 days) (Fig. 2.8f).

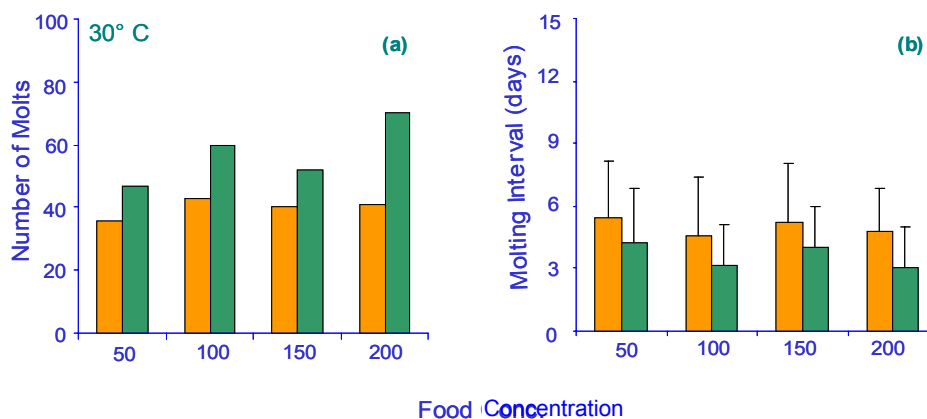


Fig. 2.19 Number of molts and molting interval of the barnacles reared for 203 days at 30° C at different food concentrations

In case of solitary individuals, maximum of 22 molts (90 days) were observed between the successive broods at 100 *Artemia* ind<sup>-1</sup> (Fig. 2.17c), whereas in paired individuals maximum of 14 molts (48 days) were observed at 50 *Artemia* ind<sup>-1</sup> (Fig. 2.18d). However the number of molts were less at higher food concentration.

## Food Consumption

The food consumption at all the concentrations was almost similar for both solitary and paired individuals (Figs. 2.17a-d and 2.18a-d). The results of the interbreeding carbon assimilation at different food concentrations indicated that, it decreased with food concentration (Table 2.4). Breeding interval was

longer at low food concentration and so were the number of larvae released (Fig. 2.18d).

Table 2.4 Average carbon assimilated between successive breeding at different food concentration, total number of broods, number of larvae produced, number of molts between two successive broods and total number of broods at all the temperatures

| 30° C | Food Conc. | C Assimilated ( $\mu\text{g C}$ ) | No. of Larvae       | No. of Molts  | Total No. of broods | Total No. of larvae | Total Molts |
|-------|------------|-----------------------------------|---------------------|---------------|---------------------|---------------------|-------------|
|       | 200        | 808 ( $\pm 615$ )                 | 1700 ( $\pm 1500$ ) | 4 ( $\pm 2$ ) | 23                  | 34700               | 70          |
|       | 150        | 622 ( $\pm 350$ )                 | 1600 ( $\pm 1700$ ) | 3 ( $\pm 2$ ) | 21                  | 31580               | 52          |
|       | 100        | 424 ( $\pm 292$ )                 | 1300 ( $\pm 1400$ ) | 4 ( $\pm 3$ ) | 19                  | 21650               | 60          |
|       | 50         | 340 ( $\pm 400$ )                 | 700 ( $\pm 600$ )   | 6 ( $\pm 5$ ) | 11                  | 7900                | 47          |

(Numbers in the parenthesis indicate the standard deviation from the average)

Regression analysis indicated that at all the food concentration the number of molts between successive broods increased with increase in assimilated carbon (Fig. 2.20e-h), whereas for larval production, significant influence was observed only in case of 100 *Artemia* ind<sup>-1</sup>. When compared with the results of the experiment 1;30° C (75 days), the production of larvae did not significantly vary with the assimilated carbon between successive broods at none of the food concentration and molting was significant only at 150 *Artemia* ind<sup>-1</sup>.

