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**Does cannibalism and predation confer
fitness advantage on *Anopheles gambiae*
sensu stricto and *Anopheles quadriannulatus*
(Diptera: Culicidae)**

**-Impact of environmental factors: Temperature and
nutrition on the interaction between *Anopheles gambiae*
sensu stricto and *Anopheles quadriannulatus* -**

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Preface:

Upon today millions of people in the tropics are struck by several lethal diseases. Vector borne diseases are responsible for many of those deaths and for that purpose only, it is already useful to study and to investigate the diseases and its vectors. Tropical Africa has always fascinated me, as a human and as a biology student. Therefore insects with human societal relevance draw my attention. Malaria is one of the major vector borne diseases in sub-Saharan Africa, responsible for millions of deaths annually, especially under children, and yet remains a neglected disease. To prevent malaria transmission vector control remains the most generally effective measure and is therefore one of the four basic technical elements of the Global Malaria Control Strategy (WHO, 2008). By controlling the vector's population it is possible to reduce the levels of transmission and subsequently to reduce the malaria morbidity and mortality. Therefore I find it valuable to study aspects of the vector's population dynamics, to understand all important mosquito habitat characteristics and mosquito behaviours. This can assist to fully understand the *Anopheles* mosquito population dynamics and enable to determine the vector adult populations. Ultimately this can lead to new sustainable vector control measures to diminish the global malaria burden.

Abstract:

In this study inter- and intra-specific interactions, among *An. gambiae* sensu stricto and *An. quadriannulatus*, two members of the *Anopheles gambiae* complex (Diptera: Culicidae), are analysed under different environmental (temperature) and nutritional conditions (quantities of larval food). The impact of temperature, food concentration and development time on larval consumption and adult wing length, as a proxy of fitness, are investigated. Temperature, food concentration and larval development time affected larval consumption as well as the size of the adult mosquitoes. Fourth instars of *An. quadriannulatus* consumed significantly more larvae of *An. gambiae* s.s. than of their con-specifics. This did not result in larger adults, however. For both mosquito species, predation and cannibalism did not have an effect on size of adult mosquitoes. There was a negative correlation between water temperature and body size of mosquitoes that engaged in cannibalism or consumed the other species. The effect of food quantify on body size was different for each temperature tested.

It is concluded that larval consumption on it self does not lead to larger adult mosquitoes. Therefore predatory and cannibalistic behaviour is thought to occur rather to diminish competition for food and space, than to acquire sufficient nutrients to develop into larger adults and is unlikely to affect fitness. The effect of predation and cannibalism on offspring could not be examined as emerging females refused to take a blood meal, and hence could not develop eggs.

Introduction:

Mosquito species of the *Anopheles gambiae* Giles complex (Diptera, Culicidae) are responsible for much of the malaria transmission in sub-Saharan Africa. Specifically *Anopheles gambiae* Giles *sensu lato* (hereafter termed *An. gambiae*) is the most important malaria vector in sub-Saharan Africa. *Anopheles quadriannulatus* and *An. gambiae*, both of the *Anopheles gambiae* Giles complex, occur in large parts of sub-Saharan Africa. *An. quadriannulatus* is not considered as a vector for malaria (White 1974, Takken et al. 2002)), due to its host preference. *An. quadriannulatus* is highly zoophilic, taking blood meals mainly from bovidae in contrast to *An. gambiae*, which is highly anthropophilic. During their aquatic stages, both species can be found to share the same larval habitats (Gimnig et al. 2001). Those natural habitats may dry up within a few days and undoubtedly lead to inter- and intra- specific competition for food and space (Koenraadt and Takken 2003). Presumably to diminish this competition and to secure survival, elder larvae prey and or cannibalise upon younger larvae. This predatory and cannibalistic behaviour may influence the outcome of competitive interactions between the two sibling species and consequently affect the adult population densities (Koenraadt and Takken 2003). To improve understanding of this behaviour and its potential ecological implications, we have studied the occurrence of predation and cannibalism within and between the two members of the *An. gambiae* complex in the laboratory under simulated natural environmental conditions. Understanding the *Anopheles* mosquito population dynamics enables to predict the vector changes in adult populations and can assess to improve vector control measures.

1. Biology of mosquitoes:

Family: Culicidae
Subfamily: - Toxorhynchitinae
- Anophelinae
- Culicinae

Every mosquito exhibits a variety of behaviours appropriate to its sex, its nutritional and reproductive requirements, and its need for secure resting sites (Clements 1992). Both sexes require sugar from plant sources, mates and resting sites. Additionally females require blood from a certain host, as a source of protein for egg development, and oviposition sites. The life cycle, feeding behaviour and reproductive behaviour of the 3 main culicid mosquito genera, *Anopheles*, *Culex* and *Aedes*, are defined in this chapter. Like other true flies, culicids exhibit complete metamorphosis, the juvenile form passes through both larval and pupal stages. The larvae are anatomically different from the adults, live in a different habitat and feed on a different type of food. Transformation to the adult takes place during the non-feeding pupal stage (Le Sueur 1988).

1.1 Life cycle:

Inseminated adult female mosquitoes fertilize the oocytes with spermatozoa, as they are ovipositing. After maturing a batch of eggs the female mosquito will look for and respond to stimuli from suitable oviposition sites (Takken & Knols 1999). For most mosquitoes the

oviposition site is a water body with particular characteristics that influences their choice. Different species are affected by particular breeding site characteristics like: pH, salinity, dissolved oxygen, pool size, flow, turbidity, presence and absence of vegetation and shade, food availability and pathogens.

- Egg:

The female mosquito may lay from 50 up to 500 eggs at once, depositing them on water or on sites that will be flooded. The eggs can be dropped individually to float on the water surface, as by females of *Anopheles*, or packed together to form a floating egg raft, as by *Culex* (Le Sueur 1988, Clements 1992). *Aedes* rather deposit their eggs on moist surfaces, often at the edge of a body of water or on a soil that will be flooded.

Each egg is protected by an egg shell, which surrounds the oocyte, egg, embryo and pharate larva. The egg shell is soft and flexible when laid but hardens after time. The shell is rigid and solid to provide mechanical support, protection and gas exchange while minimizing water loss.

Almost immediately after the eggs have been laid embryonic development starts and within one to two days to a week or more, depending on temperature, the embryo develops into a fully formed larva (Clements 1992). In most species the larva hatches once it is formed, and can survive for a few days at most in absence of water. Fully formed but not hatched *Aedes* larvae can survive for months or even years in the absence of water due to their water-proofed egg shells capable of resisting desiccation. Rainfall that inundates oviposition sites or a high tide can stimulate hatching.

- Larvae:

When the young mosquito larva hatches from the egg it is fully adapted for living in water. And three regions can be differentiated in the body of the larva: a sclerotized head, a broad thorax and a segmented abdomen (Kettle 1995). To develop and to survive it uses atmospheric oxygen for respiration and water-borne particles as food. Because of their air-breathing habit the mosquito larvae has either to live permanently close at the air/water interface, most anopheline and some culicine larvae do, or have to make frequent visits to the water surface. The anopheline larva lays parallel to the water surface and breathes through the spiracle at the last abdominal segment. Culicine larvae hang more downwards from the surface membrane by their siphons with their spiracles open to the air.

The larvae feed by collecting/ filtering food resources, mainly micro-organisms and plant tissues. Because the larvae live mainly in still water, the food particles are not brought to them. Through regular beating of their mouth brushes, the mosquito larvae generate water currents which flow towards the head. Anopheline larvae feed at the particle-rich water surface and culicine larvae feed on particles suspended in the water column.

During the larval period the mosquito larvae moult four times, passing through four instars. On the first three occasions that it leaves its cuticle the larva remains much as before. During the fourth moult the imaginal disks develop rapidly, changing the form of the larva more likely to that of an adult (Clements 1992), and the organism leaving the fourth larval skin is a pupa.

- Pupae:

The pupa remains an aquatic organism. The head and the thorax are combined into a cephalothorax, which is joined to a segmented abdomen. The pupa floats at the water surface with the top of its thorax in contact with the surface membrane. The respiration is taken over by a pair of broad trumpets dorsally placed on the cephalothorax. The culicine and anopheline

larva are very similar and can be kept apart due to the differences in shape of the respiratory trumpets. This final stage of metamorphosis can be completed within one or two days if the temperature is sufficiently high, and during this pupal stage the pupa does not feed. And finally when the adult is fully formed within the pupal cuticle, the insect rests at the water surface and starts to swallow air. This increases the internal pressure, forcing a split along the midline of the pupal thoracic cuticle (Clements 1992). Making it possible for the adult to expand out of the cuticle and to step on the surface water.

- **Adult:**

Soon after emergence the mosquito wings and legs become extended and the body cuticle begins to harden within half an hour of eclosion. The adult mosquito then flies to a shelter and rests for several hours. After the male mosquito's external genitalia have rotated, taking about one day, copulation may take place. During mating the male deposits his spermatozoa in the bursa copulatrix of the female, from which they move to the spermathecae. The role of adult male mosquito is limited to inseminating of females, and when not resting the males are either feeding on plant sugars or exhibiting a behaviour pattern that is likely to bring them into contact with females. The female mosquito once inseminated, carries in her spermathecae sufficient sperm for fertilization of all the eggs she may produce, will search for an appropriate host for her first blood meal. The blood meal, one or several, will provide the required proteins to start ovarian development and finally to mature eggs. Now the female will look for a suitable breeding site for oviposition. In tropical regions the females will engage in a continuous pattern of host searching, blood feeding, egg development and oviposition. In temperate regions the female will usually overwinter before laying her eggs.

1.2 Feeding behaviour:

Like other Diptera, mosquitoes are fluid feeders. Their mouthparts have evolved into an elongate composite proboscis, suitable for probing nectarines and, in the case of the female, adapted for piercing skin and imbibing blood from peripheral blood vessels. Both males and females use the sugar in the plant juices as a source of energy. But for the anopheline and culicine females to develop mature egg batches, proteins are required and proteins are highly concentrated in blood. Consequently the female mosquitoes engorge vertebrate blood and the proteins are incorporated into the oocytes to sustain embryo's development. The subfamily Toxorhynchitinae is an exception, females are able to reproduce feeding only on plant juices. The blood meal provides the anopheline and culicine female besides the needed proteins also a trigger that initiates endocrine events that allow her to utilize the proteins (Klowden and Briegel 1994).

Mosquitoes feed from flowers or some other plant organs to obtain plant fluids or nectar, that provides both sugars and water, important components of mosquito nutrition. The sugars are essential for the survival of males, almost certainly increase the life span of the females of many species, and have important effects upon female behaviour, physiology and fertility (Clements 1999). Plant feeding behaviour occurs right after emergence or when metabolic reserves are getting depleted. The plant juices provide an immediate energy source and permits the formation of carbohydrate and lipid reserves.

Blood feeding is required for almost all female mosquitoes to reproduce. After emergence and after each gonotrophic cycle single or multiple blood meals are needed, and for this the female mosquitoes expose blood feeding behaviour. The feeding behaviour of any mosquito species includes several aspects: (1) The identity of the vertebrate species on which it feeds in

nature, (2) whether the mosquito's host range is restricted or unrestricted, which describes the range of host species the mosquito feeds on and (3) the quantitative distribution of feeds between host species in particular localities (Clements 1999). The vertebrate hosts are primarily mammals, some mosquitoes feed also on birds and a very few feed on reptiles and amphibian. Different mosquitoes do not feed equally well from their hosts, it displays host choice. Some are very specific others are generalists. The host choice may be determined by a large number of factors, probably acting in combination, including behavioural, physiological, morphological, ecological, geographical, temporal, genetic considerations and host defensive behaviour (Lehane 2005). As a result each mosquito population has his own host-feeding pattern, the distribution of feeds taken on different vertebrate hosts. By blood-meal analysis the host-feeding patterns of *Anopheles*, *Aedes* and *Culex* are determined, but due to the large genera, information is available only for a small percentage of species. The host-feeding pattern indicates the species restricted, unrestricted or opportunistic feeding behaviour on the vertebrate classes and to which extent species related by genus share a similar pattern. For epidemiological purposes it is important to know and to understand mosquito's feeding pattern, host choice and host specificity to determine a species vector capacity. The blood-meal data obtained for *Anopheles* and *Aedes* suggests that many of its species mainly feed on mammals (Clements 1999). Because of the medical importance of *Anopheles*, most of these studies have been done in and around human habitations, and therefore on species that live in association with human populations and their domesticated animals. Only for relatively few *Culex* species their feeding patterns are known. For some species mammals are the primary hosts, for others birds, while certain feed on both. And only a small percentage *Culex* species feed on amphibians or reptiles (Clements 1999).

