Discussion
5. DISCUSSION

5.1 External features

The rostrum of *M. gangeticum* was found, short extending up to antennal peduncle, highly convex and slightly upturned. It was also found with an elevated dorsal, rostral crest with, rostral formula usually 9-11 + 1 3/4-6. The dorsal rostral teeth on elevated crest usually was observed to be closely setup with each other. The numbers of ventral teeth recorded were 4-6 numbers, which were separated from each other. However, the elevation of rostrum in *M. malcolmsonii* found much less than *M. gangeticum*. The rostral formula usually recorded 8-11 + 0-3 dorsal and 5-8 ventral. The rostral formula of both the species found different than that of *M. rosenbergii*, 12-15 dorsal and 11-14 ventral serrations were found which are separated from each others (George, 1968). The little variations in body coloration, size and external morphological character between *M. gangeticum* and *M. malcolmsonii* created confusion in the identification under field condition. *M. gangeticum* may be only recognized with the highly elevation of the dorsal crest (Kanaujia 1989; Kanaujia et al. 2005). The general morphological characters of *M. gangeticum* reported earlier are the basal crest of rostrum is highly elevated, merus and carpus of the second chela longer than the palm, rostrum with two orbital teeth on the carapace and rostral formula 10-2/4-5. More or less similar morphological characters are reported in *M. birmanicum* which is found with 12-13/5-7 rostral formula, basal crest not much elevated as in *M. gangeticum*, second chela in which merus and carpus shorter than the palm, rostrum with two orbital teeth on the carapace. Whereas three orbital teeth on the carapace was reported in *M. malcolmsonii*. Somewhat different morphological characters were reported in *M. rosenbergii* where the second chelate legs found strongly spinulated, carpus distinctly shorter than the chela, rostrum long and curved upward and rostral formula 8-13/8-13 (Kurian and Sebastian, 2002).
5.1.2 Fecundity

The number of eggs carried by a female is related to the size of prawn and is more specifically related to the weight of egg mass (Mansuri, et al., 1988). Chacko (1955) and Costa and Wanninayake (1986) reported that the fecundity of wild *M. rosenbergii* have a linear relationship to body length, carapace length, body weight and gonad weight. Similarly Mathew and Mohan (1996) also reported that the relationship between fecundity and total body length/carapace length/total weight obeys a power function with regression analysis showing positive relationship, but interestingly they observed that the egg size decreases with the increase in prawn size. The number of egg (fecundity) of *M. malcolmsonii*, *M. gangeticum* and *M. rosenbergii* were assessed by counting of the fertilized eggs carried by the different sizes of berried females in their brood sac. Minimum 7,600 eggs in females of 73 mm size and the maximum eggs 75,610 in females of 82 mm size recorded in *M. gangeticum*. Whereas the fecundity in *M. malcolmsonii* observed more or less similar with *M. gangeticum*, whereas minimum fecundity 7,200 in female of 75mm size and maximum 70,300 eggs in females of 170 mm size recorded in *M. malcolmsonii*. Ibrahim (1962) recorded 3465-63,080 eggs from the females of *M. malcolmsonii* of 54-64 mm size in the river Godavari. Rao (1986a) recorded 12,556-77,440 eggs from the female's prawns of 83-156 mm size in Kolleru Lake. A maximum fecundity 5100-83,000 eggs in *M. malcolmsonii* was reported by Mansuri et al. (1988) in females of 63-204 mm from the Ojat river (Gujarat), while Kanaujia (1989), found the fecundity of *M. gangeticum* females between 12,00-78,000 eggs of 70-195 mm size from the river Ganga around Buxur. Further, higher fecundity of 80,000 to 1,00,000 eggs has been reported in the females ranging from 150-165 mm size by George et al. (1968), from the river Cauvery. The fecundity in *M. malcolmsonii* and *M. gangeticum* recorded in the present study was found similar as reported by Kanaujia (2003) and Kanaujia et al. (2002). But the fecundity of both the species are found comparatively less than that of *M. rosenbergii*, which carry maximum 1.0-1.6 lakh eggs (Chacko, 1955). Ling (1969a) reported
that female weighing 80 gm /180 mm size total length can produce about 60,000-1,00,000 eggs.

5.1.3 Food and feeding

Prawn food constituted mainly with the organic detritus, mud and sand, crustacean / insects body parts, portion of aquatic plants, diatoms, algae etc. under natural conditions. They have been reported carnivorous as well as omnivorous in nature of feeding due to the dominance of detritus, bottom mud and sand present in their gut (Rajyalaxmi, 1968). Whereas, Ibrahim (1962) reported omnivorous in habit. Bhimachar (1965), Rao (1969), Ling (1969) and Raman (1977) designated the related prawn species including *M. malcolmsonii* as bottom feeder and omnivorous. Ibrahim (1962), Rajyalaxmi (1980), Raman (1984) and Rao (1986) on the other hand, reported *M. malcolmsonii* as an omnivorous bottom feeder. Chopra (1939) and Ibrahim (1962b) reported that the prawn consumes all types of food living or dead that comes in way, viz, mud, sand grains and derbies. *M. gangeticum M. malcolmsonii* and *M. rosenbergii* found nocturnal in habit, calm, non aggressive decapods and some times found fighting among themselves due to their strong territorial behavior. Larger one often prevents to the smaller for taking food in their won jurisdiction. Some time they become vulnerable to other at the time of food shortage and ecdysis, and prey upon the weaker due to cannibalistic in nature.

Sandifer and Smith (1978) and Daniels et al. (1992) described the feeding strategy for maintaining broodstock in indoor for extended periods. Daniels et al. (1992) stated that when commercially pelleted diets are not nutritionally sufficient, supplements such as pieces of beef liver, squid meat cut to the appropriate size and should be fed atleast twice a week. Broodstock should be fed at a daily rate of 1 to 3% of total biomass. The feeding rate should be adjusted as per food consumption. Rao (1998) reported demonstrated that *M. malcolmsonii* and *M. rosenbergii* could attain marketable size within 4-6 months under culture conditions with low inputs involving common feed of vegetable origin. Present results of experiment,
suggested that feeds prepared by using locally available common feed items are economically viable.