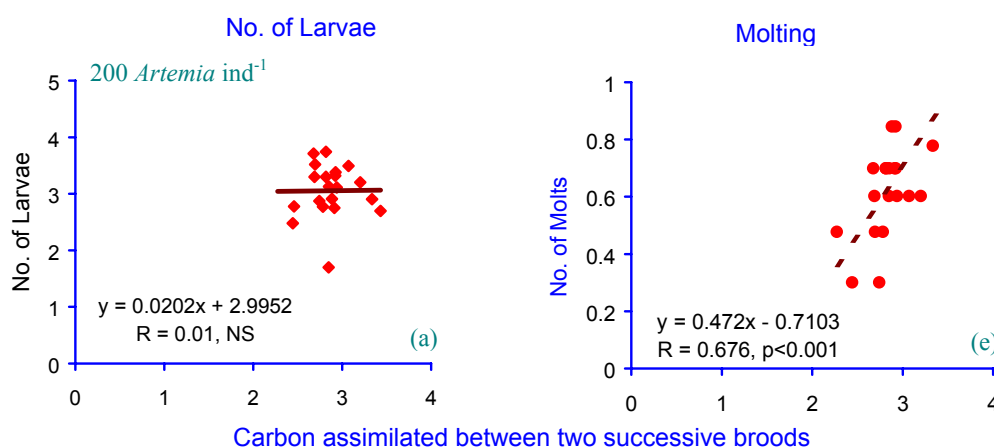


Fig. 2.20 Regression analysis between number of larvae released and the number of molts between successive breeding at different food concentration at 30° C. Ns – Not significant

