2. LITERATURE REVIEW

2.1. Taxonomic status and distribution:

The genus *Toxorhynchites* was grouped earlier under a distinct taxon in the 19th century after Robinian-Desvoidey (1827), who proposed the generic name *Megarhinus*. Later, Theobald (1901b) and Edwards (1932) included the genus *Toxorhynchites* as *Megarhinus* under *Oulicidae*. Subsequently, the genus *Toxorhynchites* was accorded subfamily status. The classification of the genus *Toxorhynchites* was based upon the adult characteristic features such as the structure of proboscis and its insertion in the head capsule and V-shaped cusitomarginal ridges of the wing (Colless 1979). Several bibliography of the genus *Toxorhynchites* were published from time to time (Trimble & Smith 1975, Fish & Beaver 1978, Steffan et al. 1980). The latest bibliography of the genus *Toxorhynchites* was the annotated bibliography of all known references to this genus, which contained 1295 references and a supplement containing an additional 452 citations (Steffan et al. 1980). This was later updated by Manning et al. (1982), Steffan (1985), and Steffan & Evenhuis (1985). The genus *Toxorhynchites* currently contains 69 valid species which have been grouped under three subgenera. Two subgenera, *Ankylorhynchus* and *Lynchiella*, occur in the New World, primarily in the Neotropical region. The largest subgenus, *Toxorhynchites*, occurs primarily throughout the Old World tropics from Africa to the Western Pacific. The species of the genus *Toxorhynchites* are amazingly similar morphologically throughout their distribution and it is very difficult to identify them at the species level (Steffan 1975).
The earliest species of the genus *Toxorhynchites* described from the Australian, Oriental and Palaeartic regions are *T. splendens* (Wiedemann 1819) from Java, *T. susulifer* and *T. amboinensis* Doleschall (1957) as *Culex* from Amsoni island in Macaku. *T. splendens* (Wiedemann 1819) had been identified and known by many names such as *Culex regius* Tennent in 1859, *Megarchinus immisericorpus* Giles in 1901, *M. sikkimensis* Giles in 1901, *M. gilesii* or *M. immisericorpus* Theobald in 1901, *M. splendens* (Wiedemann) by Giles in 1902, *T. argenteotarsis* Ludlow in 1906, *Worcesteria grata* by Banks in 1906, *M. splendens* (Wiedemann) by Bhatia and Notananda in 1953.

*T. splendens* group contains seven valid taxa including two taxa such as *T. splendens* and *T. amboinensis*. Larvae of *T. splendens* are predators of small invertebrates including larvae of mosquitoes.

*T. splendens* has been recorded from the Oriental region (Indian subcontinent, Sri Lanka, Burma, Thailand, Malaysia, Singapore, Indonesia excluding islands east of Wallace's line and Philippines), the Ethiopian, Australian and Parallactic regions, and common in coastal areas (Macdonald 1958, Thurman 1959, Yasuno & Tonn 1970). *T. christophi*, another species of this subgenus is found in the Amur Valley, USSR, 54°N latitude (Shamrai & Gutsevich 1974) and in the New World, *T. rutilus septentrionalis* extends into Canada 45°N latitude (Parker 1977). The southernmost extension is that of *T. bryvipalpis*, a widespread Ethiopian species found at 32°30'S latitude. Members of this genus are primarily inland forest dwellers although *T. splendens* is common in coastal areas. In Indian subcontinent, only *T. splendens* has been reported to breed in coastal and forest areas (Barraud 1934, Nagpal & Sharma 1987). In their survey on the mosquito species in Northeastern region of India (Assam, Arunachal Pradesh, Megalaya, and Mizoram) they found *T. splendens* to breed in the tree holes.
Adult of T. splendens is distinguished from other species by the absence of a wide pale yellow margin on the mesonotum, and presence of well-developed lateral tufts of hairs on VI to VIII abdominal segments. While the color of the lateral tufts of hair on VII abdominal segment is black, the same on VIII abdominal segment is orange. Scales on mesonotum are dull bronzy or greenish-brown in color except laterally. Some tarsal segments have white markings. T. splendens is a large species with wing-length usually 6 mm or more. One or more of the three terminal tarsal segments on fore or hind legs are without white markings (Barraud 1934).

Larval length when fully grown is 15-16 mm. Larval color is crimson or deep reddish-brown. Length of siphon-tube is 1.1-1.5 mm. Siphonal tuft has 4-8 bristles. Ventrolateral plate is one in number, sometimes divided into two. Head and chitinised parts of body in larval skin are deep rich brown in color (Barraud 1934).