1.3 Reproductive behaviour:

For mosquitoes to reproduce, male and female need to mate after emergence, upon which the female blood-feeds, matures the eggs and finally ovipositions.

- **Mating:**

After 12-36 hours after emergence, the time for males to invert their terminalia and erect their antennal fibrillae, male mosquitoes become sexually responsive to conspecific females. For the female mosquito, once a 30-60 hours non-receptive period is terminated, the virgin female flies actively in search of males, and do not resist male sexual advances (Clements 1999). For mating to occur, sequences of behaviour bring males and females nearby, insects use a variety of stimuli, volatile sex pheromones and loud sounds. To bring both culicids sexes into proximity three classes are known: swarming by males, grouping at biological significant places, as their emergence sites or around their vertebrate hosts, and the approach of isolated resting females by males (Clements 1999). Once a male has grasped a receptive female, sexual intercourse progresses, from coupling to copulation and insemination. During insemination the male deposit his spermatozoa in the bursa copulatrix of the female, from which they move to the spermathecae. In general the inseminated female carries in her spermathecae sufficient sperm to fertilize all the eggs she may produce, thus most mate only once early in their adult life. Subsequently the female mosquito will search for a appropriate host for her first blood meal to continue reproduction.

- Gonotrophic cycle:

The process of searching for a host, ingestion and digestion of a blood meal and egg maturation, followed by oviposition is repeated several times throughout the female's life and is referred to as the gonotrophic cycle (Klowden and Briegel 1994). After mating responsiveness to host cues is required for the mosquito to blood-feed. And most anautogenous females are capable to take a first blood-meal 24 to 72 hours past emergence. This reaction towards hosts can be stimulated or influenced by juvenile hormone (JH) (Clements 1999).

Females that have taken a full blood meal and commenced vitellogenesis are usually relatively inactive throughout the period of ovarian development. Their flight activity is reduced and they do not respond to host cues. The loss of responsiveness to host cues that follows engorgement is initiated immediately, and is recovered only after egg laying (Clements 1999). This pattern of responsiveness has variation, some *Anopheles* and *Aedes* species may feed multiple times before maturing and laying a batch of eggs. A nutrient deficiency can cause the mosquito to take a first blood meal to replenish their metabolic reserves before undertaking reproduction (Takken et al. 1998). Two or more days after engorging, a period with inactivity needed for ovarian development, the gravid females develop a sensitivity to oviposition-site attractants. This pre-oviposition behaviour pattern results in arrival at potential oviposition sites and indicates the suitability of such sites. The responses to the environment include the habitat, the patch or biotope, and the resource item, the particular part of the biotope on which eggs are deposited (Clements 1999). And off course between species or genera the biotope varies greatly, in the following chapter the larval habitat of *Anopheles* is enlightened.

At the oviposition site, the mature oocytes are passed into the vagina where they are fertilized by sperm cells and very soon after oviposition the oocyte chromosomes complete meiosis, transforming the oocyte into an egg. The fusion of male and female pronuclei, karyogamy, then follows (Clements 1999) and finally the eggs are laid.

2. Larval ecology of *Anopheles*:

To model and to predict mosquito species adult distribution and abundance it is important to know and to understand the dynamics and productivity of larval habitats (Gimnig et al. 2001). The female mosquitoes search for a suitable oviposition site, with specific habitat characters necessary for the larvae to survive and to develop, to deposit their eggs. Biotic and a-biotic factors in and around the aquatic habitats can affect the growth and survival of the larvae. Because of the specificity of some factors, the larval habitat characteristics can be used to distinguish species oviposition sites. The *Anopheles* larval habitat, the impact of nutrition and species interaction, predation and cannibalism are described.

2.1 Larval habitats:

Mosquito larval habitats fall into two distinct classes: phytotelmata and ground waters. Phytotelmata are bodies of water held in parts of terrestrial plants, like leaf axils, tree holes and hollowed-out fruits husks. Ground waters are predominantly still waters and include pools, fresh and saline-water marshes, puddles and even water filled hoof prints (Clements 1999). Also new larval habitats appear due to human activity, irrigated rice fields and small artificial containers such as water-storage tanks and discarded motor tyres have become an important habitat for some species. To characterize the *Anopheles* larval habitats Le Sueur and Gimnig used the following parameters: surface area (length width and depth), temperature, pH, salinity, dissolved oxygen, turbidity, shade, number of days before the habitat dried out and the presence of aquatic vegetation, mats of algae and a film on the water surface. Gimnig, 2001, studied and described the larval habitats of *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus* in western Kenya. His findings were: *An. gambiae* and *An. arabiensis* were both collected primarily from small, shallow habitats that persisted for 4-5 weeks, with the presence of algae and absence of aquatic vegetation. Habitat characteristics of both species were similar, although *An. gambiae* habitats tended to be less persistent and more likely to have algae than *An. arabiensis*. In addition, *An. arabiensis* was negatively associated with habitat surface area and positively with a higher pH. *An. funestus* habitats were rather larger, deeper, cooler, more persistent and less turbid than those of *An. gambiae* and *An. arabiensis*, and contained almost always some aquatic vegetation but no surface film. Analysis also indicated that *An. gambiae* and *An. arabiensis* were more likely to be present in the same habitat than alone and the two species were less likely to be collected together with *An. funestus* than alone (Gimnig et al. 2001). Underlining the close habitat relation between *An. gambiae* and *An. arabiensis*.

2.2 Influence of nutrition on the development of *Anopheles* larvae and its impact on adult size and fitness:

Larger females have at emergence significantly more metabolic reserves than the smaller ones (Takken et al. 1998). In experiments with restricted conditions, access to water only, small females live on average one day less than large individuals (Takken et al. 1998). Without food, larvae die within 3-4 d (Foster & Takken 2004). The metabolic reserves inherited at emergence from the egg and acquired from larval food present in the water provide the adult mosquito with a longer period to search for plant sugars and a suitable host to take a blood meal, resulting in a higher fitness. In general, well nourished larvae result in larger females,

which also exhibit a large fitness than adults resulting from malnourished larvae (Briegel, 1990; Takken et al 1998).

Fertility is the actual reproductive performance of an organism, measured by the number of viable offspring produced. Their reproductive potential is established at the end of the larval stage. And the maximum potential, the total number of eggs laid by a single female, is a function of the number of gonotrophic cycles she completes and the sizes of her egg batches. The number of gonotrophic cycles is determined by the mosquito's survival. The egg batch size is affected by several factors, such as nutritional condition, maternal body size, volume and source of blood meal. So it is important for the larva to eat adequate, mainly micro-organisms and plant tissues, to grow and to stock sufficient amount of proteins for future metamorphose and reproduction in adulthood. Conditions of the larval habitat and larval densities determine the food uptake and consequently larval growth. Low nutrient concentrations or poor food quality and high larval densities may negatively affect the body size because of competition for nutrients (Suleman 1982). And here for, large anophelines contain relatively greater quantities of metabolic reserves at emergence and have greater fecundity than small female individuals (Briegel 1990). Can this food and space competition between the larvae lead to predation and/ or cannibalism?

3. Contribution of life-cycle parameters to fitness of *Anopheles gambiae*:

In spite of the importance of *An. gambiae* as a vector for malaria parasites, many aspects of its population dynamics are not yet understood. An understanding of these dynamics and of the processes that govern the dynamics may be helpful in controlling the mosquito population densities and thus malaria transmission (Lyimo et al. 1992). The life-cycle parameters with the greatest influence on population growth are age at pupation and adult body size (Lyimo et al. 1992, Takken et al. 1998). Age at pupation and age at maturation are strongly related, having a strong effect on population growth. And a larger adult size is positively correlated with higher fecundity and longer survival (Briegel 1990 Lyimo et al. 1992, Takken et al. 1998). Temperature, larval density (Lyimo et al. 1992) and larval nutrient conditions (Takken et al. 1998) determine mainly the pupation age and adult body size. Under optimum temperature and optimum larval densities, with sufficient amount and quality of food, larger adult mosquitoes emerge with adequate metabolic reserves. For *An. gambiae* the optimum temperatures are around 27°C while for *An. quadriannulatus* these are a bit lower.

3.1 Larval condition: Nutrition and density:

Low nutrient concentrations or high larval densities during the aquatic stage hamper the growth. Consequently smaller adult mosquitoes emerge with insufficient metabolic reserves. Gimnig (2002) proved that the density of larvae significantly affected body mass and development time of males and females, but it did not affect survival. Male and female body mass declined, and development time increased as larval density increased (Gimnig et al. 2002). A threshold concentration of lipids is necessary to initiate oögenesis as documented by Briegel and Hörler (Briegel and Horler 1993) in *An. albimanus*. This was confirmed with the experiments in Takken (1998): Females with an average of 0.22 cal of lipid per female would only initiate oögenesis after a blood meal. Whereas more than half of the larger females, with an average of 0.78 cal of lipid per female, initiated oögenesis before the 1st blood meal. Consequently a strong positive correlations was found between female size and fecundity, estimated from the maximum number of eggs reported in individual egg batches. Thus in larval habitats with low larval densities and sufficient food, significantly more large anophelines develop. They contain relatively greater quantities of metabolic reserves at emergence and have greater fecundity than small female individuals (Briegel 1990). After emergence, besides the mosquito size and metabolic reserves at emergence, also the insemination rate by the male mosquito, the blood meal size and frequency of blood feeding have an effect on the reproductive development (Takken et al. 2002).

3.2 Adult parameters: Adult size, blood meal volume and frequency of blood feeding:

In experiments with restricted conditions, for example with access to water only, small adult females live on average one day less than large individuals (Takken et al. 1998). Due to the significantly lower metabolic reserves at emergence of the small females compared to the large ones. These metabolic reserves provide the larger mosquito with a longer period to search for plant sugars and a suitable host to blood feed on. The body size affects also the blood meal utilization; bigger female mosquitoes can develop mature oocytes with smaller blood meals than smaller mosquitoes (Takken et al. 1998).

The size of the blood meal has an effect on oocyte development. Only one third of small females are able to develop mature oocytes after one single large blood meal, up to 2 μ l. Some large females produce already mature oocytes with only 0.5 μ l blood meal, whereas two third will do with a 2 μ l blood meal.

Table 1: The size of the blood meal affect on oocyte development (Takken, 1998).

Blood meal	Small females	Large females
No	No vitellogenesis initiation	>50% able to deposit yolk to ovarian stage 2
0.5 μ l	50% initiated ovarian development	3% developed mature oocytes
2 μ l	33% developed mature oocytes	70% developed mature oocytes

Multiple blood meals increase the capacity of small females to mature oocytes (Takken et al. 1998). A meal of 1.5 μ l given in 2 separates amounts with a 24-h interval, induced up to 55% of small females to mature oocytes, compared to less than 5% when given the blood meal at once. A double meal on a rat induced up to 70% of females to complete oocyte maturation, whereas with 1 blood meal only, there was no maturation. Small females are seemingly not able to handle a big blood meal, 1.5 μ l or 2.0 μ l, efficiently. In contrast to large females, multiple meals did not cause a marked increase in egg maturation (Takken et al. 1998). When given the same amount of blood in 1 or 2 blood meals as enemas, the large females produced an equal number of eggs.