Labae et al. (1995) reported that a diet with a combination of animal and vegetable protein (34.8%) is more efficient feed for the growth and survival of the prawns in culture pond. Similarly, Boonyaratpalin and New (1980) reported that 15% proteinaceous diet is highly desirable formula for an economical standpoint for the first four months of rearing, but diets with 25% and 50% protein promote growth and survival significantly higher than 10 or 15% diets (Pandian and Murugadoss, 1988). Fruechtenicht (1988) found that in the larger size prawn groups, the growth increased significantly with the increasing protein levels up to 32%. Gomez et al. (1988) reported that the highest survival and growth occurs in the prawn fed with the protein with starch ratio 1:1 although, feed efficiency is highest at the protein to starch ratio of 1:3. The feed used in the present experiment, protein and carbohydrate ratio was maintained 1:1 which is at par with the experiments of Gomez et al. (1988) for achieving better growth and survival. Nakagawa (1990) investigated the effect of dietary carbohydrates on growth of *M. rosenbergii* and observed that the group receiving glucose as the carbohydrate source has low weight gain. Hence, considering the above factors, groundnut oilcake was provided as carbohydrate source in the present experiment, thus to have 35.22% of carbohydrate in the feed.

5.2 Physico-chemical Parameters

Water quality management is an important aspect in aquatic production system. Successful operation of prawn hatchery as well as grow out culture largely depends upon the water quality management. Any deviations in physico-chemical parameters of water beyond the tolerance limit affect the organism seriously under culture operation. Thus, the organisms are subjected to stress. In the present study, the biology of three larger freshwater prawns have been studied in relation with physico-chemical parameter specially with water temperature, pH, dissolved oxygen,
total hardness, total alkalinity and ammoniacal nitrogen, which have direct impact on the biology and life history of the organisms.

5.2.1 Temperature

The average water temperature during the experimental period was found to correspond with atmospheric air temperature, which reacts on water bodies quickly to change, resulting in the subsequent rise and fall of its water temperature (Welch, 1952; Dasgupta, 1993). Minimum water temperature 19.0°C and 20.0°C was recorded in the month of January during both the years. The water temperature was gradually increased from January onwards and recorded maximum 34.4°C and 35.6°C in the month of June and July. The greater light penetration with higher intensity and longer duration in a day is the main reason for higher temperature during summer and monsoon. The temperature is also considered to play an important role in physiological process to stimulate breeding mechanisms in aquatic fauna. Therefore, the breeding of the larger prawn species like *M. gangeticum*, *M. malcolmsonii* and *M. rosenbergii* observed during summer and monsoon, when the temperature was in higher range (May - October). All the metabolic and physiological activities of different life processes such as feeding, reproduction and movement are influenced by temperature. Lethal temperature for juveniles and adult prawns are 13°C and 38°C, but mortality increases rapidly at sustained temperature below 18°C and greater than 33°C (Uno et al. 1975; Armstrong, 1976).

5.2.2 pH

The pH is defined as the negative logarithm of the hydrogen ion concentration and it shows acidic or basic nature of water (Boyd, 1982). pH is an important factor in determining the productivity in aquaculture, the indirect effects of pH are more important than the direct effects. Most of the biochemical parameters of aquatic bodies are influenced by pH (Singh and Swarup, 1979). The low pH reduces the amount of dissolved inorganic phosphorus and carbon dioxide available for plankton. In such cases lime is
done to improve the productivity of water under aquaculture condition. The high pH is also not suitable, which favors rapid precipitation of phosphate, when fertilizers are applied in aquaculture system, it also increases the proportion of the total ammonical nitrogen concentration, that exists as unionized ammonia, in the toxic form (Boyd, 1979). In the present study, the pH of culture pond and FRP tanks was found within alkaline range (7.4 - 8.2) throughout the study period, Nair et al. (1989) and Sugunan (1980) recorded maximum pH value during winter followed by summer and monsoons. The variation in pH values between surface and bottom layers are due to monthly and early variation in complex buffering mechanism reported by Sikandar and Tripathi (1983) and Srivastava (1991).

5.2.3 Dissolved oxygen

Dissolved oxygen plays an important role to assess the water quality of the aquatic system. The royal commission has reported a scale for deciding the quality of water based on dissolved oxygen. The content of dissolved oxygen of 7 mg/l in water is considered as very clear, 6 mg/l moderate, 5 mg/l as doubtful and 4 mg/l or below as bad. Swingle (1968) considered concentration of D.O. below 5 mg/l as undesirable for aquaculture. Dissolved oxygen directly influenced by the rate of photosynthesis and inversely by the rate of respiration as well as rate of decomposition of the organic matter present in the aquatic ecosystem. Besides these, too high and too low water temperature also affects the saturation of dissolved oxygen to great extend. Dissolved oxygen in the present study recorded from 7.2-9.5 mg/l during two years. Which was highest during the month of March and lowest in July. The seasonal dissolved oxygen value was found higher in winter followed by summer and monsoon. Similar observations were made by Reid (1961) and Kant and Raina (1990). Oxygen content in the river water reported an inverse correlation with rainfall as reported by Srivastava (1991). Agarwal et al. (1976), Rai (1978), Sangu et al. (1983), Kant and Raina (1990) and
Srivastava (1991) have recorded highest oxygen content during winter and lowest during monsoon.

5.2.4 Total Hardness

Hardness of water caused due to the carbonate, bicarbonate, chloride and sulphate ions in association with calcium and magnesium. Sawyer and Mc Carty (1978) has categorized water into four types as soft water (0-75 mg/l as CaCO₃) moderate hard water (75 -100 mg/l as CaCO₃), hard water (150-300 mg/l as CaCO₃) and very hard water (over 300 mg/l as CaCO₃). Boyd and Walley (1975) reported that total hardness required for better growth of fish and shellfish is within the range of 20-300 mg/l as CaCO₃, but Vasquoz et al. (1989) reported that this range should be within 20-200 mg/l as CaCO₃. Further Goodwin and Hanson (1975) reported that when the hardness level is greater than 300 mg/l as CaCO₃ in water, the prawn shows retarded growth rates with high incidence of encrustation of Epistyles and precipitation of calcium carbonate. Brown et al. (1991) found that the growth of *M. rosenbergii* was maximum at hardness levels below 53 mg/l as CaCO₃ and survival was impaired at higher values.