2.2. Colonization and mass-rearing:

Virtually all biological control strategies with the exception of certain inoculation type programs, involves mass-production of biological control agent (Focks & Boston 1979). Few of the 69 known species of the genus Toxorhynchites have been successfully colonized. Trpis and Gerberg (1973) established a laboratory colony of T. brevipalpis. They reared the larvae individually in small petridishes containing 13-20 ml water in order to avoid cannibalism. Newly hatched larvae of Ae. aegypti were offered as food. They used cages of size 20 cm³ for keeping adults in which they mated readily. Mating was also observed in small cages of 15 cm³ size. Absorbent cellulose soaked in honey and water was provided as food. Furumizo et al. (1977) reported the first successful attempt to
mass-rear a colony of *T. splendens*. They started the colony with field caught adults in colony cages (61 cm$^3$). Larvae of *Ae. aegypti* and *Ae. albopictus* were offered as prey at 10 Nos. per predator. It was found that the adults paired more readily in a cage of this size, which accommodated about 4500 adults. The first batch of eggs were laid 5-8 days after adult emergence. By adapting these techniques, several thousand adults were produced on a biweekly basis. Techniques for the mass-production of species of the genus *Toxorhynchites* in the laboratory were also demonstrated by Focks and Boston (1979). Effective transportation of *Toxorhynchites* by ship was succeeded by Focks (1979a) which solved the problem of mass transportation of *Toxorhynchites* for field releases. Focks et al. (1977) found that when *T. rutilus rutilus* reared with surpluses of *Ae. aegypti* larvae, the immature development took lesser duration. Later Focks et al. (1978) reported for the first time that species of the genus *Toxorhynchites* could be reared on a commercially available food for tropical fish. The average larval period was 107.5 days as compared to 15.6 days when reared on *Ae. aegypti* larvae.

2.3 Biology:

The first general review of the biology of the genus *Toxorhynchites* was done by Horsfall (1955). Subsequently, the biology of *T. septentriomalisis* was reviewed by Hemmerlein and Crans (1968). Trimble and Smith (1975) published a detailed bibliography of *T. r. rutilus* and *T. r. septentriomalisis*. Later, Fish and Beaver (1978) published a bibliography of the aquatic fauna of bromeliads and pitcher plants, which includes many key references to the genus *Toxorhynchites*. The pertinent literature of the systematics, biology, and biological control potential of species of the genus *Toxorhynchites* was reviewed by Steffan (1975). Biology of *T.*
splendens was studied in the laboratory by Furumizo and Rudnick (1978). Steffan and Evenhuis (1981) updated the informations on the biology of the genus Toxorhynchites.

2.3.1 Egg:

Aerodynamic streamlining of eggs of the genus Toxorhynchites was related to oviposition habit of this genus (Mattingly 1969). The eggs have an outer surface that is hydrophobic. Therefore, the eggs float horizontally high on the water and is negatively thigmotactic. Eggs bounce when ejected or dropped, a property possibly related to oviposition behavior of the genus Toxorhynchites (Furumizo and Rudnick 1978). In many instances, access to the water at the oviposition site is obstructed or too small for the female to enter. In such situations, the physical properties of the egg facilitate it reaching the water surface. Egg can not withstand desiccation. Although the outer surface of the egg is hydrophobic, the inner surface is hydrophilic, which facilitates the rapid escape of the emerging first instar larva (Dodge 1964). The incubation period normally ranges from 40 to 60 hr. (Focks & Boston 1979) and is temperature dependent (Trpis 1972). Egg viability has been reported for T. brevipalpis (Bonnet & Hu 1951), T. splendens (Furumizo & Rudnick 1978), T. r. septentrionalis (Trimble 1979) and T. amboinensis (Steffan et al. 1980), and generally falls within the range of 57-100%. Furumizo and Rudnick (1978) observed egg hatching rate of 77-91%. Trimble (1979) found no relationship between female age and egg hatchability. However, Steffan et al. (1980) reported that egg viability of T. amboinensis remained constant during the first seven weeks and decreased steadily thereafter as the colony aged.
2.3.2. Larva:

2.3.2.1. Larval Habitats:

Belkin et al. (1965) described larval habitats of the genus *Toxorhynchites*. Artificial container habitats include almost an endless variety of man-made containers discarded in or near forest areas, the most common being cans, barrels, earthen pots and tyres. Macdonald (1958) reported that *T. splendens* was a common coastal species in Malaysia. It bred in Nipa palm axils, fallen coconuts, tree holes and artificial containers. This report was further corroborated by Cheong and Ganapathipillai (1964).

Tree holes and bamboo stumps are considered to be the most common natural container habitats. The attractive characteristics of the tree hole habitat for mosquito breeding were described by Belkin et al. (1965). *T. r. rutilus* and *T. r. septentrionalis* were found to breed in deciduous forest areas (Jenkins and Carpenter 1946). Vertical distribution of the breeding of many *Toxorhynchites* species was studied by Corbet (1961). His study reflected the height preference of the genus *Toxorhynchites* for oviposition, which in turn avoided the interspecies competition and habitat overlapping. Corbet (1961, 1963) reported that *T. kajmosi* bred at levels ranged from 30 to 50 ft. However, another species *T. brevipalpis conradi* preferred ground level in the same area. Species composition of the bamboo tree hole and species interactions were studied elaborately by Macdonald (1958, 1960). His study has given impetus to understand the complexity of the bamboo tree hole habitats. Another larval habitat studied elaborately was habitats in pitcher plants (*Nepenthes* spp.) (Beaver & Fish 1978). Thienemann (1932) recorded 25 insect species, of which eleven species were of the genus *Toxorhynchites*. Beaver (1979) studied the complexity of pitcher plant habitat and its
characteristics conducive for the breeding of a variety of insects including the genus Toxorhynchites.