In conclusion, it has been observed that large females, emerging with sufficient reserves, are able to engorge and use higher blood volumes more efficient. Capable to spend most of their energy derived from a blood meal on reproduction, they can complete ovarian development with one blood meal. Small females however, require a first blood meal to build up the protein reserves, and successive meals will be needed to develop mature oocytes. From this it can be derived that the fitness of adult mosquitoes (contribution to future generations) is determined by larval nutrition. Hence, when predation and cannibalism are common, one wonders about the fitness effects of this type of nutrition.

3.3 Species interaction: predation and cannibalism:

The above mentioned information shows how larval and adult nutrition affect adult fitness. As predation is a common feature in anophelinae (Koenraadt & Takken 2003), it would be interesting to see whether this behaviour contributes to adult fitness. Throughout great parts of anopheline species habitat, several species are known to coexist (Coetzee, Craig & LeSueur 2000). And when the larvae develop in the same water pool competition in and between species can occur. Older larvae, third or fourth instars, can prey or cannibalise on younger first or second instars (Koenraadt et al 2004). They may predate on larva of a different species or engage in cannibalism, feeding on conspecifics larvae. Individuals of the same life stage cannibalize each other rarely, whereas large differences in size between larvae may result in frequent cannibalisation of the smaller larvae (Sherratt and Church 1994). First instar larvae are only able to eat from larval corpses. In comparison to elder larvae, third or fourth instar larva are able to execute this behaviour on living larvae.

As in previous studies on competition among culicine species, the effects of inter and intra-specific competition are manifold. In single and mixed populations effects on either or both species can range from prolonging the development time to reduction in size of emergent adults, inhibition of egg hatching, distorted sex ratios or displacement of one species by

another (Schneider et al. 2000). This is mainly mediated by rivalry for food resources and/ or space.

Koenraadt confirmed that predation by the fourth instar larvae of the sibling species, *An. gambiae s.s.*, *An. quadriannulatus* and *An. arabiensis*, on first instars of the other occurred readily under experimental conditions (Koenraadt and Takken 2003). In these studies (Koenraadt and Takken 2003, Koenraadt et al. 2004), it was recorded that cannibalism and predation were not enhanced as a result of low food quantities. No significant effect of the amount of food was found on larval survival to L3. But in 2004, Koenraadt found that smaller tray size (simulating crowding) increased mortality, while it didn't have an effect on the larval development rate to L3 (Koenraadt et al. 2004). This competition for space might play an important role, as the larval densities used in these studies are relatively high compared with densities found in natural habitats. But when densities in a breeding site become higher as the habitat shrinks through drying out, cannibalism and predation might influence the outcome of competitive interaction between these sibling species, affecting their adult population densities (Koenraadt and Takken 2003).

4. Goal and objectives:

In this study the intra and inter-specific competition in and between two members of the *Anopheles* Giles complex, *An. gambiae* s. s. and *An. quadriannulatus*, is investigated. *An. gambiae* being highly anthropophilic and considered as the most important malaria vector in Sub-tropical Africa and *An. quadriannulatus* being zoophilic and not regarded as a vector for the *Plasmodium* parasite. This distinction in host choice and consequently in vectorial capacity as well as their nearly undistinguishable morphological characteristics, are the main reason to exploit these specific *Anopheles* species in this fitness research.

4.1 Goal:

To assess the impact of predation and cannibalism on the fitness of *An. gambiae* s. s. and *An. quadriannulatus*, measured as adult size.

To determine the influence of the interactions between environmental factors, temperature and nutrition, and the predatory behaviour on fitness of *An. gambiae* s. s. and *An. quadriannulatus*.

4.2 Research questions:

- I. Do larval consumption; predation and cannibalism, food concentration and temperature confer fitness advantages, expressed on adult body size?
- II. Does predation occur more frequently than cannibalism under similar temperature and nutrition conditions?
- III. Do the *An. gambiae* larvae cannibalise and/ or predate more than the *An. quadriannulatus* larvae do?
- IV. Is cannibalism and/ or predation affected by nutrition concentrations?
- V. Is cannibalism and/ or predation affected by temperature?
- VI. Does the pupation time effect adult wing length?

5. Materials and methods:

5.1 Mosquitoes:

For all experiments, first and fourth instar larvae of laboratory-adapted strains of *An. gambiae* s.s. (Suakoko) and *An. quadriannulatus* (Sangwe) were used. Strains originated from Liberia and Zimbabwe, respectively. Both species are reared in climate-controlled rooms, at 27°C and 80% relative humidity, with a 12 hour photoperiod at the Laboratory of Entomology, Wageningen University, the Netherlands. Adult *An. gambiae* females were fed twice per week on human blood and *An. quadriannulatus* females daily on cow blood and both species were kept in 30 cubic cm cages provided with 6% sucrose solution as maintenance diet. First instar larvae were collected out of the colony and reared in 0.5-L trays filled with tap water and fed Tetramin® fish-food. Experiments were conducted in separate climate-controlled rooms.

5.2 Preliminary experiments:

First a basic rearing experiment was completed, to acquire the life cycle parameters and ecology, under the selected rearing conditions, of the larvae.

50 First instar larvae/ cup were reared under 24°C, 27°C and 30°C, respectively and each temperature was tested with four different food regimes: no food, 0.1mg Tetramin®/ larvae/ day, 0.1mg Tetramin®/ larvae/ for the first 3 days and 0.3mg Tetramin®/ larvae/ from day 4 on and finally 0.1mg Tetramin®/ larvae/ for the first 3 days and 0.5mg Tetramin®/ larvae/ from day 4 on. The experiments were repeated twice for both species. In total 3*4*2= 24 cups and 1200 *An. gambiae* larvae and 1200 *An. quadriannulatus* larvae were used.

Table 1: Overview experimental variables (temperature and food concentration)

		Tetramin ®/ larvae			
		0 mg	0.1 mg / day	0.1/ 0.3 mg / day	0.1/ 0.5 mg / day
Temperature in °C	24 °C				
	27 °C				
	30 °C				

5.3 Predation:

Fourth instar *An. gambiae* larvae prey on first instar *An. quadriannulatus* larvae and vice versa. Similar to the preliminary experiment, the same temperature and food gradient are used except for the treatment, no food. As no vital fourth instar larvae can be reared without any food. In plastic cups, 15 by 10 by 5cm containing 500 ml distilled water, 30 predatory fourth instar larvae are reared. Under the same temperature and nutritional conditions as the experiment they were placed in. When they reached fourth instar stage, the L4's were placed individually in cups of 5cm Ø and 3.5cm high, containing 50 ml distilled water (Koenraadt and Takken 2003) and ten first instar larvae of the other species, potential prey. Once the L4 larvae evolved into the non-eating pupal stage, the pupae were removed from the cups and reared separately for fitness analysis. The remaining L1 larvae were counted, before removing and killing them with hot water. The pupae were placed in a glass eppendorf tube containing some water and sealed off with a wad of cotton with 6 % glucose water. When the mosquito emerged, the water was drained out to prevent the mosquito from drowning. The female mosquitoes were given a human arm to blood feed on, 48 hours after their emergence. To test their fitness, the females were killed 48 hours after their blood meal, killed by freezing them half an hour. Whereupon they were dissected, to count the formed eggs and secondly to measure the length of their left wing (Lyimo et al. 1992). Similarly male mosquitoes were killed to measure their left wing length. These predation experiments were repeated ten times for each predator/ prey combination. In a period of 4 months, 10 L1 larvae * 9 cups *10 repeats = 900 L1 larvae and 1 L4 larvae * 9 cups *10 repeats = 90 L4 larvae were used for each species, *An. gambiae s. s.* and *An. quadriannulatus*.

5.4 Cannibalism:

The experiments to examine the effects of adult size caused by cannibalism were executed in a similar procedure as the predation experiment but with a single difference, the L4 and L1 larvae are of the same species, to induce cannibalism.

Simultaneously with both predation and cannibalism experiments a control cup was added to each setting, containing only ten L1 larvae and no L4 larvae. The control serves to provide proof of the occurrence of predation and cannibalism and can be used to compare survival and growth rate.

6. Data analysis:

6.1 Data collection:

To count the rate of predation and cannibalism, the number of disappeared L1 larvae were counted, if the body of missing larvae could not be found, it's expected to be eaten by the elder larvae. Dead first instar larvae were removed from the cup and excluded from the results.

To detect the possible fitness advantage of the predatory or cannibalistic fourth instar larvae, the wing length was measured to the nearest μm using a binocular equipped with a measuring ocular. Wing length and adult size are correlated with fitness (Lyimo et al. 1992) and consequently wing length is compared to discover fitness differences. A second method to measure fitness was implemented, counting the number of eggs, in the first gonotrophic cycle, produced by the blood fed female mosquitoes.

6.2 Statistical analysis:

The statistical analysis was performed with SPSS 15.0.

- Preliminary experiment:

With a general linear model, univariate analysis of variance, the effects of two fixed factors on a dependable variable, wing length, were examined. The individual influences and the interactions between the variables were analysed.

- Predatory and cannibalistic experiments:

Similar to the preliminary experiments, a general linear model, univariate analysis of variance was used. A third variable, larvae consumed, was included to these analysis, to test the individual variables for fitness advantages. The univariate analysis of variance was also used to test if larval consumption, predation/ cannibalism, is affected by temperature and food concentration. In all tests a confidence interval of 5% is used. To test if predation occurs more frequently than cannibalism under similar temperature and nutrition conditions, a non parametric test with two independent samples; Mann-Whitney test was applied. The same test was used to test if *An. gambiae* larvae cannibalise and/ or predate more than the *An. quadriannulatus* larvae do. The error bars in all graphs have a confidence interval of 95%.

7. Results:

7.1 Preliminary experiments:

In this experiment on the effect of predation and cannibalism on body size and fitness, we first examined the survival of larvae of both *An. gambiae* and *An. quadriannulatus* under different quantities of food and at different temperatures. This provided background information on development rate and survival. The data were used to select the food quantities for the predation/cannibalism experiments.

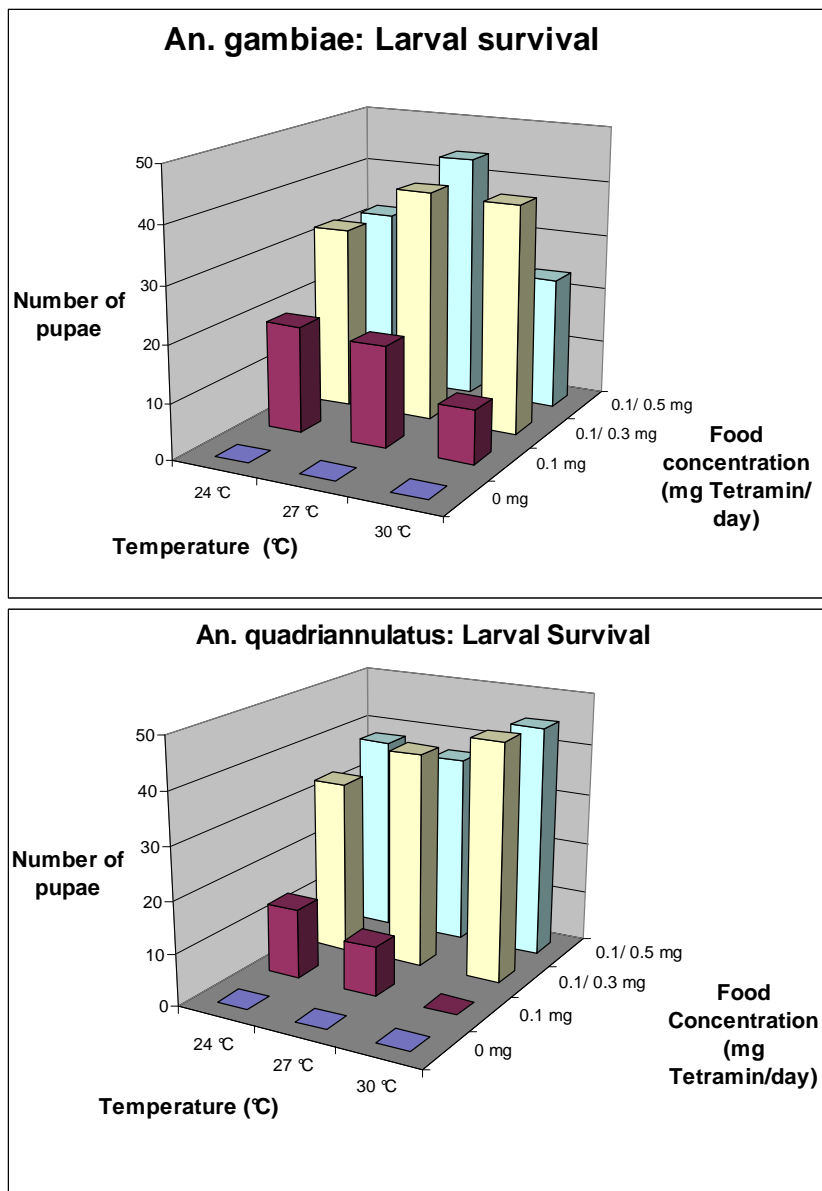


Fig. 1: The average number of larvae (*An. gambiae* above and *An. quadriannulatus* below) to reach the pupal stage, under the different temperature and food concentrations.