5.2.5 Ammoniacal Nitrogen

Dissolved nitrogen compounds (ammonia, nitrate and nitrite) are the most important water quality parameters in culture system. Build-up of ammonia or nitrite indicates that one or both groups of nitrifying bacteria responsible for their conversion to nitrate are present in insufficient numbers or water quality conditions are not suitable for their growth. Ammonia and nitrites can cause severe mortality in *M. rosenbergii* larvae. Sub lethal concentration of these two compounds can cause cessation in feeding, retardation of growth, or increased susceptibility to parasites and disease in prawn (Armstrong et al. 1976).

Parry (1960) reported that the principal form of nitrogen excreted by crustaceans is ammonia. The proportion of ammonia as unionized ammonia
increases with increasing pH, salinity and temperature (Emerson et al. 1975). Smith and Piper (1975) found that high ambient levels of unionized ammonia affect osmoregulation and oxygen transport in aquatic species and sub lethal levels cause pathological changes in different organs and tissue.

5.3 Age and Growth pattern

To generate broodstock, juveniles of *M. gangeticum* *M. malcolmsonii* and *M. rosenbergii* having size 0.41gm, 0.58gm and 0.40gm respectively were stocked in the pond for twelve months of rearing period. Rajyalaxmi et al. (1979) reported fastest growth in prawn under culture condition during the mid February to May. Under such conditions, males grow faster to attain larger size than the females (Henderson and Mathai, 1910; Ibrahim, 1962; Rajyalakshmi, 1968,1980; Kurian and Sebastian, 2002). The size of males up to 230 mm and females 133 mm of the *M. malcolmsonii* were recorded by Henderson and Mathai (1910), whereas Ibrahim (1962) recorded the size of males up to 230 mm and females 197 mm in the river Godavari. George et al. (1998) made a record of 165 mm females with 150 gm weight and 200 mm males with 170 gm weight in river Cauvery. Fully grown males, 240 mm in total length and 160 gm in weight and females 159 mm in length and 90 gm weight were recorded by Rajyalakshmi (1968) in river Godavari. However, Kurian and Sebastian (2002) reported males with 240 mm and females 210 mm size. More or less similar growth rate in *M. malcolmsonii* males (190mm) and females (170mm) were recorded in the present study. A comparative study of *M. malcolmsonii* from river Hoogly and Godavari made by Rajyalakshmi (1980) indicated the five years group in males and four-year group in females in river Godavari, whereas three years group in males and two years in females in Hoogly river. Similar observations have also been made by Raman (1984) from the river Mahanadi at Cuttack. In the present study, the growth rate in *M. gangeticum* recorded similar to *M. malcolmsonii*. The growth rate in *M. gangeticum* males recorded from 65 – 225mm, whereas in females it ranged from 65-190 mm. Tiwari and Holthuis (1996) reported the length of adult males between 165 and 188.5 mm and
adult females around 130mm. Whereas Bate (1868) recorded the total length 6 inches (150mm) in *M. gangeticum*. Brown (1957) examined and categorized the extent of growth in different parts of life and concluded that in most of the animals, the specific growth rate is highest in early life, but slowed down with the increasing age. The specific growth rate was recorded highest in younger stage and declined slowly with the age and index at zero (Minto, 1908; Brown, 1957; Rajyalakshmi, 1966). The growth rate in crustacean depends upon the three factors i.e. environment (mostly water temperature), food and water quality. Animals takes food and develop the body tissue, since its body is covered with hard chitinuous exoskeleton, it remains constant during the interval of molting. The size of animal expands only at the time of molting when body covering becomes soft and elastic. The different growth rates in two different sexes reported in prawns revealed that the males grow faster than the females (Rajyalakshmi, 1980).

5.3.1 Maturation and occurrence of berried prawns

*M. gangeticum* and *M. malcolmsonii* was found to attain maturity at sizes 75mm and above in the stretch of the river Ganga, whereas *M. malcolmsonii* recorded at 83mm in Hooghly, 68mm in Godavari river system and 81-85 mm in Kolleru lake (Rajyalakshmi, 1980; Rao, 1987). However, Ibrahim (1982), Ahemad (1984, 1999), Patel et al. (1984) and George et al. (1968) have recorded a smaller size range from 41-58 mm in the river Godavari, Mahanadi, Cauvery and Ojat (Gujarat). Mohapatra (2001) recorded the maturity at 60mm and above under pond condition of *M. malcolmsonii* and *M. rosenbergii*. The testicular maturity in *M. gangeticum* and *M. malcolmsonii* are attained at 75mm, whereas, secondary sexual characters in males are recorded with the presence of appendix masculina observed on the endopodes of the second pleopods at 60mm size. The occurrence of berried females in both the species during last week of May indicated maturation and breeding of the prawn, which continue till the end of October. The number of berried females recorded more during the month of August and September, which was found peak period for their breeding.
However, prolong breeding period of nine months from April to December with peak during August to November was observed in Kolleru lake by Rao (1986). Ibrahim (1962) made similar observation in the river Godavari. However, this period was restricted to 6th month from May to October in river Mahanadi and Ganga as well as under pond condition (Kanaujia, 1989, 1999; Kanaujia and Mohanty, 1994; Mohapatra, 2001). Rao (1986, 1991), Kanaujia et al., (1999) and Mohapatra (2001) studied the maturity stages of ovary in *M. malcolmsonii* and *M. rosenbergii*, and reported four stages of the ovarian development based on the colour and size of the ovary in relation with carapace cavity and diameter of the ova. Rao (1986 and 1991) and Rajyalaxmi (1961) studies the maturity stages of ovary in *M. malcolmsonii* and *M. rosenbergii* and reported that the ovaries in both the species are paired, elongated and flattered structures, situated on the dorsal side of the stomach and hepato-pancreas and limited to cephalothorax. The multiple breeding has been recorded in three *Macrobrachium* species, just after spawning ovary is observed in stage one. The embryonic development proceeded inside the fertilized egg in brood chamber. On the other hand, ovarian development inside the ovary took place. Just after hatching of zoea stage I, the spent female observed with matured ovary, which is ready for pre-mating molt corresponding to breeding and spawning. As the ova are already matured in side the ovary. Therefore, prawns breed and spawned three to four times within one season. In the present study the breeding of two prawn species *M. gangeticum* and *M. malcolmsonii* was recorded from May to October as evident from the occurrence of berried females in the catch. But occurrence of breeding in different river system as well as lakes like Chilka in Orissa and Kolleru lake in Andhra Pradesh, depends upon the climatic conditions mostly water temperature. The prawns may also induce for breeding under controlled condition by maintaining water temperature, food and water quality as reported by (Kanaujia 1998, 1999; Kanaujia et al., 1999a; Mohapatra, 2001; Mohanty, 2003). In the present study, the breeding process in three species found similar with those of other workers recorded in *M. rosenbergii*, *M. malcolmsonii* and *M. gangeticum* (Ling and
Although, breeding and embryonic development of three species take place in freshwater under river system, the newly hatched zoea stage I drifted out along with water current and reached to the estuary and complete their larval stages in brackish water. Which has been also reported by Ibrahim (1962), Rajyalakshmi (1980); Rao, (1986), Kanaujia (1989, 1999) in $M.\ malcolmsonii$ and Kanaujia, et al.(2001) and Kanaujia (2003) in $M.\ gangeticum$. Kanaujia et al., (1999), reported mating and spawning in $M.\ malcolmsonii$ year around under captivity by maintaining water temperature. Mating behavior in three species observed similar with those of $M.\ gangeticum$, $M.\ malcolmsonii$ and $M.\ rosenbergii$ studied earlier by Rao (1965), Ling (1969a) and Mohapatra (2001). These species observed to breed four times and the number of eggs in first breeding was found less, which increases moderately in second and third breeding and reduces again in fourth breeding. The freshly spawned eggs of $M.\ malcolmsonii$ are yellow and in $M.\ gangeticum$ green yellow in colour. Whereas, it has been reported orange yellow colour in $M.\ rosenbergii$, (Rajyalakshmi 1960; Uno and Sao, 1969; Kanaujia, 1998, 1999, 2003). The fertilized eggs carried by the females were undergone for embryonic development till the hatching of zoea stage I. Which appeared on 12-13 days in $M.\ gangeticum$, 12-15 days in $M.\ malcolmsonii$ whereas in giant freshwater prawn $M.\ rosenbergii$ reported longer period for incubation and embryonic development 18-25 days (Uno and Sao, 1969; Kanaujia, et al., 2002). The hatching period in $M.\ malcolmsonii$ started mostly during night (24 hrs) and completed before morning (5 -6 hrs). This period was prolonged till to the morning (12 hrs) in $M.\ gangeticum$ and much longer in $M.\ rosenbergii$ where hatching start during night (24 hrs) and completing in 2nd night or 2nd day (24-36 hrs) (Ling, 1969; Fujimura and Okamoto, 1972; Kanaujia, 2003; Kanaujia, et al., 2001). However, this period may fluctuate further depending upon the water temperature reported by New and Singholka (1985); Kanaujia (1998, 1999) and Kanaujia and Mohanty (1992).
5.4 Larval Rearing