Laird (1947) reported that larvae of T. inornatus tolerated highly polluted water with more organic debris and with a pH range of 5.5 - 7. However, it was reported that larvae of T. r. septentrionalis died when placed in sewage water with chlorine content of 0.3 ppm (Jenkins and Carpenter 1946). In general, most of the natural habitats are found to have highly polluted water with organic debris. So it is assumed that larvae of the genus Toxorhynchites like other tree hole breeding mosquitoes should have developed tolerance to heavy organic pollution. Studies of Muspratt (1951) in the laboratory indicated that larval mortality may be due to adverse changes of surface film and bacterial contamination.

2.3.2.2. Predation and feeding:

Instars of the genus Toxorhynchites are predaceous and mosquito larvae constituted their chief prey. However, they were also found to feed on other prey including larvae of Chironomidae, Tipulidae, small nymphs of Dragonflies and Syrphid (Breland 1949, Jenkins 1964). The predatory behavior of larvae of the genus Toxorhynchites is basically opportunistic. They do not search for prey; instead they ambush prey as it comes within their range. Swimming towards prey seems to be an exception (Breland 1949). Trpis (1972) found that larvae of T. brevipalpis predated prey efficiently at high temperature, with more prey consumed at 30-32°C. He found that each larva destroyed an average of 154-358 larvae of Aedes aegypti during its development. The effect of illumination on the predatory habits was studied by Goma (1964) and there seemed to be little effect of illumination on predation. Feeding rate was found to be adjusted in response to low
prey densities (Lounibos 1979). Corbet (1963) reported that fourth instar larvae of T. brevipalpis conradti slowed their metabolism and feeding in response to food scarcity. Fourth instar larvae of T. rufulus, after a period of starvation, resumed feeding at an initially low rate (McCray 1965). It is also not known whether predation of different instars of the genus Toxorhynchites could differ with different genera of prey mosquitoes such as Oulex, Anopheles and Aedes. Blumberg and Swiraki (1974) have pointed out the influence of quality of food on food selection and feeding rate. Such differences in the quality of food may also have definite influence on growth and survival of insects. The rate of predation of larvae of T. splendidus was studied by Paine (1934). An average of 9.2, 10.4, 13 and 91.6 larvae of Oulex spp. were consumed by first, second, third and fourth instar of T. splendidus respectively. Chan (1968) reported that total number of Ae. aegypti larvae consumed by four larval instars of T. splendidus were 6.7, 8.5, 34, and 83.9 respectively. Larvae of T. splendidus when provided with respective instars of Aedes spp., consumed an average of 8, 13, 33 and 96 prey (Furumizo and Rudnick 1978). Prey consumption by larvae of the genus Toxorhynchites involves many variables (Jenkins 1964). Hemmerlein and Crans (1968) reviewed the biology of T. r. septentrionalis including prey consumption. Beaver (1979) studied the low minimum requirement of prey for fourth instar of T. klossi in relation to possible alternative food availability. The geographic variations in the development time and predation of the larvae of the genus Toxorhynchites were attributed to variance in the temperature. These possible biological differences in relation to climate and environment seem important (Trimble & Smith 1978).
2.3.2.3. Cannibalism:

All instars of the genus *Toxorhynchites* are reported to be cannibalistic (Furumizo & Rudnick 1978, Steffan et al. 1980, Steffan & Evenhuis 1981). Cannibalism is considered to be the most important behavioral trait of this genus because this may affect the stable prey-predator equilibrium under the lower threshold level and yield of adult in mass-rearing. Banks (1908) and Jenkins & Carpenter (1946) did not observe cannibalism in their laboratory studies on *Toxorhynchites* spp. But latter studies showed that larvae of the genus *Toxorhynchites* were cannibalistic only if prey were unavailable (Horsfall 1955, Williams et al. 1961). It was reported that when larvae of the genus *Toxorhynchites* approached each other head on, they avoided cannibalism (Breland 1949, Muspratt 1951). Furumizo and Rudnick (1978) described the aggressive ritual behavior of the larvae of the genus *Toxorhynchites*. The backward swimming of larvae of the genus *Toxorhynchites* makes them highly cannibalistic. Cannibalism was observed when large numbers of larvae of the genus *Toxorhynchites* were placed in the same container, even when abundant quantities of prey were provided (Green 1905, Jenkins & Carpenter 1946). Field observations revealed the presence of one or two larvae of the genus *Toxorhynchites* per container. This may be due to the peculiar oviposition habit of this genus. This may also be due to cannibalism when prey is in less number. Presence of more than one predator per container, frequently observed in the field conditions supported the belief that though cannibalism is frequently observed in laboratory conditions when prey density was less, in field condition the predator larvae were found to coexist in a single container (Corbet & Griffiths 1963, Trpis 1973, Lounibos 1979). It was also observed that presence of floating surface debris in the
container habitats reduced cannibalism (Lounibos 1979). Egg cannibalism was also reported in the species of the genus *Toxorhynchites* (Gerberg 1985).