Without any food no single *An. gambiae* or *An. quadriannulatus* larva, starting with fifty first instars, is able to develop into pupal stage regardless of the temperature conditions (Fig. 1 and Table 2). The majority of larvae die within three or four days, but at 24°C several larvae were able to grow to third instar stage, an *An. gambiae* individual survived up to 22 days and up to 28 days for an *An. quadriannulatus* larva. These individuals must have eaten small parts of the remaining corpses for them to survive this long, as they are too small to actively predate and to swallow an entire contemporary larva. The successful development of larvae to pupae of neither *An. gambiae* nor *An. quadriannulatus* is significantly affected by temperature, $P=0.077$ and $P=0.693$ respectively. But different food concentrations do have a significant effect on the number of larvae to pupate, $P<0.001$ for both species (Table 3 & 4). Namely, a higher food quantity per larva results in more larvae that pupate. Even when the lowest food concentration, 0mg, is left out of the analysis, the effect remains significant ($P<0.001$). Table 2 clearly shows that more larvae pupate when having 0.3mg and 0.5mg of food per day than 0.1mg; however the difference between 0.3mg and 0.5mg remains limited. The interaction between temperature and food concentration is insignificant to affect the number of larvae to pupate, with P values of 0.159 for *An. gambiae* and 0.240 for *An. quadriannulatus* (Table 3 & 4).

Temperature and food concentration have a significant affect on pupation time of both *Anopheles* species, all P values are smaller than 0.001. The higher the temperature and nutrient level, the faster larvae develop into pupae.

Table 2: The average number of larvae to reach the pupal stage \pm standard deviation and the average time the larvae needs to reach the pupal stage \pm standard deviation, under the different temperature and food concentrations.

	N of replicates	N of larvae at start	Average number of pupae \pm S.D.			
<i>An. gambiae</i>			0 mg	0.1 mg	0.1/ 0.3 mg	0.1/ 0.5 mg
24 °C	3	50	0 \pm 0	22.0 \pm 13	32.7 \pm 5.5	34.7 \pm 6.1
27 °C	3	50	0 \pm 0	18.7 \pm 9.0	43.0 \pm 3.6	45 \pm 3.5
30 °C	3	50	0 \pm 0	8.0 \pm 7.0	41.3 \pm 1.5	30.3 \pm 13.1
<i>An. quadriannulatus</i>						
24 °C	3	50	0 \pm 0	19.0 \pm 13.5	37.3 \pm 12.4	38.3 \pm 15.0
27 °C	3	50	0 \pm 0	7.0 \pm 6.1	38.7 \pm 9.0	37.7 \pm 6.8
30 °C	3	50	0 \pm 0	0 \pm 0	43.7 \pm 4.5	42.3 \pm 7.6

	Average pupation time (day) \pm S.D.		
<i>An. gambiae</i>	0.1 mg	0.1/ 0.3 mg	0.1/ 0.5 mg
24 °C	13.3 \pm 0.6	10.3 \pm 1.5	9.3 \pm 0.6
27 °C	10.7 \pm 1.5	8.3 \pm 1.2	9.0 \pm 1.0
30 °C	11.7 \pm 1.5	8.3 \pm 1.2	8.7 \pm 1.5
<i>An. quadriannulatus</i>			
24 °C	14.3 \pm 1.2	10.3 \pm 0.6	9.3 \pm 0.6
27 °C	12.3 \pm 0.6	8.7 \pm 0.6	7.7 \pm 0.6
30 °C	/	9.7 \pm 1.5	8.0 \pm 1.0

7.2 Experiments to investigate the effect of predation and cannibalism:

Table 5 gives an overview of the data of the predatory and cannibalistic experiments. The data is ordered in four distinctive groups, *An. gambiae* predation (G-Q), *An. quadriannulatus* predation (Q-G), *An. gambiae* cannibalism (G-G) and *An. quadriannulatus* cannibalism (Q-Q). The first part of the table contains; temperature, food concentrations, the total number of repeats per experiment, the average number of larvae that the fourth instars consumed, the minimum, maximum and standard deviation of the number of larvae consumed and the number of emerged fourth instars and its percentage. The second part is ordered similarly to the first part and describes for both sexes, the number of individuals, their average wing length and the minimum, maximum and standard deviation of the wing lengths.

On average a fourth instar larvae of *An. gambiae* consume 1.32 *An. quadriannulatus* first instars and cannibalises 1.5 first instars. A fourth instar larvae of *An. quadriannulatus* consumes on average 2.87 *An. gambiae* first instars and cannibalises 1.29 first instars. The adult *An. quadriannulatus* mosquitoes have significant larger wing sizes than the *An. gambiae* mosquitoes have ($P < 0.001$) (Figure 2 and Table 6). *An. quadriannulatus* mosquitoes have average wing lengths of 3.10 ± 0.02 mm and the *An. gambiae* mosquitoes 2.73 ± 0.13 mm. The *An. gambiae* larvae that consumed one or more first instars, not taking predation nor cannibalism in consideration, are significant larger than the *An. gambiae* larvae that did not consume any first instar larvae ($P = 0.002$). This effect was not seen in *An. quadriannulatus*, the *An. quadriannulatus* adult mosquitoes, that consumed first instar larvae in their larval stage, were not significant larger than the ones that did not consume any first instar larvae ($P = 0.704$).

In both species the female mosquitoes are significantly larger than the males. $P = 0.003$ for *An. gambiae* and $P < 0.001$ for *An. quadriannulatus* (Tables 9 & 10).

When *An. gambiae* is reared with only 0.1mg Tetramin®/ larvae/ day and under 27°C and 30°C, the rate of emergence is less than 50% (Table 5). Low rates of emergence, 18.2% and 44.4%, occurred for *An. gambiae* predation when reared under 27°C with 0.1mg food and 30°C with 0.1mg, respectively. *An. gambiae* cannibalism had similar low emerging rates, 46.4% and 36.4%, when reared under 27°C with 0.1mg and 30°C with 0.1mg, respectively.

Table 5: Overview of the experiments done to investigate the effect of predation and cannibalism of *An. gambiae* and *An. quadriannulatus*. Raised at different temperatures and nutrient concentrations.

	Temp. °C	Food concentration	N	Average <u>no. of</u> larvae consumed	Min.	Max.	SD	Emerged L4	
								N	%
Predation									
G-Q	24	0.1	10	2.43	0	5	1.78	9	90
	24	0.3	10	4.3	0	9	3.06	10	100
	24	0.5	10	0.1	0	1	0.32	10	100
	27	0.1	11	1.45	0	8	2.25	2	18.2
	27	0.3	10	0.3	0	2	0.68	9	90
	27	0.5	10	1.5	0	3	0.97	9	90
	30	0.1	9	0.67	0	3	1.12	4	44.4
	30	0.3	9	0.33	0	2	0.71	5	55.6
	30	0.5	11	0.82	0	5	1.54	9	81.8
				1.32					
Q-G									
	24	0.1	10	4.8	0	9	2.97	10	100
	24	0.3	11	0.82	0	4	1.40	10	90.9
	24	0.5	10	1.9	0	5	1.45	8	80
	27	0.1	16	6.19	3	10	2.69	13	81.3
	27	0.3	11	1.18	0	4	1.47	9	81.8
	27	0.5	11	2.64	0	6	2.29	11	100
	30	0.1	11	1.73	0	4	1.27	11	100
	30	0.3	11	4.64	0	9	3.67	10	90.9
	30	0.5	11	1.91	0	6	1.92	8	72.7
				2.87					
Cannibalism									
G-G	24	0.1	10	0.2	0	1	0.42	9	90
	24	0.3	15	3.4	0	10	3.11	13	86.7
	24	0.5	10	0.2	0	1	0.42	8	80
	27	0.1	13	0.77	0	4	1.36	6	46.2
	27	0.3	11	2.45	0	6	1.97	11	100
	27	0.5	10	3.3	0	9	3.20	10	100
	30	0.1	11	1.0	0	2	0.78	4	36.4
	30	0.3	15	0.87	0	2	0.92	13	86.7
	30	0.5	10	1.3	0	3	1.42	8	80
				1.50					
Q-Q									
	24	0.1	11	1.55	0	6	1.97	11	100
	24	0.3	14	0.64	0	2	0.75	14	100
	24	0.5	11	1.73	0	9	2.61	11	100
	27	0.1	11	1.09	0	5	1.58	8	72.7
	27	0.3	14	1.03	0	4	1.24	14	100
	27	0.5	11	1.64	0	3	0.92	11	100
	30	0.1	11	1.36	0	3	1.03	11	100
	30	0.3	11	1.03	0	4	1.41	9	81.8
	30	0.5	11	1.64	1	4	1.03	8	72.7
				1.29					

Table 5: (Continued)

	Female Wing size					Male Wing size				
	N	mm	Min.	Max.	SD	N	mm	Min.	Max.	SD
Predation										
G-Q	7	2.87	2.72	3.04	0.13	2	2.66	2.64	2.68	0.03
	6	2.77	2.60	2.96	0.14	4	2.55	2.28	2.72	0.19
	3	2.91	2.84	3.00	0.08	7	2.79	2.64	3.04	0.15
	1	2.48	-	-	-	1	2.52	-	-	-
	6	2.80	2.72	2.88	0.62	3	2.63	2.52	2.68	0.09
	5	2.90	2.84	2.96	0.46	4	2.71	2.68	2.76	0.04
	4	2.71	2.68	2.76	0.08	0	-	-	-	-
	4	2.59	2.56	2.64	0.04	1	2.44	-	-	-
	3	2.76	2.68	2.84	0.08	6	2.67	2.60	2.80	0.08
		2.75					2.62			
Q-G	4	3.11	2.76	3.48	0.35	6	3.03	2.68	3.28	0.27
	6	3.24	3.04	3.60	0.21	4	3.20	2.96	3.48	0.21
	4	3.31	2.96	3.60	0.27	4	3.08	2.96	3.24	0.12
	6	3.22	3.04	3.56	0.21	7	3.00	2.64	3.36	0.25
	5	3.14	3.00	3.24	0.10	4	3.00	2.88	3.12	0.10
	4	3.34	3.16	3.52	0.15	7	3.10	2.92	3.28	0.12
	7	3.03	2.80	3.16	0.12	4	3.10	2.92	3.24	0.14
	6	3.22	3.04	3.48	0.15	4	3.00	2.92	3.04	0.06
	3	3.09	2.84	3.36	0.26	5	2.85	2.68	2.96	0.14
		3.19					3.04			
Cannibalism										
G-G	6	2.68	2.40	2.92	0.18	3	2.59	2.52	5.64	0.06
	6	2.91	2.76	3.04	0.11	7	2.89	2.72	2.96	0.09
	6	2.72	2.68	2.80	0.05	2	2.66	2.52	2.80	0.20
	5	2.62	2.56	2.68	0.05	1	2.52	-	-	-
	6	2.90	2.76	3.04	0.09	5	2.71	2.64	2.80	0.02
	5	2.92	2.76	3.08	0.12	5	2.84	2.76	2.96	0.08
	2	2.72	2.68	2.76	0.06	2	2.34	2.28	2.40	0.08
	9	2.59	2.48	2.72	0.08	4	2.51	2.48	2.56	0.04
	4	2.66	2.52	2.76	0.10	4	2.57	2.48	2.68	0.11
		2.75					2.63			
Q-Q	3	3.17	2.90	3.44	0.26	8	3.24	2.96	3.44	0.17
	7	3.16	2.84	3.56	0.26	7	2.92	2.60	3.28	0.23
	8	3.40	2.92	3.56	0.22	3	3.16	3.00	3.36	0.18
	3	3.07	3.00	3.16	0.08	5	3.06	2.68	3.28	0.25
	4	3.07	3.00	3.20	0.09	10	2.84	2.52	3.12	0.21
	8	3.35	3.16	3.44	0.11	3	3.20	3.12	3.28	0.08
	6	3.03	2.56	3.16	0.23	5	3.01	2.92	3.08	0.07
	6	3.06	2.84	3.28	0.17	3	2.87	2.60	3.04	0.23
	5	3.09	2.88	3.36	0.19	3	2.87	2.76	2.92	0.09
		3.16					3.02			