Freshwater prawn farming has been taken up the shape of an industry throughout in the world with different prawn species available in different regions. However, the major constraint faced for its development is the availability of adequate quantity of quality seed (New and Singholka, 1985; New and Valenti, 2000; Kutty et al., 2000). The Indian fishery farmers and entrepreneurs are facing similar problems. Although prawn seed are available in natural waters to be utilized for stocking in ponds but it has many disadvantages such as greater variations in stage and size (Suharto, et al., 1980), contamination of seed with pathogens and mixed lot of various species. It is also difficult to get sufficient required quantity of seed during stocking period (Kanaujia, 2003). Keeping all the factors in view, the production of seed on commercial scale in hatchery became utmost importance.

The newly hatched zoea stage I larvae of three *Macrobrachium* species found transparent or translucent and have the spot of red and blue chromatophores during early stage. However, the colour of chromatophore found to fluctuate on some portion of the body followed with their development. The larvae of *M. malcolmsonii* during early stages appeared with churning movement, whereas, in later stage, they moved along the side of the tank as well as in the water column. The movement of the advanced stages of larvae appeared to be moderate. The observations made in the present study of *M. malcolmsonii* are found similar with those of other workers (Rao, 1991; Kanaujia and Mohanty, 1992). However, the movement of the larvae of *M. gangeticum* is found more active which is similar to that of *M. rosenbergii* reported by Fujimura, (1966), Kanaujia et al., (2001) and Kanaujia, (2003). The number of larval stages in *Macrobrachium* species is non-synchronous in nature, depends on several factors (Sandifer and Smith, 1979). In the three species several molts with eleven distinct larval stages are recorded to attain post larval stages, which is in agreement with the reports of Rao,(1991), Kanaujia and Mohanty (1992) and Mohapatra (2001). But the number of larval stages in *M. malcolmsonii* was reported 16
by Kewalramani et al. (1971) and eleven stages by several other workers (Anon, 1990; Rao, 1991; Kanaujia, 1998, 1999; Yadav, 1992; Sharma, 1994; Kanaujia and Mohanty, 1992; Mitra, 2001; Mohapatra, 2001). The stocking density, food and water qualities are important factors, which influence the larval growth and post larval production under hatchery conditions.

5.4.1 Water Quality

Successful operation of a prawn hatchery with proper management of the water quality parameters has been suggested by New and Singholka (1985), Kanaujia and Mohanty (1992) and New and Valenti (2000). The chemical composition of the larval rearing medium during hatchery operation undergoes enormous changes, which are not visible. The quality of water deteriorates during larval rearing mainly due to accumulation of metabolic wastes released by the larvae, live food organism particularly Artemia nauplii and decomposition of unutilized feed settled at the bottom of the larval rearing tank. The deterioration of water quality increased considerably and exerts stress on the larvae make them susceptible to various diseases and thus resulting in their mass mortality. Hence, to avoid all these unwanted factors, airlift bio-filter re-circulatory system adopted in the present study appears to be useful and effective tools for maintaining congenial water quality in the hatchery, particularly effective control of the excreted ammonia (Kanaujia and Mohanty, 1992; Kanaujia et al., 1998b). The technique adopted for the larval rearing in the present study, showed promising result which have realized various benefits of airlift biofilter recirculatory system during hatchery operation of the prawns reported by several workers (Minamizava and Morizane, 1970; Sandifer and Smith, 1974; Smith et al., 1976; Minasveta, 1982; Lee, 1982; Aquacop, 1983; Natali, 1989; Chen et al., 1991; Minasveta et al., 1991; Millamena et al., 1991; Ng et al., 1992; Kanaujia and Mohanty, 1992). Aquacop (1983a) recorded 100% survival of the larvae of M. rosenbergii till the occurrence of first post larvae while adopting re-circulatory system similarly. Vasudevappa...
et al. (1997) recorded good survival and production of post-larvae and suggested effective use of bio-filter re-circulation system in inland regions; under controlled water quality condition with the use of suitable water heater to maintain the temperature. Kanaujia and Mohanty (1992) reported that the airlift bio-filter re-circulatory system is most effective and efficient in successful metamorphosis of post larvae of *M. malcolmsonii* over water renewal and green water system. Airlift biofilter recirculatory system is commonly adopted by various workers in the operation of prawn hatchery and reported promising result. They have also suggested used of this system may also reduce the cost of seed production in inland regions (Singholka and Sukapunt, 1982; Chauvez and Remirez, 1983; Ong, 1983; Vu-Zung-Tion, 1989; Valenti and Daniels, 2000). They have also reported that the larvae in this system metamorphosed to post-larvae earlier than that of the water exchange system. Similar results were obtained in the present study while adopting the airlift bio-filter re-circulatory system in larval rearing trails of *M. gangeticum, M. malcolmsonii* and *M. rosenbergii*. Effectiveness of the airlift biofilter recirculatory systems provides good water quality for larval culture, has been well established. Natali (1989) found post-larva metamorphosis of complete batch of *M. rosenbergii* without any water exchange; the system controls the concentration of ammonia, nitrate, nitrite and COD within the safe levels. Therefore, larval metamorphosis occurs effectively. The various water quality parameters such as salinity, temperature, pH, total hardness, total alkalinity, dissolved oxygen, ammoniacal nitrogen, nitrite nitrogen etc. required to be maintain within the desired level for effective larval rearing.