2.3.2.4. *Prepupal compulsive killing behavior*:

Killing behavior of fourth instar of the genus *Toxorhynchites* has been reported for 7 species (Steffan & Evenhuis 1981). Three distinct types of predatory behavior were observed in most of the *Toxorhynchites* spp.: (1) complete consumption of prey, (2) partial consumption of prey, and (3) killing without consuming any part of the prey (Trimble & Smith 1978). The compulsive killing behavior is apparently similar to normal predatory behavior except that the dead larva is not consumed (Crans and Slaff 1977). First preliminary field observation on the compulsive killing behavior was reported by Taylor (1989). Chan (1968) reported that all instars of *T. splendens* displayed the compulsive killing behavior both before and after each molt but it was most pronounced 2-3 days before pupation. The most generally accepted theory to explain this behavior of the genus *Toxorhynchites* is protection of the relatively vulnerable pupa from predation by fourth instar of its own kind (Corbet & Griffiths 1963, Lounibos 1979). Killing behavior was rarely noted until the predator reached the body weight of 30-33 mg. Both the numbers of prey killed but not eaten and the length of the killing phase increased and were linearly correlated with increase in prey density. Killing behavior was greatly reduced at 12 prey per predator (Lounibos 1979). Russo (1983, 1986) described this behavior as a separate artifact different from normal feeding.
2.3.2.5 Time to starvation death:

Time to starvation death of instars of genus *Toxorhynchites* is an important parameter in biological control consideration. The fourth instar is the most resistant to starvation. It was reported that fourth instar of *T. splendens* could withstand starvation for about 7 weeks (Paine 1934, Chan 1968). In another study it was found that the limit of starvation period for normal recovery of *T. splendens* larvae was 3 or 4 weeks only (Muspratt 1951). Purumizo and Rudnick (1978) reported that the first and second instars of *T. splendens* would die within 2 days without food. When first, second, third and fourth instars of *T. r. rutilus* were fed with larvae of *Ae. aegypti* 0, 4, 9 and 13 days respectively before the test began, their respective survival was 7, 8, 18 and 59 days without food (Focks & Hall 1977). This adaptive character has also been observed in other species of the genus *Toxorhynchites* (Steffan & Evenhuis 1981).

2.3.2.6 Larval development:

Larval development of the genus *Toxorhynchites* is related to prey density, feeding behavior, and temperature (Trpis 1972, Trimble & Smith 1978). In general, higher the prey density, more rapid is the development time. On the otherhand, the immature developmental time is inversely related to temperature. Paine (1934) reported that larval duration ranged from 16 to 134 days depending on food availability. Chan (1968) found that *T. splendens* completed immature development in 39.2 days in the laboratory. The life studies of *T. r. septentrinalis* in the laboratory revealed that the duration from egg to pupae was 21.8 days (Crans & Slaff 1977). Mean duration from egg hatch to adult emergence of *T. splendens* was 21.5 days (Purumizo & Rudnick 1978), which was shorter than the duration
reported by Steffan et al. (1980) for T. amboinensis. This difference was related to high predator-prey ratio maintained by Furumizo and Rudnick (1978). In T. brevipalpis, minimum prey required for completion of the fourth instar development was between 60 and 70% of its total consumption during the larval period (Vongtangswad and Trpis 1980).

2.3.2.7. Functional responses:

Many mathematical models have been proposed to describe the predator-prey relationship and reviewed by Hassell (1978). These models could be considered satisfactory in that the behavior they predict can be classified in a way similar to the one observed in the real world. The predator-prey interaction was first explained by Solomon (1949). He called the response of the predator to change in prey density as functional response. The another terminology which he used was numerical response which relates to the change in predator number with change in prey density. Behavior associated with tracking prey density, such as functional response, can be directly related to components of predator’s fitness. Functional response generally has been considered from the standpoint of the impact of predation on prey population (Murdoch & Oaten 1975). The concept of the functional response of predators has been reviewed by many people (Murdoch & Oaten 1975, Hassel et al. 1976, Van Lentern & Bakker 1976). The importance of the functional response as insect predator measure was highlighted by Hassel et al. (1976). Changes in the character of the predator resulting in changes in the functional response is called developmental response (Murdoch 1973). Functional response is determined by two parameters namely \( (1) \) attack rate or rate of successful search \( (a') \) (the proportion of prey killed
per unit of time by a predator) and (2) handling time ($T_h$) (time spent in pursuing, subduing, eating and digesting each prey by a predator) (Holling 1959, Hassell et al. 1976). The value of "a'" and "$T_h$" vary with the relative sizes of predator and prey and the predatory efficiency. Thompson (1975) measured "a'" and "$T_h$" for older nymphal instars of the damsel fly Ischnura elegans attacking different size classes of Darphini megalus. McArdle and Lawton (1979) measured "a'" and "$T_h$" for instars of Notonecta glauca attacking four full size classes of D. magna. Venkateson and Sivaraman (1984) reported that five instars of the water bug, Diplonychus indicus, when exposed to four instars of Ae. aegypti and Cx. quinquefasciatus at varying densities, showed the type II functional response with the increasing attack rate (a') and corresponding decreasing handling time ($T_h$). Largest instar of predator killed maximum number of smallest prey (first instar) and vice versa of the both prey species; later predator instar showed more successful attack rate and shorter handling time than the earlier instars. Sherratt and Tikasingh (1989) found Type-II functional response of Holling (1959) and Rogers (1972) in instars of T. moctezumae when prey constituted Ae. aegypti. They reported higher searching rate in later instars than earlier instars and conversely higher handling time for early instar predator and late instar prey.