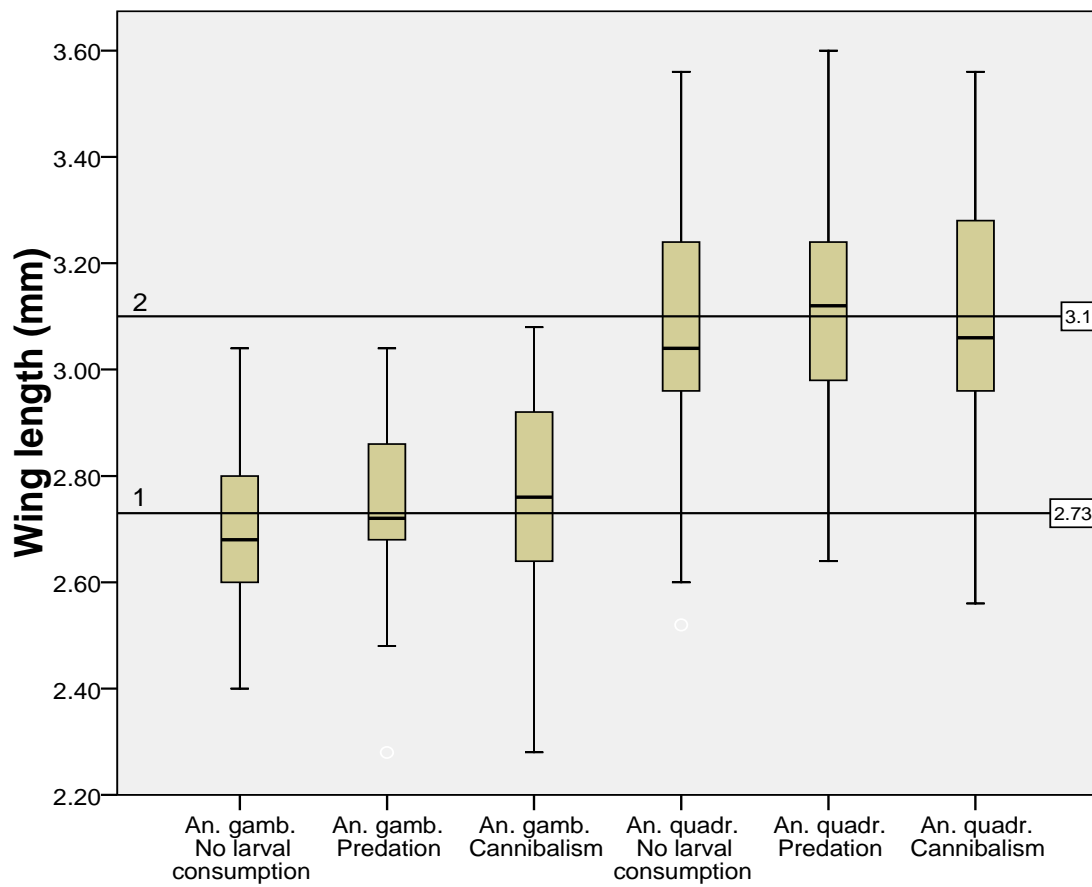


Fig. 2: The wing lengths of *An. gambiae* and *An. quadriannulatus* raised as larvae, that did not consume first instar larvae or that did prey on another species or did carry out cannibalism. The horizontal lines, 1 and 2, are the mean wing lengths of *An. gambiae* and *An. quadriannulatus*, respectively. Error bars: standard error of the mean.

7.2.1 Do larval consumption, food concentration and temperature confer fitness advantages, expressed on adult body size?

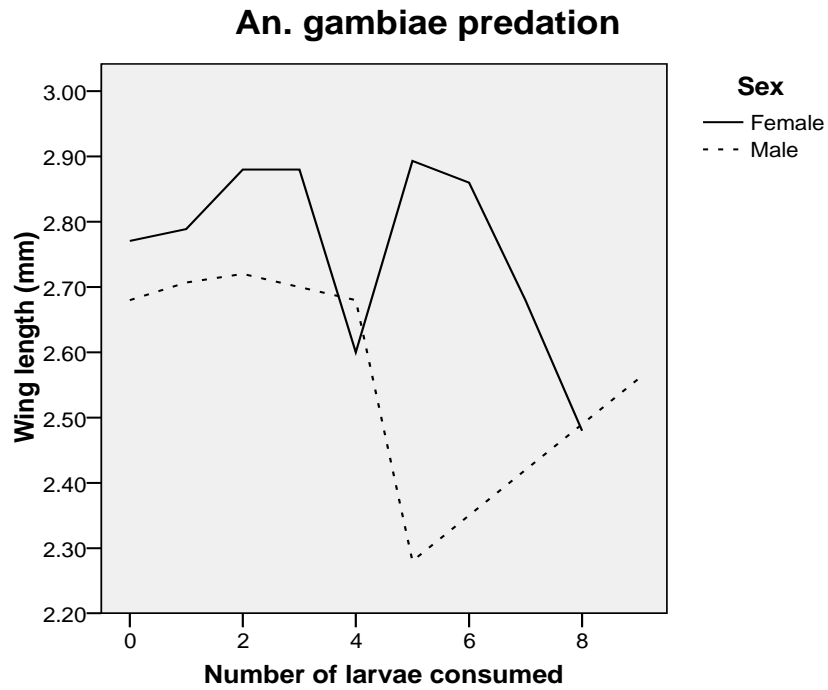


Fig. 3: The effect of larval predation on wing length of *An. gambiae* mosquitoes.

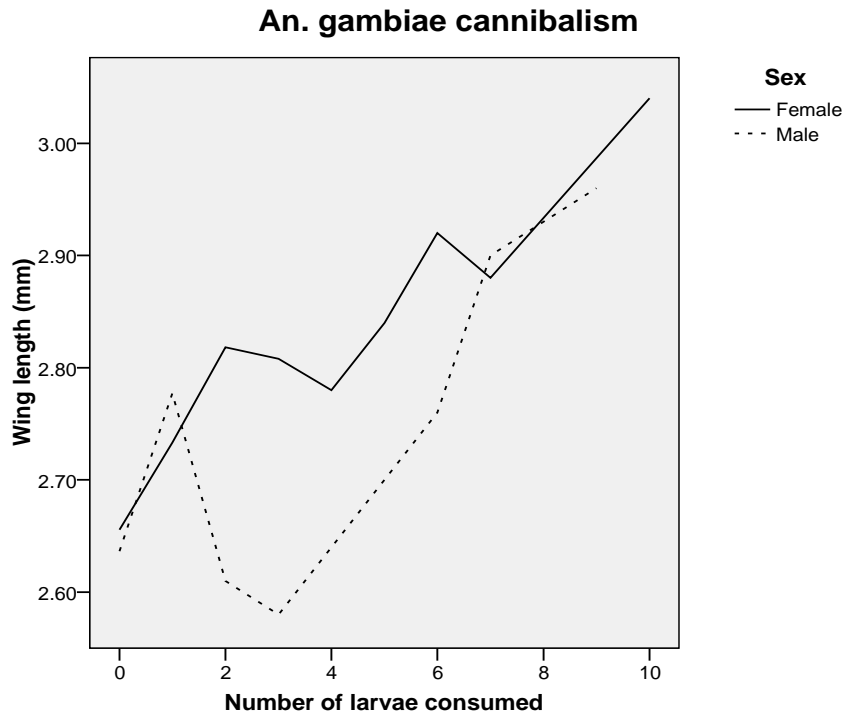


Fig. 4: The effect of cannibalism on larvae on wing length of *An. gambiae* mosquitoes.

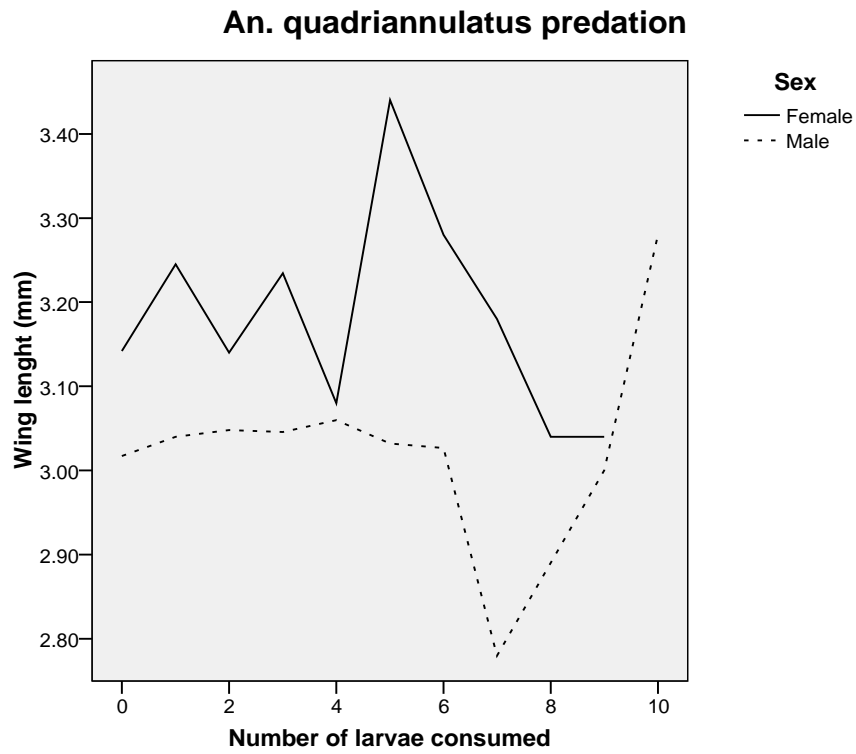


Fig. 5: The effect of larval predation on wing length of *An. quadriannulatus* mosquitoes.

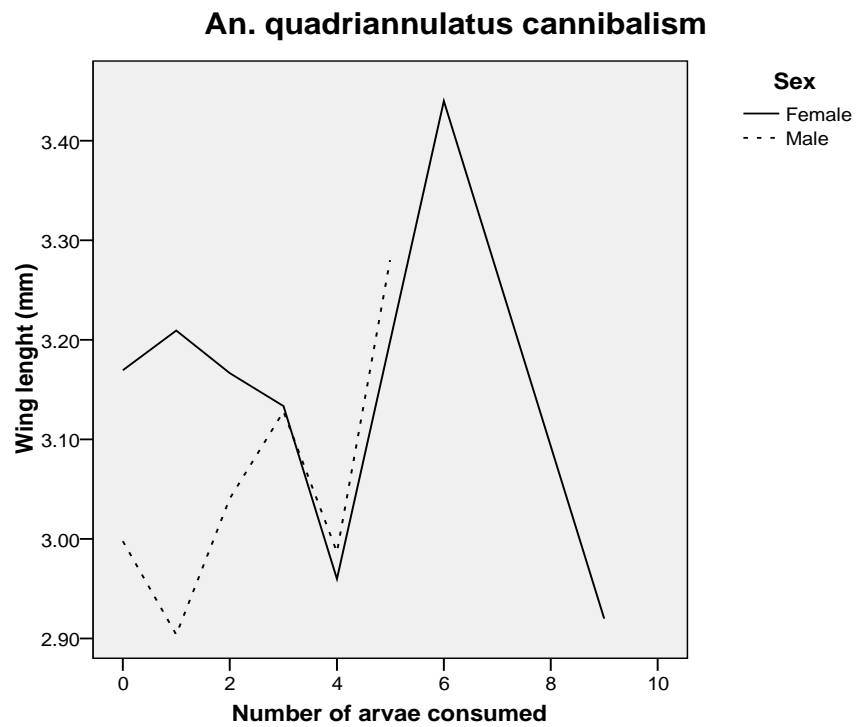


Fig. 6: The effect of cannibalism on larvae on wing length of *An. quadriannulatus* mosquitoes.