### 5.4.1.1 Salinity

Salinity indicates the total concentration of all the ions in brackish water medium. The source of salinity is either from marine salts or salts of terrestrial region or both being used for larval rearing. The marine salts are predominated with chloride and sulphide ions, while the land base salts are usually carbonates and bicarbonates. The quality and quantity of salts
significantly influenced the growth and development of the larvae. Adults *M. gangeticum*, *M. malcolmsonii* and *M. rosenbergii* are hyper tonic of environment as they inhabit in freshwater but the osmoragulation particularly during the larval stages takes ions from the environment and restricts ions loss from their body. Therefore, the larvae need a suitable range of salinity for their optimum development (Kanaujia and Mohanty, 1992; Mitra, 2001; Kanaujia et al., 2001). In the present study, salinity varied from 12 – 18 ppt in 6 trials of *M. gangeticum* showing relatively higher variation as the optimum range of salinity for this species reported 12 – 16 ppt (Kanaujia et al., 2001). The variation in salinity in *M. malcolmsonii* in six trails varied from 12 – 20 ppt as the optimum salinity range reported in higher range 18 – 20 ppt, whereas salinity range in *M. rosenbergii* recorded 10-16 ppt (Kanaujia and Mohanty, 1992). Ling and Merican (1961) and Fujimura (1966) reported that the larval rearing of *M. rosenbergii* occurs in brackish water, since the larvae are euryhaline. The salinity range during larval culture of *M. rosenbergii* varies from 10 to 18 ppt (Brock, 1993). Lower salinity range may prolong the metamorphosis duration with poor survival rate considerably (Rao, 1991; Kanaujia and Mohanty, 1992; Mitra, 2001).

### 5.4.1.2 Temperature

Prawns are temperature dependent cold-blooded animals. The temperature of water regulates the metabolism and growth of various larval stages of prawn. Rao (1991), and Kanaujia and Mohanty (1992, 1999) have recommended a favorable temperature range 28-31°C for optimum larval growth and development of *M malcolmsonii* and 24-34°C for *M rosenbergii*. New and Singholika (1982) and Diaz and Ohno (1986) have reported that the temperature above 35°C and less than 24°C may result retarded growth and mortality of the larvae of *M rosenbergii*. According to Boyd (1990), a sudden change in the ambient water temperature over 2°C may cause thermal shock and even death of the larvae. In the present study, the temperature recorded in *M gangeticum* trails, range from 29-30°C, in *M malcolmsonii* 29.8-32.2°C and *M. rosenbergii* 27.5 - 31.1°C. The variations
in temperature found very narrow range in the present study, which was recorded twice daily during morning and evening hrs to know the maximum and minimum temperature during the rearing trails for effective management. Indulkar (1996) reported that the temperature ranging from 27-29.5°C yields 8-20% better survival rate and 16-18 days early occurrence of post larvae. Gibson (1975) reported optimum temperature range 28-31°C for *M. rosenbergii* during the larval rearing.

### 5.4.1.3 pH

Most of the biological parameters of aquatic water bodies are influenced by pH. It is defined as the negative logarithim of the hydrogen ion concentration and it shows acidic or basic nature of water (Boyd, 1990). The medium pH has been recognized as important parameters during larval rearing of all the *Macrobrachium* spp. It measures the hydrogen ion concentration in the water and therefore, serves as indicator of acidity and alkalinity. Due to the buffering nature of the saline water, the pH fluctuations are comparatively less than that of freshwater. The pH values between 6.5-9.0 in larval rearing of freshwater carps and prawn has been considered favorable (Ellis, 1937). The pH in water is generally affected by the concentration of free carbon dioxide, bi-carbonate and carbonate ions. It is greatly influenced by the presence of residual feed, larval metabolites and molting shells present in the water medium of the freshwater prawn hatchery. High pH increases the un-ionized fraction of ammonia and thereby increased the toxicity of ammonia in water. Low pH decreases the utilization of the oxygen by larvae and increases the toxicity of nitrite and hydrogen sulphide (Chandraprakash and Reddy, 1993). In the present study the increase in pH during the larval rearing period was observed and maintained within the range of 7.5 – 7.9 by periodic application of calculated amount of calcium sulphate and calcium hydrogen phosphate in biological filter tank of the rearing trials which is suggested by Kanaujia and Mohany (1993) for larval rearing trails of *M. malcolmsonii*. The total dissolved ammonia affects the survival particularly during molting when the pH of the
water is high. Therefore, to avoid possible toxicity of ammonia in prawn hatchery Kanaujia and Mohanty (1992) suggested maintaining the water pH within the range of 7.5 – 8.5. New and Singholka (1985) have reported a suitable range of pH between 7.5 and 8.5 in the water during larval rearing of giant freshwater prawn *M. rosenbergii*. In the present study, pH maintained in all the 12 trails for seed production of three species was slightly lower as compared to the other workers reported earlier. However, the initial higher pH was observed in the trails, which did not continue for longer period to affect the larval growth due to the application of calcium sulphate and calcium hydrogen phosphate in the bio-filter tanks.