2.3.2.8. **Prey behavior in response to predation:**

Antipredator response of prey is an important factor which influences the predator-prey relationships. In general, prey respond to the initial presence of predators by various avoidance behaviors such as reducing movement or change in using the foraging area etc. There have been a number of reports of various responses of prey to
predator (Edmunds 1974, Morse 1980, Jeffries & Lawton 1984 for reviews). All these studies were based on behavior, cues and the adaptive significance of the resulting patterns. Workers interested in the adaptive basis of antipredator behaviors have often found a rough correspondence between prey response and predation risk, i.e., prey show a strong response to dangerous predators and a relatively weak response to less dangerous predators. The susceptibility of prey to a given predator also differs, more susceptible prey typically show a stronger antipredator response. These studies are restricted only to qualitative relationships between predation risk and response and lack quantitative informations. An exception was the work of Sih (1982) who made quantitative assessment to examine the relationship between predation risk and prey response. In this he quantified prey responses to predator. *Ae. aegypti* and *Cx. quinquefasciatus* were found to avoid the predator *Notonecta* spp. by spending more time near the edges of experimental containers and their movement was less. By this way they were found to reduce predation risk (Sih 1979, 1982). Less prey movement reduced the chances of encountering predator and conspicuousness to predator (Murphy & Mendenhall 1973, Sih 1979).

2.3.2.9. Prey selection:

Several models of prey choice by predators have shown how the profitability of a prey can be maximized by predators adopting to particular foraging strategies, i.e., time to feed, the place of feeding, the feeding technique or the type of prey chosen depending on their density (Schoner 1971, Ealen & Ealen 1975, Pyke et al. 1977, Krebs 1978 for reviews). According to optimal foraging theory, the major influencing factor on the prey choice of a predator is the profitability of the prey, and this is a function of net energy
gained from the prey after spending energy and time in pursuing, catching and ingesting activities. For maximizing net energy gain, the predator should specialize on the most profitable abundant prey item as it involves less energy and time. If the abundance of that prey type decreases, the predator should include the next most profitable prey in its diet. The most appropriate way to test this is to offer prey of a given species but from various size classes to the predator. Thus, other factors which influence the prey choice other than prey size, such as palatability, conspicuousness are eliminated.

2.3.3. Pupa:

The pupal stage of the genus *Toxorhynchites* is considered as the most vulnerable to predation by other predators and cannibalism by fourth instar of its own kind (Corbet & Griffiths 1963). However, pupae of the genus *Toxorhynchites* can escape predation by submerging under water for several minutes. Pupal duration is reported to last for 3-7 days (Jenkins & Carpenter 1946).

2.3.4. Adult:

2.3.4.1. Pre-reproductive period:

Oogenesis:

Females of the genus *Toxorhynchites* are not capable of taking blood meals and autogeny is obligatory. The follicles (oocytes) develop asynchronously and high degree of oosorption is noted in young females. This anomalous pattern of oogenesis is related to the peculiar life history of the sub-family *Toxorhynchitinae* (Watts & Smith 1978). In nature, container breeding mosquitoes such as *Ae. aegypti*, *Ae. albopictus* and *Cq. quinquefasciatus* colonize quickly and their short life cycle makes control by conventional methods
difficult. The two requirements for the effective check on the breeding of these mosquitoes by a predator such as the ability to oviposit in available habitats and distribution of eggs over a number of sites and over a relatively longer period are fulfilled by the species of the genus *Toxorhynchites* (Watts & Smith 1978, Trimble 1979). These characters of the genus *Toxorhynchites* tend to reduce crowding that could deplete available prey and result in cannibalism. In the genus *Toxorhynchites*, these conditions are met by continuous production of mature follicles and the adoption of a more primitive asynchronous pattern of oogenesis. In contrast to other mosquitoes, members of the genus *Toxorhynchites* exhibit interruptible oviposition behavior.

**Pre-oviposition period:**

In *T. splendens*, oviposition occurred 5-7 days after adult emergence (Pocks & Hall 1977, Furumizo & Rudnick 1978, Steffan et al. 1980). Paine (1934) reported that females of *T. splendens* imported into Fiji usually oviposited one week after emergence. Observation by the same worker in Hawaii showed a minimum pre-oviposition period of 4 days. Observations of pre-oviposition periods of other species ranged from 5 to 12 days; the usual period is considered to be 7 days (Steffan & Evenhuis 1981).