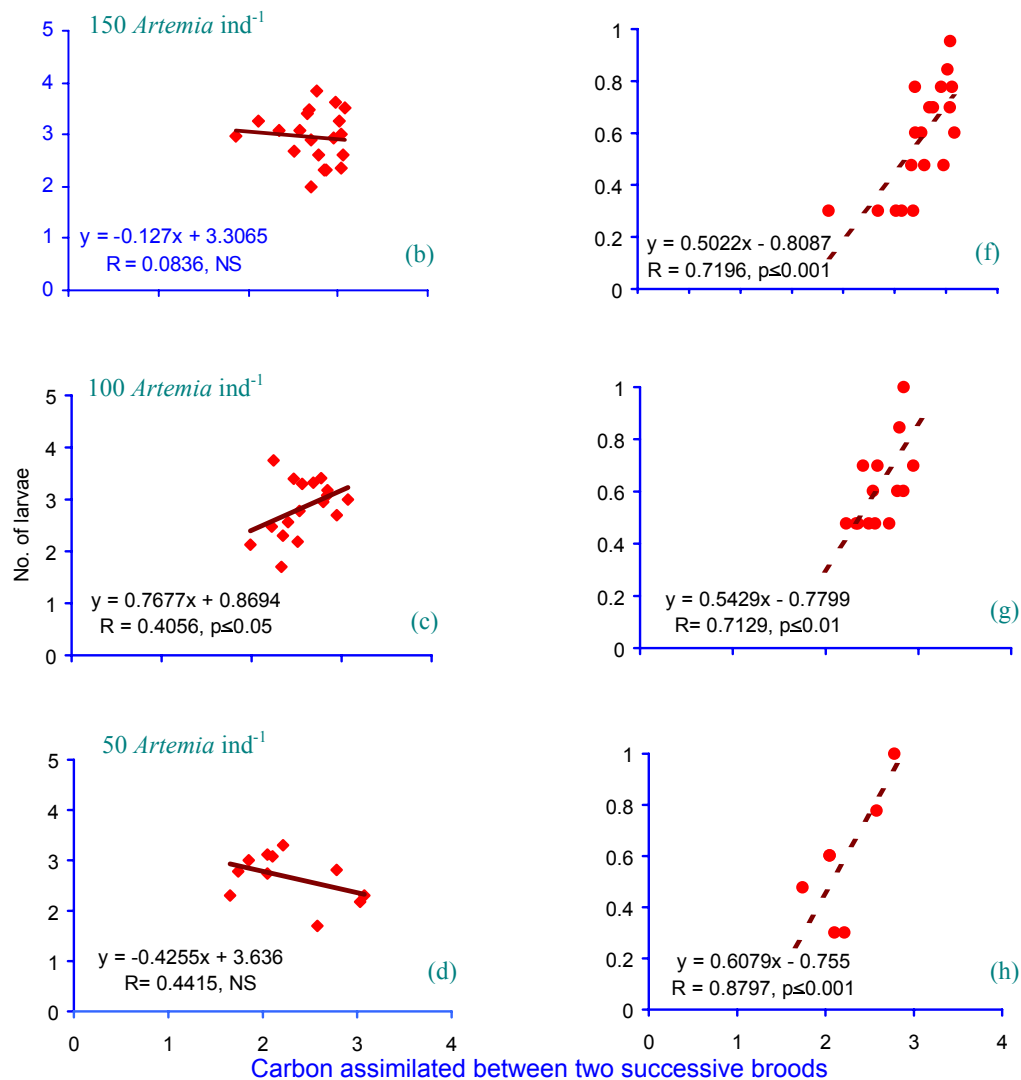


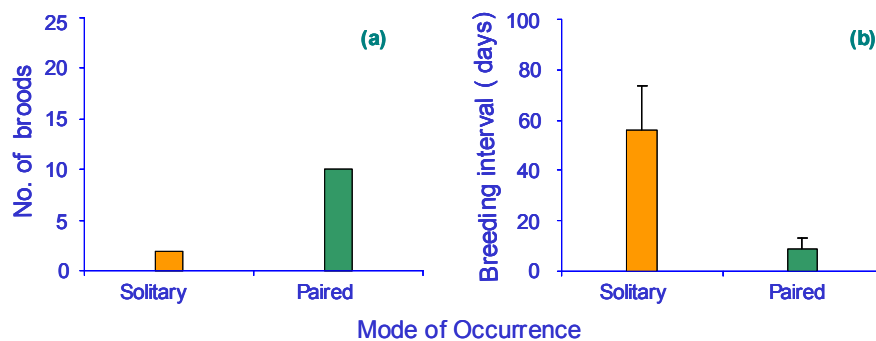
Fig. 2.20 Regression analysis between number of larvae released and the number of molts between successive breeding at different food concentration at 30° C. NS – Not significant

### 2.3.3 Breeding and Molting (Experiment 3)

#### Breeding

The observations on the breeding of barnacles raised in the laboratory indicated that in case of solitary individuals the interval of self-fertilization was comparatively higher (Fig. 2.21b) than the barnacles used in the experiment 1

(collected from wild) (Fig. 2.1f). However in case of paired condition this interval was slightly shorter (Figs. 2.21b and 2.1f). The barnacles raised in the laboratory had higher breeding frequency than the individuals used in experiment 1 (in case of paired condition). In paired individuals the number of larvae released were high ( $3000 \text{ brood}^{-1}$ ) in laboratory raised barnacles, whereas in solitary conditions the number of larvae were less.



## Molting

*Balanus amphitrite* raised in the laboratory showed slightly low molting interval ( $2.7 \pm 1.6$  days) (Fig. 2.22b) throughout the experiment when compared to the individuals brought from the field ( $3.2 \pm 1.9$  days) (Fig. 2.8f).

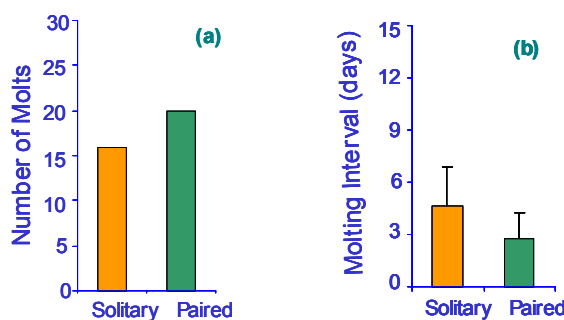


Fig. 2.22 Number of molts and molting interval for solitary and paired *B. amphitrite* raised in the laboratory

### 2.3.4 Field observations of gonads

The barnacles with ripe ovaries were found round the year, however it varied with the seasons (Fig. 2.23). The percentage of individuals with ripe ovaries was high during late post-monsoon and early pre-monsoon months. During monsoon months this percentage was low.

Maximum percent of ripe ovary were found during Dec '98 to March '99 (50 to 37%) followed by Dec '97 and Jan '99 (31 and 33%). Percentage of ripe ovaries was minimum during late pre-monsoon (April and May; 1997: 4 and 9%, 1998: 12 and 17%, 1999: 9 and 8%) and early monsoon months (June and July; 1997: 14 and 18%, 1998: 4 and 0%, 1999: 12 and 7%).

It was observed that barnacles occurring as solitary individuals also showed ripe ovaries, however their percentage was comparatively low than those observed in paired individuals (Fig. 2.23). The minimum size at which mature brood encountered were 3.9 mm of rostrum carinal basal diameter during post-monsoon season. During monsoon minimum size at which mature brood was encountered was 6.2 mm of rostrum carinal basal diameter.