5.4.1.4 Dissolved Oxygen

Dissolved oxygen reported to be the most important physico-chemical parameters of the rearing medium which influences the growth and production of the larvae through its indirect impact on optimum feed consumption and metabolism. The optimum levels of dissolved oxygen in the larval rearing medium of prawns commonly maintained through water aeration with the use of aerators / air compressor / air blower (Brock, 1993). To maintain the desirable level of dissolved oxygen in larval rearing trials, the accumulation of unutilized feed to be avoided with the provision for providing appropriate quantity of feed to the larvae and cleaning of the tank bottom at regular intervals. Dissolved oxygen is important not only for respiration but also for maintenance of most favorable chemical and hygienic environmental conditions of the larval rearing medium. In the present study, the oxygen varied from 5.0 – 5.9 mg/l which is found a very narrow range. The wide variations in dissolved oxygen were reported by Mohapatra (2001), with wide variation of climatic temperature and disruption of power failure. Since, temperature and salinity both influence the oxygen solubility in water, the use of airlift bio-filter re-circulatory system maintained the dissolved oxygen within the range in all the trails. However, the mean value recorded at weakly intervals was not significantly different in the trails. Kanaujia and Mohanty (1992) and Kanaujia (1998, 1999) recorded better
production of *M. malcolmsonii* post larvae by maintaining the dissolved oxygen level between 4.73 – 6.24 mg/l and suggested as optimum range for the seed production of *M. malcolmsonii*.

### 5.4.1.5 Total Hardness

Total hardness affects the growth of the larvae and mineralization of carapace. The optimum total hardness level needed for larval metamorphosis was reported within the range of 3800 – 5200 mg/l in *M. malcolmsonii* (Kanaujia and Mohanty, 1992). Mohapatra (2001) recorded total hardness level within 2020-2220 mg/l as calcium carbonate in the comparative larval rearing study of *M. malcolmsonii* and *M. rosenbergii* and found within the desired level. In the present study, the total hardness ranged form 3230 – 3298 mg/l in larval rearing trials of *M. gangeticum*, *M. malcolmsonii* and *M. rosenbergii* during two years. Which is found similar with the report of Mohapatra (2001) recorded in *M. malcolmsonii* and *M. rosenbergii*. However, it differ form those of Kanaujia and Mohanty (1992), they have recorded higher-level hardness 3800 – 5200 mg/l in *M. malcolmsonii*. Thus, it can be concluded that among the three *Macrobrachium* species under larval rearing investigation, *M. gangeticum* shows better efficiency in terms of growth, reproduction, larval development, salinity requirement, duration of larval cycle etc. than those of *M. malcolmsonii* and *M. rosenbergii* under the similar environmental condition.

### 5.4.1.6 Total Alkalinity

Total alkalinity denotes the quantity of acid consuming constituents present in the water. In natural water bi-carbonates and carbonates are the main alkaline sources, which determines the pH of water. Water with low alkalinity recorded with low buffering action, which leads too wide range of fluctuation in pH value. High alkalinity increases the pH and leads to cause the larval mortality. Alkalinity range from 50 – 100 mg/l have been reported the desirable for *M. rosenbergii* larvae (Chandraprakash and Reddy, 1993). During the course of present study, the total alkalinity of the larval rearing
medium ranged from 75.4 – 95.2 mg/l which is found to be a desirable level as reported above in *M. rosenbergii*.

5.4.1.7 Ammoniacal Nitrogen

Ammonia is the second most important water quality parameter after oxygen as it is one of the most toxic factor to the cultural organisms. Ammonia although toxic to the prawn larvae, originates in larval rearing medium through excretion by the larvae and presence of algal blooms as well (Chin and Chen, 1987). Ammonia exists in water in two forms, namely, un-ionized ammonia (NH₃⁺) and ammonium ion (NH₄⁺). The rise in pH and temperature of water increases the concentration of un-ionized fraction of ammonia. Compared to ammonium ions the un-ionized ammonia is highly toxic to the larvae. The total ammonia (NH₃⁺ + NH₄⁺) less than 0.12 ppm in the rearing medium is considered ‘safe’ for prawn larvae (Kanaujia and Mohanty, 1992; Kanaujia, 1998, 1999). The efficacy of re-circulatory systems with the provision of biological filter controlling the level of dissolved ammonia within an acceptable limit has been reported by several workers (Menasveta, 1982; Horsch, 1984; Hoergenson, 1985; Chen et al., 1991; Ng et al., 1992; Kanaujia and Mohanty, 1993). In the present study, the initial accumulation of excretory ammonia was found in all the trials during first week of rearing. However, it has been controlled effectively through the operation of airlift re-circulatory system with the provision of bio-filter and kept it within 0.073 – 0.109 mg/l, which was much below the ‘safe level’. Such trend of ammonia levels in the rearing trails suggests that improper conditioning of the biological filter has accumulated total ammonia in the rearing medium initially, and which was reduced after the conditioning of the bio-filter unit, thereby stimulating the nitrification rate, which corroborates with the views of Kanaujia and Mohanty (1992) and Mohapatra (2001). Interestingly, the average ammonia content was significantly lower in some of the trials, which might be due to the in effective nitrification of chelated ammonia in the rearing trials in the presence of Na-EDTA.
5.4.2 Larval food and feeding

Freshwater prawn larvae eat continuously but they do not actively search for food, therefore, Moller (1978) has suggested that the food density is critically important to be maintained as per the density of the prawn larvae. Ling and Uno (1987) suggested that the food density reduces following the growth of the larvae. Therefore, suitable food, proper and timely feeding and water quality management are the important factors for successful larval rearing under hatchery operation. Therefore, among them, food is found important inputs in running cost of hatchery operation. The expensive live food *Artemia* nauplii are mostly used in most of the hatcheries of the world in combination with formulated supplementary feed (Goodwin and Hanson, 1975). In the present study the larvae of *M. gangeticum* and *M. rosenbergii* were fed with *Artemia* nauplii alone up to 7 days to *M. malcolmsonii* larvae and up to 15 days. Thereafter, food was provided in combination with egg custard and mussel meat as per feeding scheduled maintained. Initially, larvae were fed with *Artemia* nauplii twice daily for one week followed by four times a day during rest period. Once few post larvae occurred in the tanks, egg custard and mussel meat were provided ad-libitum till the end of rearing period. As discussed earlier, the food and feeding propensity of the larvae of three species was observed; the larvae of *M. gangeticum* and *M. rosenbergii* feed voraciously, grow and to attain the metamorphosed post larvae stages within 22-32 days. This nature of feeding was not observed in of *M malcolmsonii* larvae. Dugan et al. (1975) emphasized that better growth rate in the larvae of *Macrobrachium* spp. should be at the feeding frequency of three to four times a day. However, feeding frequency of four times/day in the present study has been found ideal for better utilization under prevailing condition of the larval rearing.