2.3.4.2. **Mating:**

Laboratory observation showed that mating in *Toxorhynchites* occurred 2-3 days after adult emergence (Pocks & Hall 1977). The mating of the genus *Toxorhynchites* was unique that male either flew to and pounced on a resting female or intercepted the female in flight and attempted to copulate from the rear, then adults in
copulation fell to the cage floor. Trpis and Gerberg (1973) reported that adults of T. brevipalpis, when kept in cages of size 0.2 m³ mated readily.

2.3.4.3. Oviposition:

Oviposition behavior:

Oviposition in the genus Toxorhynchites is aerial in nature which is one of the key aspects of the biology of the genus Toxorhynchites (Steffan & Evenhuis 1981). The ovipositing female normally flies in oblique loop of 3-12 cm in diameter, 5-10 cm above the water surface. A single egg is forcibly ejected on the downward flight. The female then repeats the pattern or departs the oviposition site. Females could deposit up to 10 eggs in a period of 1 minute before resting and were observed to oviposit at least 3 times within 1 hr. (Steffan & Evenhuis 1981). Furumizo and Rudnick (1978) reported that females did not touch the water surface immediately before or during oviposition. This indicated the presence of hygrogroceptors to detect potential aquatic breeding sites. The same authors also observed that females of T. splendens never oviposited while resting on the water surface.

Aerial oviposition by members of the genus Toxorhynchites has several selective advantages. It allows oviposition in obstructed container habitats such as obstructed tree holes and bored bamboo where the female could not have free access to the water surface (Steffan & Evenhuis 1981). The deposition of a single egg after relatively elaborate flight pattern is also of selective advantage to the survival of the predaceous larvae. The female is more likely to oviposit in several breeding sites, thus reducing danger of cannibalism among her progeny. Aerial oviposition may also reduce likelihood of capture by predators, especially spiders, which frequently congregate around tree holes (Trimble 1979). Confinement
of adults to a relatively small cage prevented any prolonged flight of a dispersal nature, which may be an obligatory prerequisite to subsequent oviposition.

**Oviposition rhythm:**

Oviposition activities of females of *T. splendens* at night was observed by Furumizo and Rudnick (1978). But others reported oviposition in the afternoon hours (Paine 1934, Steffan et al. 1980). There was a defined diel peak in the oviposition of *T. r. moctezuma* in and near forests of Trinidad, and >68% of eggs were laid between 8 and 10 hr. after sunrise (Chadee et al. 1987).

**Oviposition period:**

Laboratory studies indicated that the oviposition period ranged from 27 to 95 days (Steffan & Evenhuis 1981). Steffan et al. (1980) observed oviposition period of 85-95 days. This is much longer than the 27 days reported for *T. splendens* (Furumizo & Rudnick, 1978).

**Fecundity:**

Hu (1955) reported that females of *T. amboinensis* and *T. brevipalpis* oviposited over 400 eggs each in laboratory colonies. Initial individual fecundity averaged 14.3 eggs/day. After 77 days, fecundity decreased to 4.8 eggs/day. Fecundity of 40-50 eggs during a two day period (Banks 1908) and 35 eggs during an eight day period (Newkirk 1947) were reported. Focks et al. (1977) reported that females of *T. r. rutilus* laid on an average 1 egg/day. Focks and Hall (1977) tried to increase the egg production by rearing in the outdoor cages, but got only 0.83 eggs/female/day. Trimble (1979) reported that the proportion of females laying eggs varied from 0.45 to 0.82
on first five days of oviposition and then decreased to an average of 0.3. It was postulated that initial relatively high fecundity in the vicinity of adult emergence would take advantage of nearby development sites, but subsequent dispersal could reduce crowding at the original emergence site and ensure that other available sites are colonized.

Oviposition site preference:

Informations on the oviposition site preference of the genus Toxorhynchites and the existence of oviposition stimuli such as the presence or past presence of prey could be important in biological control by members of the genus Toxorhynchites. The finding of Focks and Hall (1977) revealed that T. r. rutilus laid 247 eggs in jars containing colony water which was 302 times more than the eggs laid in jars containing well water. Generally, the genus Toxorhynchites prefers to oviposit in well-shaded habitats (Arredondo-Bernal & Rayes-Villanuera 1989). In Thailand, Yasuno and Tonn (1970) reported that eggs of T. splendens were deposited singly or in groups of less than ten. Eggs were oviposited mostly in water jars, especially in jars containing rain water and lot of debris which were found to support other aquatic organisms. There were vertical variations in the oviposition site selection among the species of the genus Toxorhynchites. Species of the genus Toxorhynchites preferred to oviposit in dark brown water and preferred black oviposition jars (Steffan & Evenhuis 1981). However, Chadie (1985) reported that water drums received 60% of the total eggs laid by T. moctezuma. Habitat selection initially appears to be by visual cues and modified by
hygroscopic responses to presence of water vapor (Steffan & Evenhuis 1981, Benzson et al. 1988). Oviposition was inhibited in T. brevipalpis at temperature below 21°C (Muspratt 1951).