The figures 3-6 show the relationship between the number of larvae consumed, and wing size (as a proxy for fitness). Only cannibalising *An. gambiae* larvae show a trend, cannibalising on first instars does confer here in larger adult wing size. When the effect of larval consumption on wing length is analysed separately, without the effects of temperature and food concentration, just cannibalising *An. gambiae* larvae have a significant larger wing length (P = 0.038). Predating *An. gambiae* and *An. quadriannulatus* larvae and cannibalising *An. quadriannulatus* larvae have no significant adult wing length advantage over larvae that do not consume first instar larvae. With respectable P values of 0.343, 0.860 and 0.742.

When the analysis is done including temperature and food concentration (Univariate Analysis of Variance), the effect of larval consumption on wing length remains insignificant for the predating and cannibalising *An. gambiae* and *An. quadriannulatus* larvae.

* *An. gambiae* predation:

When a single fourth instar *An. gambiae* larva has the possibility to prey on first instar larvae of *An. quadriannulatus*, no single variable significantly influences its adult size. The predation of *An. quadriannulatus* L1 has no significant effect on adult size (P= 0.512). And the intensity (expressed as the no. of larvae consumed per fourth instar) of predation is not augmented if the predating *An. gambiae* larvae have received less food (P= 0.149). Similarly as larval consumption, temperature and food concentration have no significant effect on wing length, with P values of 0.311 and 0.133, respectively (Table 11). But the variables food concentration and larvae consumed do show an interaction (P= 0.033). If the predatory larva is given 0.1mg or 0.3mg Tetramin®, the number of larvae consumed is positively correlated with wing length (Fig. 7). This correlation is stronger for 0.1mg than for 0.3mg. For 0.5mg Tetramin® no correlation with wing length appears. There is no interaction found between temperature * food concentration (P= 0.181), temperature * larvae consumed (P= 0.788) and temperature * food concentration * larvae consumed (P= 0.958).

* *An. gambiae* cannibalism:

Similar to *An. gambiae* larvae that engaged in predation, cannibalism on first instar larvae by *An. gambiae* has no significant effect on wing length (P=0.880). Cannibalising *An. gambiae* larvae consume significantly more larvae when given more food (P= 0.022), but this does not result in a larger body size of adult mosquitoes. Chapter 7.2.4 discusses further the effects of nutrient concentration and temperature on the occurrence of predation and cannibalism on first instar larvae.

In the cannibalistic experiment with *An. gambiae*, temperature and food concentration do show a notable effect on the adult body size. Temperature has a negative correlation with wing length; the larvae in the experiment under 24°C and 27°C are significantly larger than the individuals of 30°C (Table 12 & Fig. 8). Food concentration has a positive effect on the adult wing length, the *An. gambiae* larva who have received 0.3mg and 0.5mg are significantly larger than those who only got 0.1mg Tetramin® (Table 12 & Fig. 9). The interaction between the two variables, food concentration and temperature, has also a significant effect on adult wing length (Table 12 & Fig. 10). Figure 10 shows a positive influence of food quantity and a negative for temperature on wing size, except for the interaction between 0.3mg and 0.5mg, 24°C and 27°C. The variable combinations temperature* larvae consumed, food concentration * larvae consumed and temperature * food concentration * larvae consumed have no interaction, with P values 0.745, 0.182 and 0.473, respectively.

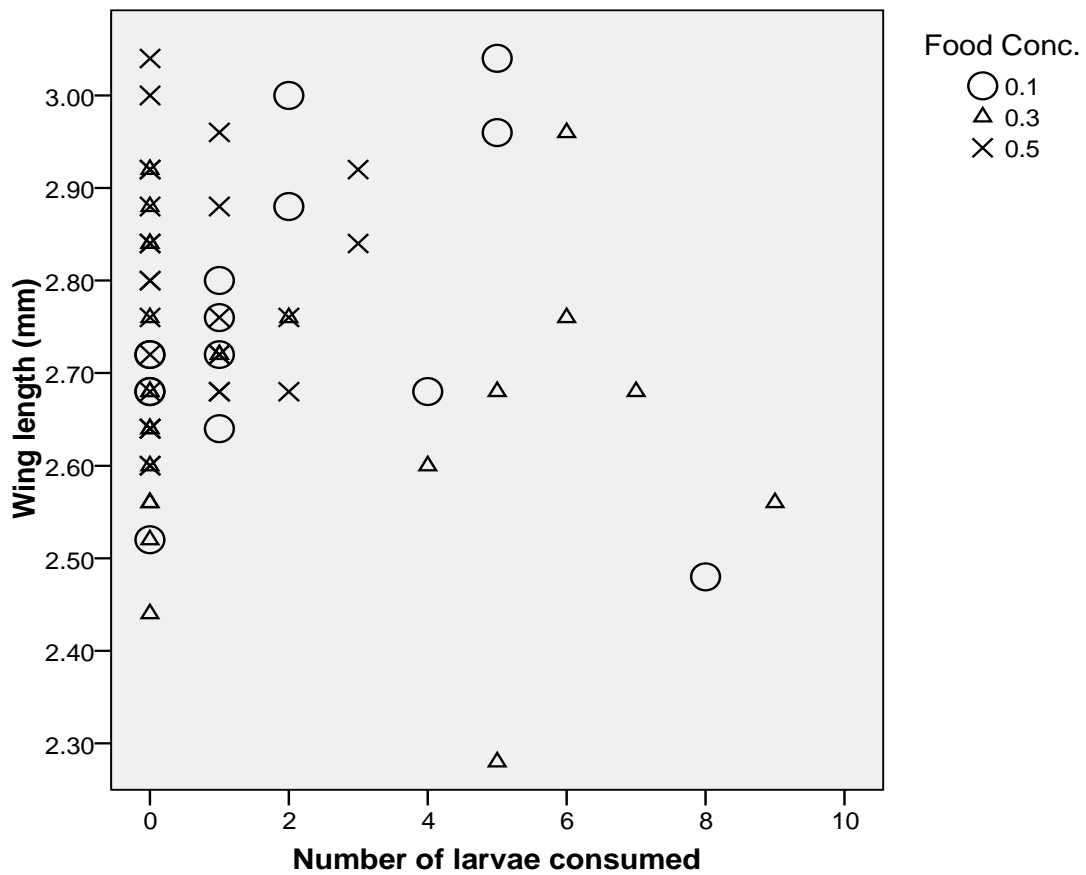


Fig. 7: The effect of number of larvae consumed on wing length of *An. gambiae* larvae that preyed on *An. quadriannulatus*, at different food concentrations.

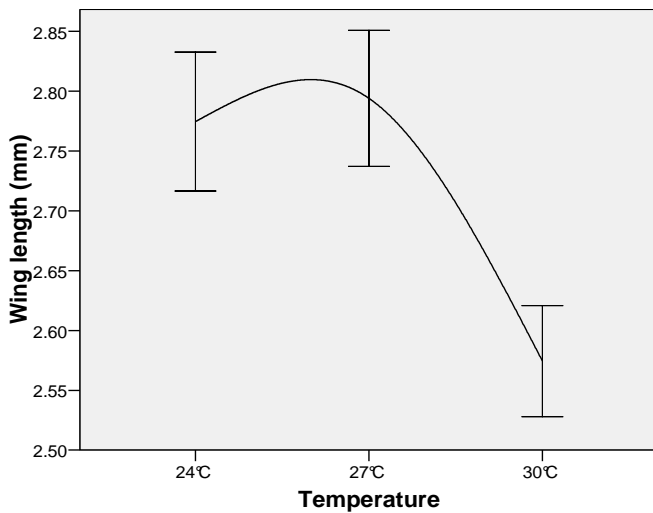


Fig. 8: The effect of temperature on wing length of cannibalising *An. gambiae* larvae. Error bars: confidence interval of 95%.

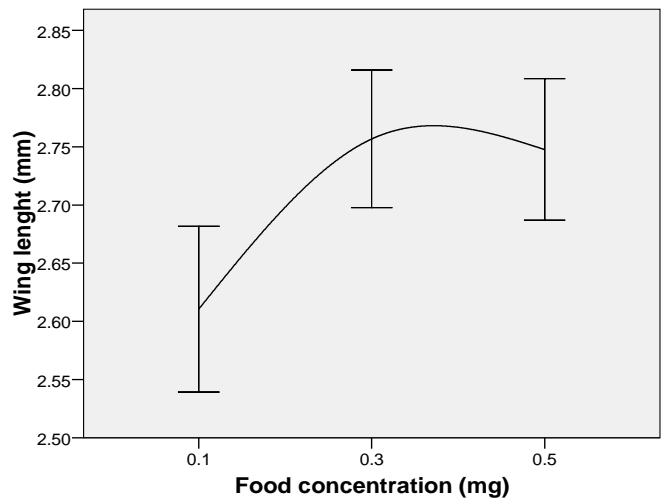


Fig. 9: The effect of nutrient concentration on wing length of cannibalising *An. gambiae* larvae. Error bars: confidence interval of 95%.

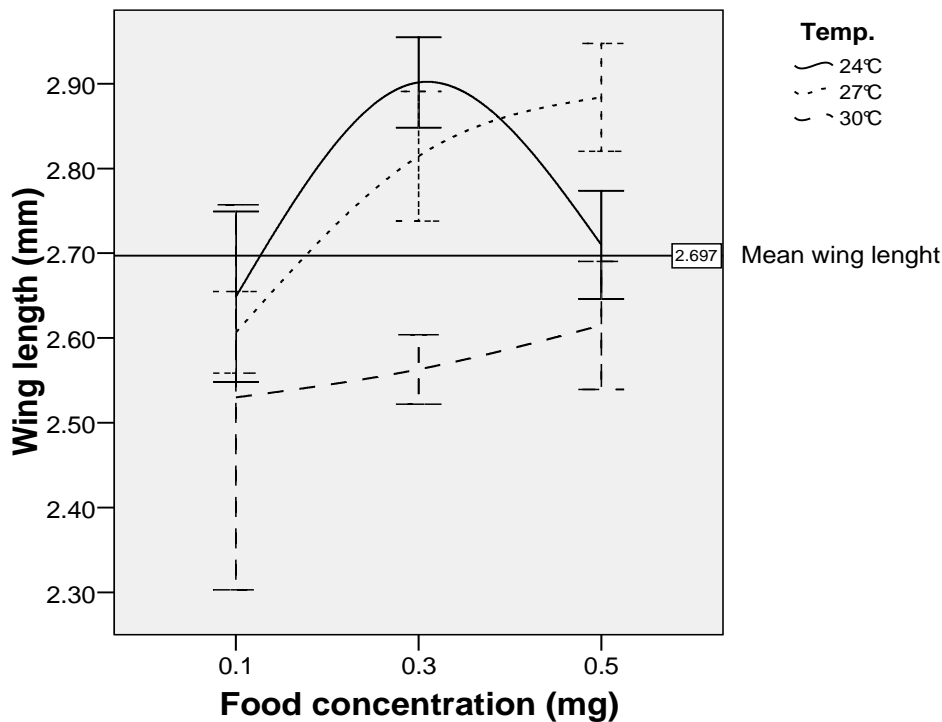


Fig. 10: The effect of food concentration on wing length of *An. gambiae* larvae that cannibalised on *An. gambiae*, at different temperatures. Error bars: confidence interval of 95%.