In the present study, it has been observed that the acceptance of *Artemia* nauplii was better than the supplementary diets (egg custard and
mussel meat). Optimum feed intake by the larvae and the size of food particles reported to contribute an important role for larval growth (Sharma, 1994). The suitable size of food particles for different stages of the larvae of *M. malcolmsonii*, has been suggested by Kanaujia and Mohanty (1992) and Kanaujia (1998,1999). In the present study, the feed was prepared by fine mincing the food materials in fine particles by passing through appropriate sieves. The method of preparation of the desired size of food particles was suggested by Ling (1969), Dugan et al (1975), Menasveta and Piyatiratitvokul (1990), which was found very efficient and effective method for feeding the larvae in the present experiment. Present study clearly indicated that the appropriate size of food particles and strict schedule of feeding 4 times/day proved to be useful for good survival, uniform growth and development of the larvae of these two species, which corroborates with the observation of Kanaujia and Mohanty (1992) and Raje and Joshi (1992).

5.4.3 Larval Growth and Metamorphosis

There are several factors, which are directly or indirectly affect the growth and development of prawn larvae under culture system. Stocking density is an important factor for larval rearing and subsequent seed production. Different authors have suggested different stocking density for larval rearing of *M. rosenbergii* (Ling and Costello, 1976; Sick and Beaty, 1979; Hsiech et al, 1989; New, 1990; Rao and Tripathi, 1993). Beside that food and feeding, water temperature, water quality and management of husbandry play important role in growth and development of larvae. Rao and Tripathi (1993) have suggested that larval growth and metamorphosis are related with water temperature, water quality and feed, which need to be critically monitored on every day in order to obtain better growth. The larvae of *M rosenbergii* reported to attain post larval stage within 22-32 days after hatching at optimum water temperature and salinity 25.2-35.8°C and 14-16ppt respectively. In the present study, the growth of larvae of *M. gangeticum*, *M. malcolmsonii* and *M. rosenbergii* initiated with more or less
similar size (1.81, 1.87 and 1.92mm) at stage I and size varied from 8.31-11.79 while attaining to post larval stage between 22 and 50 days respectively. In between these stages the variations were recorded in progressive increase in size and duration for subsequent stages in three species. The larval development in *M. gangeticum* and *M. rosenbergii* in all the trails during two years progressed at the same pace. But the growth and rate of metamorphosis was delayed in case of *M. malcolmsonii*. The growth and development of *M. malcolmsonii* have also been studied by Kanaujia and Mohanty (1992) and recorded 1.65-2.40mm size of stage I larvae, the progressive growth increment from stage I to stage VI is rather slow but increases rapidly from stage VI onward. The stage V takes 10 – 15 days to attain stage VI. Thereafter, the growth rate and time taken by each subsequent stages is quite short and more or less similar, except stage XI, which takes little longer times to attain post larval stage. Similar observation were obtained in the present studies of *M. malcolmsonii*, however, it differed from that of *M. gangeticum*, in which the subsequent development of larval stages from stage I to post larvae observed similar to that of *M. rosenbergii*. Which takes 22-32 day to attain the post larval stage of a complete batch. More or less similar duration was recorded in the present study of *M. gangeticum*. According to Rao and Tripathi (1993), the larvae of *M. rosenbergii* normally takes 1 to 4 days to reach I stage to next stages. The larval growth and metamorphosis recorded to vary considerably between the outdoor (Yard condition) and indoors conditions. However a synchronous larval development is reported under both the system up to zoeal stage IV only. The observations in the present study in three species indicated that the transition of individual larvae from stage I to next stage occurred synchronously up to stage IV. Overlapping of stages was recorded during later stages (after stage V and above). The overlapping stage was more significant in *M. malcolmsonii* than those of *M. gangeticum* and *M. rosenbergii*. These observations are corroborate with the earlier records of Kanaujia and Mohanty (1992) and Rao and Tripathi (1993), in which they have reported that the number of larval stages increased from 2-3 in early
part of the cycle (1-15 days). But as the cycle progress into its second half (day 16-25 above) the duration in range of stage is increased to 3-5 days.

In the present study, the duration of larval cycle of *M. malcolmsonii* was kept for 60 days whereas in *M. gangeticum* and *M. rosenbergii* it was 50 days. But the occurrence of first post larva in *M. malcolmsonii* was recorded within 40-42 day, *M. gangeticum* on 22-24 day and *M. rosenbergii* on 30-31 day. The occurrence of first post larvae in most of the reports varied from each other's. Kanaujia and Mohanty (1992) obtained first few post larvae in *M. malcolmsonii* within 40 to 53 days in natural brackish water and on 41st day in artificial seawater. Whereas, Mohapatra (2001) obtained first post larvae of *M. malcolmsonii* within 39-42 days, Sankolli et al. (1994) recorded first post larvae within 22-35 days and Rao (1991) observed them on 52nd day. But Yadav (1993) recorded them on 46-72 days respectively. The duration for the larval cycle of a batch varied considerably in different freshwater prawn species. In the present study duration for one cycle was kept 50 days in *M. gangeticum* and *M. rosenbergii* where 95% larvae metamorphosed into post larvae. Whereas, in *M. malcolmsonii* it was kept for 60 day where 80% larvae metamorphosed into post larvae.