Adult longevity:

Newkirk (1947) reported male and female life span of 27 and 33 days respectively in T. splendens. The adult life span of 42 days was reported for T. splendens (Gerberg & Trpis 1973). Furumizo and Rudnick (1978) estimated an adult life span of 28–35 days. Focks et al. (1977) reported female survival of 49 days in T. r. rutilus. A maximum survival of 120 days of T. amboinensis was reported by Steffan et al. (1980). All the above workers observed gradual mortality of adults which appeared to fit Slobodkin’s (1962) Type II survivorship curve.

Dispersal:

Relatively little is known about dispersal of adults of the genus Toxorhynchites. Adults rest on trunks of trees and on vegetation near oviposition sites (Macdonald 1957, Sato & Arita 1968, Ramalingam 1974). Paine (1934) observed that dispersal of the introduced T. splendens was hampered by dense undergrowth. Macdonald (1958) reported that the coastal distribution of T. splendens and its propensity for ovipositing and breeding in artificial containers facilitated its spread throughout coastal areas of the Oriental region. Nakagawa (1963) reported that ten years after the introduction of T. amboinensis into Hawaiian island, this species spread to Oahu island of 303 miles. It has since spread to other major islands in Hawaiian chain. Likewise, Engber et al. (1978) reported possible accidental spread of T. amboinensis from American
Samoa to the northern coast of Upolu, Western Samoa. The distance between these two islands is 72.5 km. Although *T. splendens* has been reported windblown several miles between islands of the Fiji group, Engber *et al.* (1978) discounted the possibility of natural dispersal.

2.4. Predator–prey population interaction:

Predator–prey population interaction in nature should be a sustained one for the effective control of prey population below threshold level. O’Flynn (1975) and O’Flynn & Craig (1982) studied the population interaction of *T. brevialpis* and *Ae. aegypti* in colony cages of size 1 m$^3$. They reported that the interaction between populations of these two species continued for about six months. A classical predator–prey relationship was witnessed between the natural population of *T. brevialpis* and *Ae. aegypti* in a tyre dump in Dar es Salaam, Tanzania (Trpis 1973). There was a marked seasonal fluctuations in population density with increase in *Ae. aegypti* population preceding increase of *T. brevialpis* population. The existence of complexity in nature is found to affect the predator–prey system and this was studied by Huffaker (1958) and O’Flynn and Craig (1982). Increase in complexity of breeding habitats is believed to increase the survival of both predator and prey. Studies of *T. brevialpis* and *Ae. aegypti* predator–prey system amply demonstrated that the predator–prey system is influenced in many ways by the structural complexity of the environment. They are (1) effect of complexity on distribution of eggs among the sites and (2) its effect on predation rate within the site. The complexity may be increased by leaves and other debris. The observation of Flynn and Craig (1982) on the experimental continuous breeding populations of
T. brevipalpis and *Ae. aegypti* in room-sized cages elucidated the sustained effect of the structural complexity in keeping the predator-prey interrelationships going on at the low population level.

2.5. Insecticide susceptibility:

Much work has been done on the cumulative effects of insecticides on the biology of vertebrate predators (DeBach 1974). Nonetheless, little is known about insecticide accumulation in predaceous insects (Van Halteren 1971). Sublethal dosages of insecticides may affect insect population by affecting survival and reproductive ability. Wiedl (1977) offered fourth instar larvae of *Ae. aegypti* previously exposed to dieldrin at 0.03 and 0.09 ppm to larvae of *T. brevipalpis*. He found adverse effect on daily food consumption, accompanied by an increase in the time of pupation. Focks et al. (1979b) indicated that *Ae. aegypti* was twice as susceptible as that of *T. r. rutilus* to organophosphorus insecticides tested and that *T. r. rutilus* adults were 16 times more susceptible to permethrin.