* *An. quadriannulatus* predation:

Temperature is the only variable to significantly affect adult wing length in the *An. quadriannulatus* predation experiments ($P=0.044$) (Table 13). The temperature is negatively correlated with wing length (Fig. 11). Neither predation on *An. gambiae* L1 ($P=0.648$) nor food concentration ($P=0.701$) has an effect on wing length and neither is there an interaction between the three variables. With P values of 0.158, 0.437, 0.994 and 0.202, respectively (Table 13).

* *An. quadriannulatus* cannibalism:

Similar to all the other predatory experiments, larval consumption has no significant effect on wing length ($P=0.267$). Both temperature ($P=0.023$) and food concentration ($P=0.027$) significantly affect the adult wing length of cannibalising *An. quadriannulatus* larva (Table 15). As in the *An. quadriannulatus* predation experiments, temperature is negatively correlated with wing length (Fig. 12). Food concentration has a more hyperbole shaped curve, with intermediate wing lengths at 0.1mg, a low wing size at 0.3mg and a larger wing length at 0.5mg Tetramin® (Fig 13).

There is no interaction between the three variables. P values are insignificant between food concentration and temperature ($P=0.331$), likewise for temperature * larvae consumed ($P=0.472$), food concentration * larvae consumed ($P=0.831$) and temperature * food concentration * larvae consumed ($P=0.906$) (Table 14).

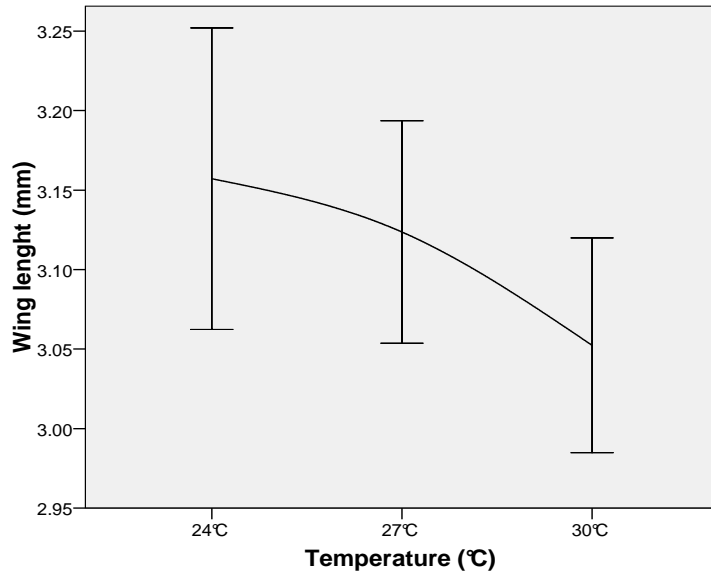


Fig. 11: The effect of temperature on wing length of predating *An. quadriannulatus* fourth instars that prey on *An. gambiae*. Error bars: confidence interval of 95%.

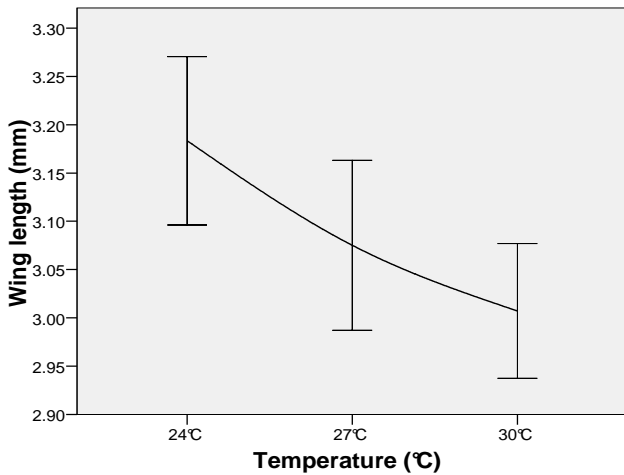


Fig. 12: The effect of temperature on wing length of cannibalising *An. quadriannulatus* larvae. Error bars: confidence interval of 95%.

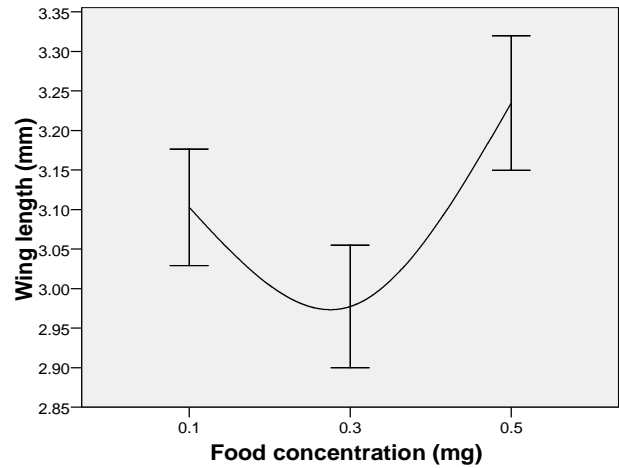


Fig. 13: The effect of food concentration on wing length of cannibalising *An. quadriannulatus* larvae. Error bars: confidence interval of 95%.

7.2.2 Does predation occur more frequently than cannibalism under similar temperature and nutrition conditions?

The predatory (Pr) and cannibalistic (Can) experiments with the same variables, temperature and food concentration are analysed for any difference in larval consumption with a Mann-Whitney test. The cases that differ significantly are described. Figures 14 and 15 give an overview of the differences between the amount of predation and cannibalism for both *Anopheles* species.

Table 15: Overview of the P values of a Mann-Whitney test: to compare the intensity of larval consumption between predation and cannibalism, at similar temperatures and nutrient concentrations.

Predation/ Cannibalism	Temperature	Food concentration	P Value
An. gambiae	24°C	0.1 mg	P= 0.001
	24°C	0.3 mg	P= 0.557
	24°C	0.5 mg	P= 0.542
	27°C	0.1 mg	P= 0.158
	27°C	0.3 mg	P= 0.004
	27°C	0.5 mg	P= 0.261
	30°C	0.1 mg	P= 0.239
	30°C	0.3 mg	P= 0.138
	30°C	0.5 mg	P= 0.368
An. quadriannulatus	24°C	0.1 mg	P= 0.015
	24°C	0.3 mg	P= 0.737
	24°C	0.5 mg	P= 0.310
	27°C	0.1 mg	P= 0.001
	27°C	0.3 mg	P= 0.907
	27°C	0.5 mg	P= 0.480
	30°C	0.1 mg	P= 0.496
	30°C	0.3 mg	P=0.014
	30°C	0.5 mg	P= 0.917

* *An. gambiae*: Pr - Can, 24°C and 0.1mg of Tetramin: P= 0.001
Predation occurs significantly more than cannibalism at 24°C and with 0.1mg Tetramin® fish food (Table 16).

* *An. gambiae*: Pr - Can, 27°C and 0.3mg of Tetramin: P=0.004
When the L4 are given 0.3mg of fish food and at 27°C, cannibalism occurs significantly more than predation (Table 17).

* *An. quadriannulatus*: Pr - Can, 24°C and 0.1mg of Tetramin: P= 0.015
An. quadriannulatus consumes significant more *An. gambiae* first instars than it cannibalizes *An. quadriannulatus* first instars at 24°C and when given 0.1mg fish food (Table 18).

* *An. quadriannulatus*: Pr - Can, 27°C and 0.1mg of Tetramin: P< 0.001
Predation occurs significant more than cannibalism at 27°C and with 0.1mg food (Table 19).

* *An. quadriannulatus*: Pr - Can, 30°C and 0.3mg of Tetramin: P= 0.014

As in the other *An. quadriannulatus* cases, 24°C and 0.1mg and for 27°C and 0.1mg, so does predation occurs significant more than cannibalism at 30°C and with 0.3mg fish food (Table 20).

For the species *An. gambiae*, two comparisons have a significant difference in larval consumption between predation and cannibalism. In one of the occasions predation occurred more frequently and in the other cannibalism did. The seven remaining experiments did not show any particular dissimilarity (Fig. 14). *An. quadriannulatus* has three comparisons with a significant difference, and predation occurred more often than cannibalism in all of the three cases. Still six out of nine comparisons remain indifferent (Fig. 15).

In addition the 5 specific groups were analyzed for difference in wing length. Only for *An. gambiae* Pr - Can, at 24°C and with 0.1mg fish food there was also a significant different wing size (Fig 16). *An. gambiae* predating larvae, at 24°C and with 0.1mg fish food, have a significant larger adult body size than *An. gambiae* cannibalizing larvae (P= 0.019).

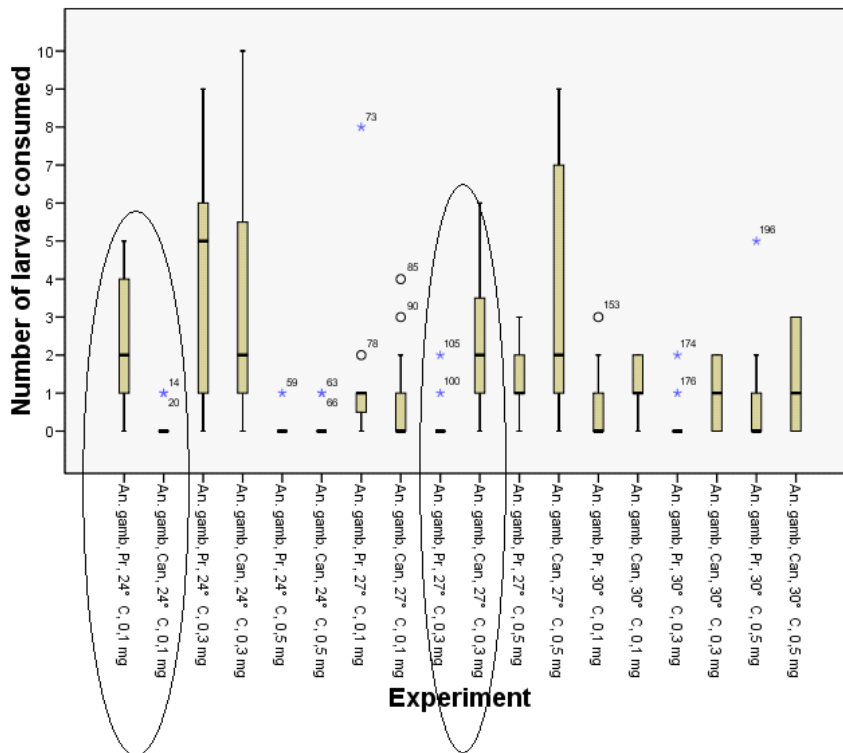


Fig. 14: Overview of pair wise comparison of all experiments done with predation and cannibalism on the number of larvae consumed by *An. gambiae* with the question does predation occur more frequently than cannibalism?