5.4.4 Molting Frequency

Growth of freshwater prawn as well as most of the crustaceans depends on the frequency of their molting. According to Rao and Tripathi (1993), visible growth in prawns is registered only at the time of molting, which occurred immediately after all the stages of life cycle. The frequency of molting is dependent on the age, quality, and quantity of food consumed and environmental parameters. Molting process is also evident in larval stages, the number of larval stages and the frequency of molts by the larvae to metamorphose into post larval stages significantly varied reported by a number of workers. Eight number of larval stages in *M. rosenbergii* have been reported by Ling (1969), eleven by Uno and Sao (1969) and seventeen by Gomez and Kashahara (1987). The total number of mots
undertaken by the larvae to attain each stage varied. Similarly Kewalramani et al. (1971) first time recorded 15 molt and 16th zoeal stages in *M. malcolmsonii* to reach post larval stage. Thereafter Sankolli et al. (1934) obtained post larvae after passing through 18-20 molts, later on many workers recorded eleven distinct zoeal stages before attaining post larval stage in *M. malcolmsonii* (Rao, 1991; Kanaujia and Mohanty, 1992; Yacav, 1992; Qureshi 1994; Mohapatra, 2001).

The observations made in the present study found similar with the earlier, recorded in the prawns *M. gangeticum*, *M. malcolmsonii* and *M. rosenbergii* passed through 11 distinct larval stages, however, the number of molts in three species found some what similar, where the larvae stage I passed through one molt at each stage from stage I to IV, and 3-8 molts between stage V-VI, thereafter the molting frequency recorded one or two at each stages to reach post larval stage in *M. gangeticum* and *M. rosenbergii*. These observations was found different from that of *M. malcolmsonii*, where 6-13 molts were reported in V-VI stage minimum 2-3 molts in subsequent stages (Uno and Sao, 1969). Such factors can be attributed strongly to the variation of food and genetically variation during the development of larval cycle in different *Macrobrachium* spp. Which are also overlapping from stages during larval metamorphosis.

5.4.5 Larval Survival

In the present study, larvae were stocked at the rate of 50 larvae/l in all the trials during two years. However, the survival of the post larvae in all those trails at the end while concluding the experiments ranged from 61.09±10.60-70.35±6.70% in *M. gangeticum* during first and second year. Whereas *M. malcolmsonii* it was recorded slightly less which ranged from 58.46±7.52-66.73±9.42% and maximum survival was recorded in *M. rosenbergii* from 73.44±8.52-76.77±8.76. The survival of post larvae in prawn hatchery mostly depends upon the management of husbandry. Therefore, the initial stocking at higher rate may not be considered for
computing the survival while obtaining the post larval production at the end of hatchery operation. Stocking density of 40 larvae/litre has been recommended for best growth and survival (Sick and Beaty, 1979; Ling and Costillo, 1976).

5.4.6 Post Larva Production

Present study revealed that most of the larvae in *M. gangeticum* metamorphosed into post larvae within 50 days of larval cycle. At the end of experiments the number of post larvae recorded during first and second years, were maximum 6663 and 5090 respectively in *M. gangeticum*. Whereas post larvae production in *M. malcolmsonii* was recorded maximum 2376 followed by 2143 respectively and *M. rosenbergii* it varied 5-68 followed by 4337 respectively. Rao (1991), Kanaujia and Mohanty (1992) reported the duration of 60 days or more for the 80-90% metamorphosis of complete batch of larvae of *M. malcolmsonii*. Whereas, in the present study, the duration of 50 days for *M. gangeticum* recorded to be much shorter than the *M. malcolmsonii* and similar to *M. rosenbergii*. The wide variation in duration of post larval metamorphosis in *Macrobrachium* species has been reported by several workers (Malecha, 1983; Kanaujia and Mohanty, 1992; Sankaran and Nair, 1992; Kanaujia, 1998, 1999; Kurian and Sebastian, 2002; Mohapatra, 2001). 46-63 days variation in *M. malcolmsonii* at various larval stages recorded to be more pronounced as compared to *M. rosenbergii* and *M. gangeticum* where the whole batch of larvae may attained post larval stage within 32-45 days and 22-45 days respectively (Ling, 1964, 1969a; Kanaujia et al., 2001; Kanaujia, 2003). The instars duration between the stages were monitored through molting, which took place for all the larvae un-informally in a healthy population. However, the delay in molting either due to genital region or unhealthy condition of the female prawns influences the instars duration (Sankaran and Nair, 1992). In the present study, the growth rate is more or less uniform in all three species during early zoeal stages (I - V) and it is in agreement with the findings of Yadav (1993) and Chaudhury (1995). The occurrence of second
to fifth larval stages simultaneously in the larval culture of *M. rosenbergii* suggested that the feed density, size of particle, water exchange and husbandry must be adjusted periodically to accommodate this variation in larval stages (Malecha, 1983). The long duration (22-50 days in *M. gangeticum* 37-63 days *M. malcolmsonii* and 30-50 days in *M. rosenbergii*) occurring in the metamorphosis of larvae into post larvae in two species necessitated their removal from the larval rearing tank in order to avoid cannibalism as well as cost for providing expansive feed. Therefore, a specially designed "string shells" was successfully used to harvest the post larvae daily without disturbing and stressing the larval population (Kanaujia et al., 2002). The use of "string shell" for the removal of post larvae from the larval rearing tanks were not only useful for harvesting the post larvae but also suitable for providing hiding space for the advance larvae of stage XI which were almost ready to metamorphose into post larvae (Kanaujia et al., 2002). Further the trend of post larval metamorphosis recorded through harvesting of post larvae on every day on occurrence of 1st post larvae onwards showed fluctuation in post-larvae production in all the units, which was observed decreasing trend of post larval metamorphosis at later stage of the cycle.

A suitable and effective larval rearing medium is the key in success of large-scale prawn seed production. All the trails during two years in present study resulted in successful metamorphosis of the post larvae. Although, the appearance of first post larvae was noticed earlier in *M. gangeticum* in all the six trails where the total number of post larvae produced were also recorded maximum as compared to post larvae produced in the trails of *M. malcolmsonii* and *M. rosenbergii*. The post larval production per litre (PL/L) and percentage survival also followed the same trend as evident the trails of *M. gangeticum* gave better results in every respect than the trails of *M. malcolmsonii* and *M. rosenbergii*. However, the major differences among the trails between two species with regard to survival and post larval production and duration for the larval cycle indicated the genetically difference in the three *Macrobrachium* species. Further the
mode of preparation for larval food and rearing medium may also have a bearing on the larval growth. It needs further investigation on the ingredient composition for various larval stages for better growth of *M. malcolmsonii* larvae so that duration of larval cycle may reduce and seed may be produced in mass scale. Further the maximum post larval production with higher survival rate in shorter duration in *M. gangeticum* indicated for the inclusion of one more candidate *Macrobrachium* species in freshwater prawn farming.