2.6. Control trials using *Toxorhynchites*:

The use of the genus *Toxorhynchites* as a potential control agent against container breeding mosquitoes was known as early as 1920. Studies conducted in the field revealed that species of the genus *Toxorhynchites* might be useful in the biological control of container and tree-hole breeding mosquitoes. *Ae. scuttelaris* and *Ae. pseudoscutellaris* were controlled in Fiji and American Samoa respectively by the introduction of *T. splendens* (Paine 1934, Peterson 1956). An equilibrium between the predator and prey such as *Ae. aegypti* and *Ae. polynesiensis* was established at lower level by
the introduction of *T. amboinensis* to Tahiti from American Samoa in 1975 (Pichon & Riviere 1975). The breeding of *Ae. hacketi* Edwards, an important vector of simian malaria was drastically reduced by the presence of *T. splendens* in Malaysia (Cheong & Ganapathipillai 1964). By marking, releasing and recapturing adults of *T. brevipalpis*, Trpis (1973a) estimated that 3459 adults were present in a habitat covering 1 ha. The population of adults of *Ae. aegypti*, the larvae of which formed the principle prey for *T. brevipalpis* was estimated from the biting rate and was 570 in the same area at the same time. Thus, the predator population was about six times that of the prey. While studying the interaction between a species of the genus *Toxorhynchites* and *Ae. aegypti* in containers, it was observed that fewer larvae of prey was found in predestine places containing predator larvae than in those without (Trpis 1973b). Monthly changes in the number of predator larvae followed the same pattern as those of the prey, peak numbers occurring about a month later than those of the prey. It was found that the delayed effect of the genus *Toxorhynchites* upon its prey population at the beginning of the rainy season was due to slow larval development than the larvae of prey. It was suggested that this could be overcome by making mass releases of adults from laboratory colonies at the beginning of the rainy season. Workers in Thailand (Yasuno & Tonn 1970), Tanzania (Trpis, 1973) and Florida, U.S.A (Focks et al. 1980) have monitored natural populations of mosquito larvae and concluded that the genus *Toxorhynchites* was effective in reducing larval population densities of *Ae. aegypti*. Releases of eggs of the genus *Toxorhynchites* into urban areas of the island of Maarten, in the Caribbean, by Gerberg and Visser (1978) and releases of fourth instar *T. splendens* larvae into urban areas of Sa-med, Thailand by Vongtangswad et al. (1983) have reduced the population density of *Ae. aegypti*.
In Western Samoa observations confirmed that *T. amboinensis* could be effective in checking the breeding of *Ae. aegypti* in habitats such as disused tyres (Pillai 1976). Reviere (1978) have summarized the results of the introduction of *T. amboinensis* from Tutuila (American Samoa) to Tahiti in 1975 to control *Ae. polynesiensis* and *Ae. aegypti*. *T. amboinensis*, a close relative of *T. splendens* was reared in Tahiti and up to the time of reporting 50,000 adults had been released in Tahiti and neighboring islands. Entomological studies showed that *T. amboinensis* became well established in Tahiti and Moorea and perhaps in Maiao and Mangareva and the vector species were actively controlled. A preliminary field trial on the Caribbean island of St. Marsten demonstrated the feasibility of using *T. brevipalpis* against *Ae. aegypti* (Gerberg & Visser 1978). Sixteen days after the introduction of *T. brevipalpis* eggs in to all breeding containers of *Ae. aegypti*, all 21 houses sampled did not show *Ae. aegypti* breeding and the house index dropped to nil. An experiment carried out at Sea Horse key island in 1978 (Quarterly report of Entomological Research, United states department of Agriculture 1978) indicated that the genus *Toxorhynchites* significantly reduced larval densities of *Ae. aegypti* and *Cx. quinquefasciatus* in the field. Several observations were made from the Sea-Horse key island experiment in the context of using members of the genus *Toxorhynchites* as biological control agents against mosquitoes breeding in artificial containers. Firstly the high densities of late stage predators observed in this experiment tend to discount the notion that the utility of the genus *Toxorhynchites* as a biocontrol agent will be limited by cannibalism. Secondly, the ability of *T. r. rutilus* to control *Ae. aegypti* on Sea Horse key island was enhanced by the artificially induced stability of the breeding containers over
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a period of time, a fact which allowed the accumulation of predator larvae. In situations where the containers regularly and frequently dry out, the accumulation of predators seldom occurs and the high rate of increase of *Ae. aegypti* would result in a lower degree of control. Finally *T. r. rutulus* could control *Ae. aegypti* on Sea Horse key islands because tyres were the primary breeding sites and thus the larval habitats of both predator and prey coincided. Lounibos (1979) in his study found that the prevalence of *T. brevipalpis* was influenced by seasonal changes. Prey density in selected bamboo traps was reduced more than two or three fold by the presence of predator larvae during periods of peak abundance. The work of Annis et al. (1989) has showed that weekly release of first instar of *T. splendens* into household water storage containers did not control *Ae. aegypti*. When *T. splendens* larvae were released in coastal villages of Pondicherry, India, *T. splendens* brought down considerably the number of containers supporting the breeding of *Ae. aegypti* and *Armigerus subalbatus* (Viswam et al. 1990). Biweekly release of 5 to 10 second or third instars of *T. amboinensis* for 7 months failed to control *Ae. aegypti* and *Ae. albopictus* in containers in Java, Indonesia. The reason given for the failure was due to the multiple larval habitats in this rural area. (Annis et al. 1990)

2.7. Integrated vector management:

Knipling (1966) suggested an integrated approach to managing insect population through the application of insecticides and then maintaining the pest population density at low level. In an effort to control *Ae. aegypti*, insecticides were used as the primary means of control. In all such programs the control obtained was rather poor.
This was attributed mainly to the difficulty in the application of the insecticides in vast micro breeding areas of *Ae. aegypti*. So several workers proposed the use of integrated control as a mean to more effectively reduce the size of pest population (Stern *et al.* 1959, DeBach 1974). The theory of integrated control suggests the use of a variety of techniques in a combined and compatible manner to reduce pest population to acceptable level (Metcalf & Luckman 1975). One such approach is the combined use of insecticides and a predator. Focks *et al.* (1986) operationally applied this concept by initially reducing a localized population of adults of *Ae. aegypti* by ULV application of malathion. Then the remaining population of this species was further reduced by releasing adult of *T. amboinensis* (Doleshall).