The cluster dendrograms and the NMDS plots for ripe ovary (stage IV) in case of paired individuals is presented in Figure 2.24 a,b. Two prominent clusters were observed. It was observed that late post-monsoon and early pre-monsoon months (29-50% ripe ovary) formed one cluster and rest of the months (4-23% ripe ovary) were grouped into one cluster (Fig. 2.24a,b).



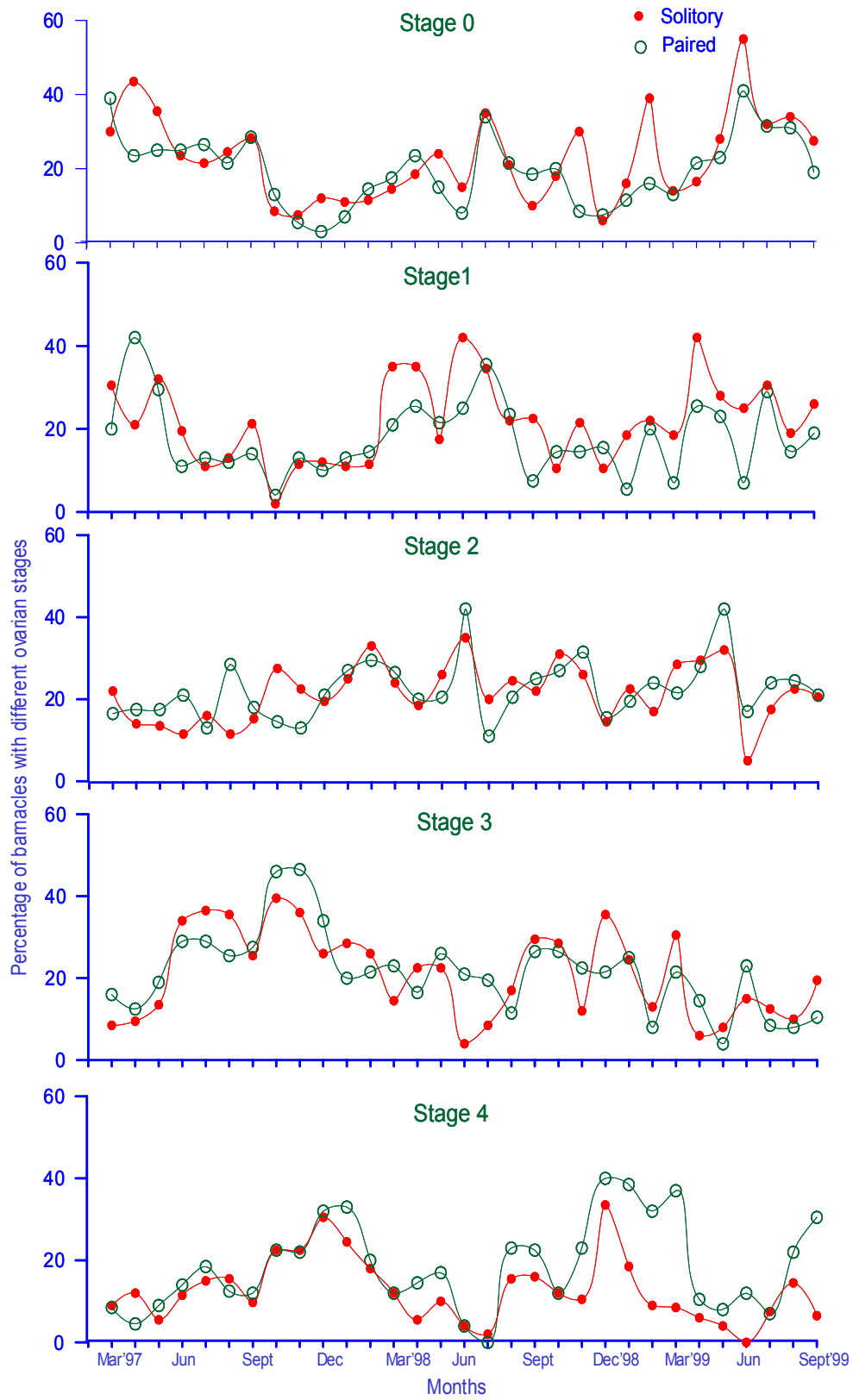
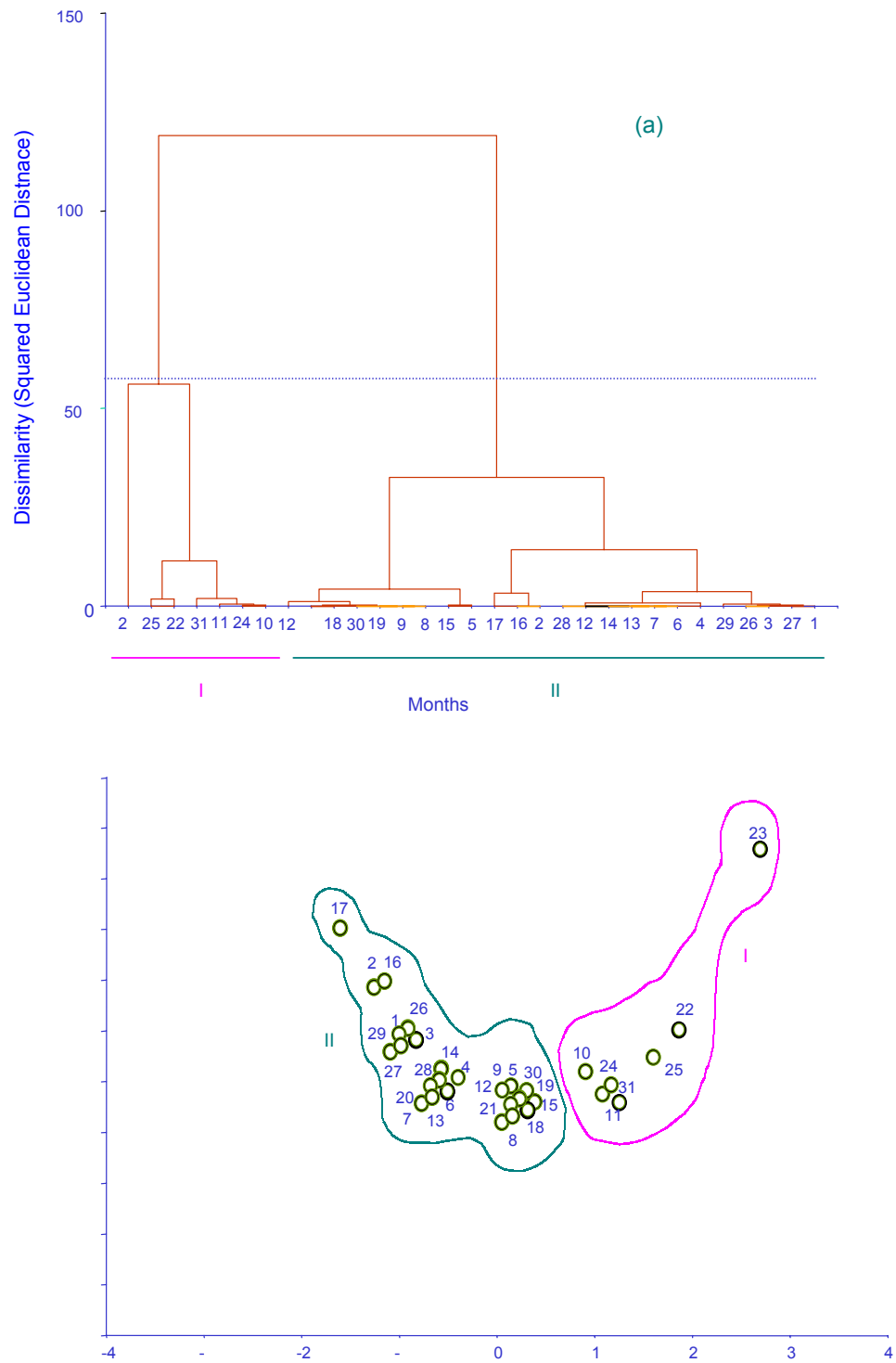


Fig. 2.23 Monthly variation in the percentage of barnacles (solitary and paired) with different stages of ovary



1- Mar'97; 2- April'97; 3- May'97; 4- June'97; 5- July'97; 6- Aug'97; 7- Sept'97; 8- Oct'97; 9- Nov'97;  
 10- Dec'97; 11- Jan'98; 12- Feb'98; 13- Mar'98; 14- Apr'98; 15- May'98; 16- June'98; 17- July'98; 18- Aug'98;  
 19- Sept'98; 20- Oct'98; 21- Nov'98; 22- Dec'98; 23- Jan'99; 24- Feb'99; 25- Mar'99; 26- Apr'99; 27- May'99;  
 28- June'99; 29- July'99; 30- Aug'99; 31- Sept'99

Fig. 2.24 Dendrogram and the non-metric multi dimensional scaling (NMDS) diagram for the stage 4 ovary of *B. amphitrite*

## 2.4 Discussion

*Balanus amphitrite* has euryhaline and eurythermal survival and breeding capability. It can breed at temperatures as low as 15° C (Crisp and Costlow 1963; Iwaki 1981; Anil 1991). In Indian waters, *Balanus amphitrite* is known to breed round the year (Karande 1967). Though exchange of sperms between adjacent individuals leading to cross-fertilization is a thumb rule in this organism, incidences of self-fertilization are also observed. The individuals that were kept solitary in this study released larvae. However, the breeding interval was long (43 days). Such self-fertilization is stated to occur under circumstances when gametogenesis is completed without the occurrence of copulation (Barnes and Crisp 1956; Patel and Crisp 1961). At all the rearing temperatures and food concentrations molting was observed. The number of molts were high at 30° C and decreased with a decrease in temperature and it increased with an increase in the food concentration. The breeding frequency also increased with an increase in food concentration. The increase in food concentration positively influenced the molting and breeding at all the temperatures.

It has been shown that embryonic development is a function of temperature in several barnacle species e.g. *Balanus amphitrite* var. *denticulata*, *Elminius modestus*, *Balanus balanoides* (Crisp 1959; Patel and Crisp 1960; Crisp and Costlow 1963). Thus the nutritional condition and temperature seem to play an important role in breeding and molting process in barnacles. In this study it was observed that at lower temperature breeding was frequent, whereas it was not so in the case of molting.

Wu and Levings (1978) observed in *Balanus glandula* that large amount of energy is lost in respiration. The second most important budget item was egg production, followed in decreasing order by shell production, production of body tissue and molting. They also reported that oxygen consumption rate to increase with increase in temperature (0-25° C) for egg masses, naupliar embryos and ovarian tissue. The maximum number of molts at higher temperature can be attributed to adequate energy allocation for growth.

The frequency of breeding at any given temperature increased with an increase in the food concentration. However at 20° C, more number of larvae were released at 50 *Artemia* ind<sup>-1</sup> (9290; Table 2.1) than at 200 *Artemia* ind<sup>-1</sup> (2829; Table 2.1). But the breeding interval at 200 *Artemia* ind<sup>-1</sup> was shorter (Fig. 2.1f) when compared to 50 *Artemia* ind<sup>-1</sup> (Fig. 2.1f).

In case of solitary individuals, feeding rate was almost similar at all the food concentrations and temperatures except at 50 *Artemia* ind<sup>-1</sup>, where low feeding was observed. Low feeding can possibly be due to poor sensitization of the presence of food by the barnacle or inability to capture food at that density.

Barnes and Barnes (1967) observed in *Balanus balanoides* that interruption of food supply leads to regression of ovarian tissue, also rate of development of ovarian tissue is clearly under close metabolic control and in turn is dependent upon food supply. At many instances the liberation of brood is accompanied by a molt. It was reported that the intermolt period of ovigerous barnacles is longer than the unfertilized ones owing to presence of egg masses in the mantle cavity (Patel and Crisp 1961). Low molting and breeding

was observed at low food concentration. However maximum number of larvae were observed at 50 *Artemia* ind<sup>-1</sup> at 20° C. Hence the decrease in molting can be attributed to the presence of egg masses in the mantle cavity, for which the animal may delay its molting. Thus the energy gained between a longer breeding interval seems to be utilized for sustaining the egg masses and produce higher number of larvae.

It was observed that during or prior to larval release the feeding rate decreased. There could be two possible explanations. (1) There may be actual halting of feeding activity during breeding (2) The interval of observation period was 24h and within this period the adult could have fed on its own larvae. However the incidence of reduced feeding a day prior to release of larvae indicates the first reason for reduced feeding a greater probability.

The observations on the gonad index of the field collected *Balanus amphitrite* indicated that the minimum size at which mature broods (stage IV ovary) observed varied with the seasons (3.9 mm RCB in post-monsoon and 6.2mm RCB during monsoon) and can be used as a point for conditioning of adults in the environment.