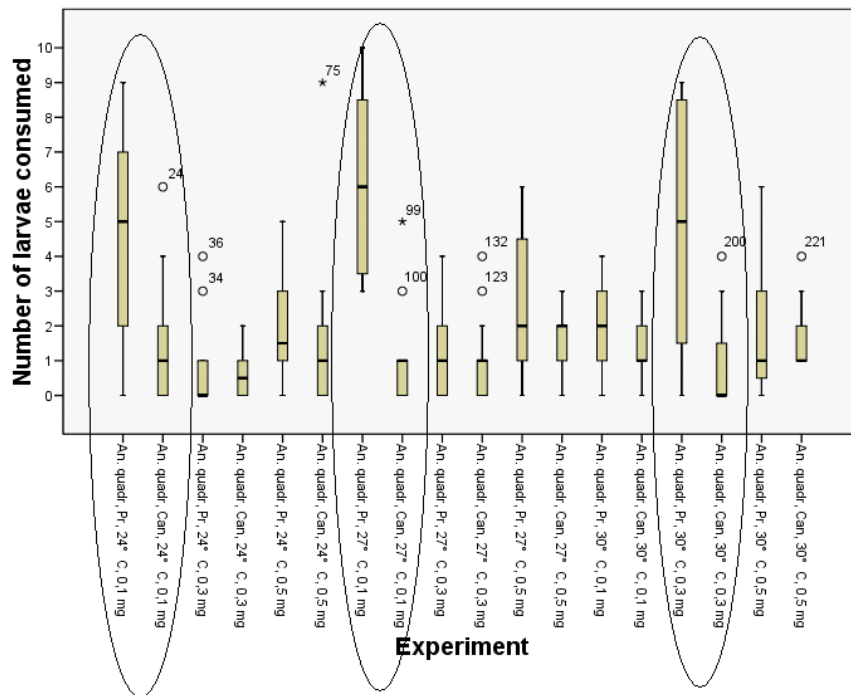


Fig. 15: Overview of the pair wise comparison of all experiments done with predation and cannibalism on the number of larvae consumed by *An. quadriannulatus* with the question does predation occur more frequently than cannibalism?

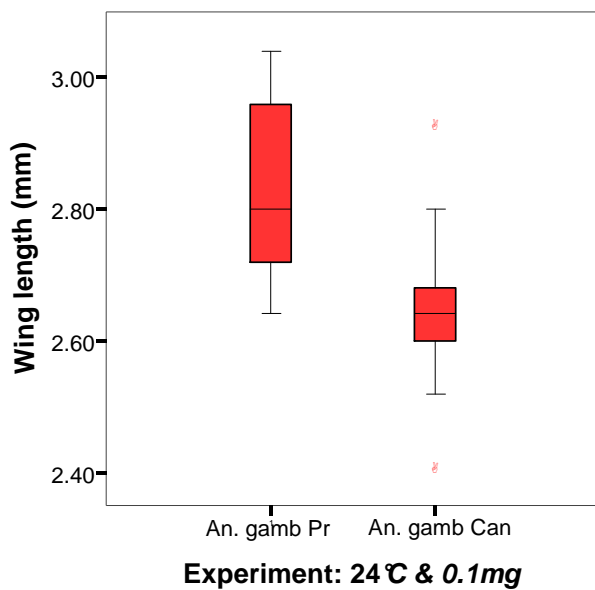


Fig. 16: Wing length of predating and cannibalising *An. gambiae* larvae under 24°C & 0.1mg of Tetramin®.

7.2.3 Do *An. gambiae* larvae cannibalise and/ or predate more than *An. quadriannulatus* larvae?

* Predation:

An. quadriannulatus L4 larvae consumes significantly more larvae than *An. gambiae* L4 larvae do ($P < 0.001$) (Table 21 & Fig. 17). In these experimental settings, on average each *An. gambiae* larva consumes 1.33 *An. quadriannulatus* first instars and *An. quadriannulatus* L4 larva consumes 3.02 *An. gambiae* first instars. Temperature and nutrient concentration do affect the amount of predation, this is discussed in chapter 7.2.4 and 7.2.5.

* Cannibalism:

When engaged in cannibalism, both species do not differ in the number of larvae they consume ($P = 0.893$) (Table 22 & Fig. 18). On average, in these experimental settings, each *An. gambiae* larva consumes 1.54 *An. gambiae* first instars and *An. quadriannulatus* L4 larvae cannibalises 1.27 *An. quadriannulatus* first instars. The amount of larvae the two species cannibalise is also dependent on the amount of food and the temperature conditions the fourth instars received.

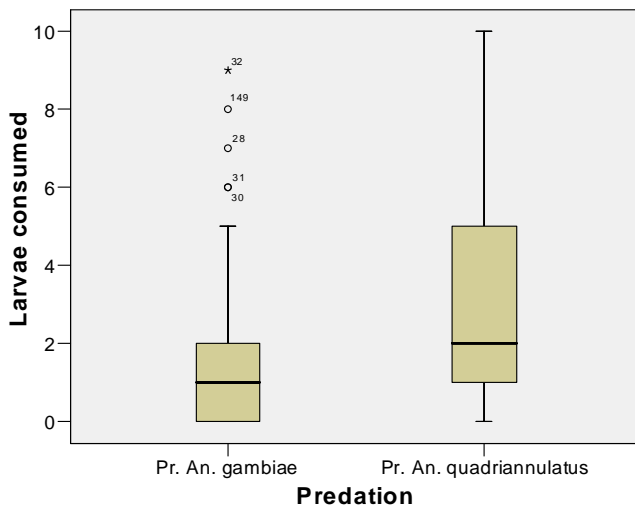


Fig. 17: The intensity of larval predation of *An. gambiae* and *An. quadriannulatus* fourth instars expressed as the number of first instars consumed. Error bars: confidence interval of 95%.

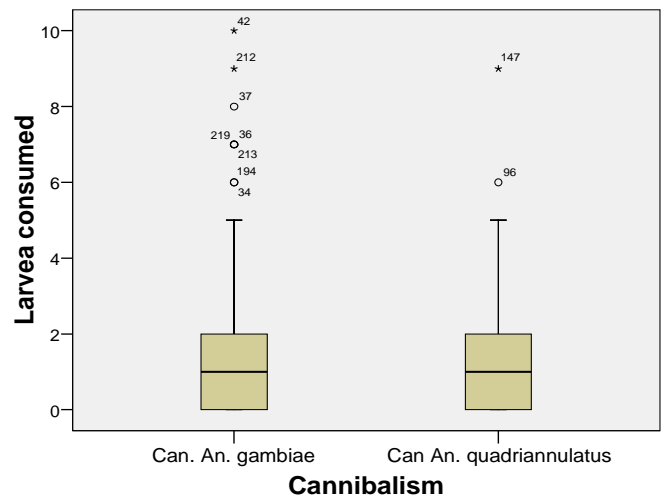


Fig. 18: The intensity of cannibalism of *An. gambiae* and *An. quadriannulatus* fourth instars expressed as the number of larvae consumed. Error bars: confidence interval of 95%.

7.2.4 Is cannibalism and/ or predation affected by the concentration of fish food and temperature?

* *An. gambiae* predation:

Temperature significantly affects the predation rate of fourth instar *An. gambiae* larva on first instar *An. quadriannulatus* larva ($P=0.001$) (Table 23). Temperature is negatively correlated with the number of larvae consumed (Fig. 19). Food concentration had no adequate effect on larval consumption ($P=0.154$). But both variables do have an interaction ($P<0.001$) between 24°C and both other temperatures at 0.3mg and 0.5mg (Fig. 20).

* *An. gambiae* cannibalism:

For cannibalising 4th instars of *An. gambiae*, in stead of temperature ($P=0.058$), food concentration has a significant effect on larval consumption ($P=0.001$) (Table 24). Figure 21 shows a positive correlation between food concentration and larvae consumed, but at 0.5mg fewer larvae are consumed than at 0.3mg Tetramin®. There is also an interaction between the two variables ($P<0.001$). Figure 22 shows the interactions between temperature and food concentration. Illustrating that under several different temperatures and food concentration, the interaction between the two variables differs and consequently has another effect on larval consumption.

In table 5 and Fig. 23 it is perceptible that the cannibalising *An. gambiae* larvae that only received 0.1mg Tetramin® did not consume more than 4 *An. gambiae* first instar larvae. There is also a trend visible, when the cannibalising *An. gambiae* larvae consume 4 or more first instar larvae, the larvae grow into larger adults. In this specific part of the date, cannibalism resulted in significant larger adult mosquitoes ($P= 0.010$) but food was insignificant to affect wing size ($P= 0.403$) (Table 25).

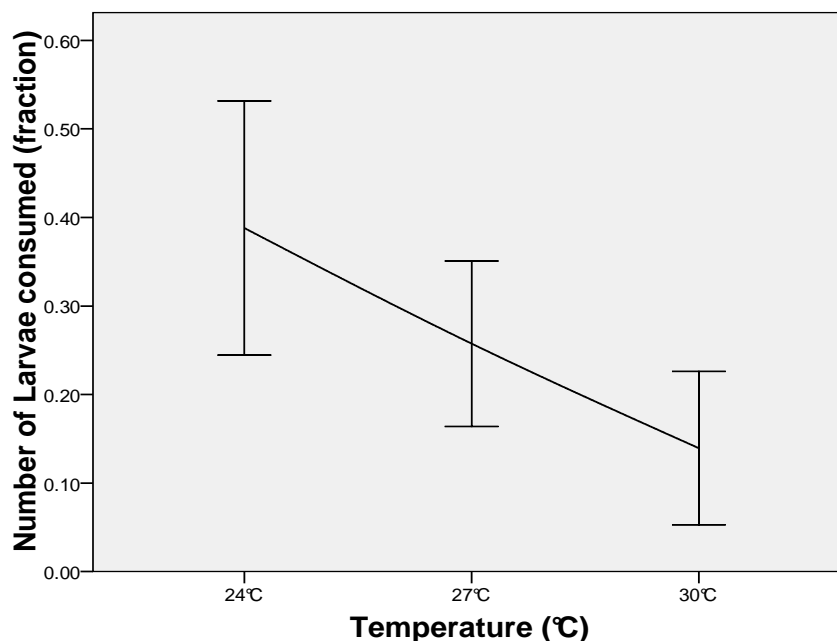


Fig. 19: The effect of temperature on predation by *An. gambiae* fourth instar. Error bars: confidence interval of 95%.

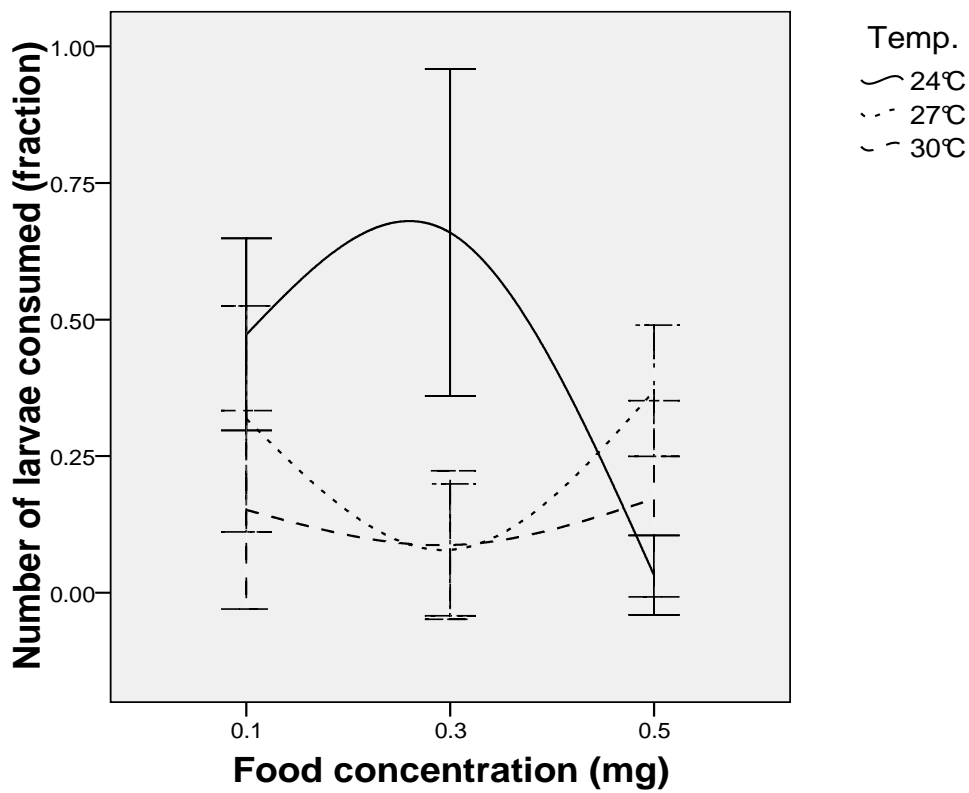


Fig. 20: The effect of the interaction between temperature and nutrient concentration, on predation by *An. gambiae* fourth instars. Error bars: confidence interval of 95%.

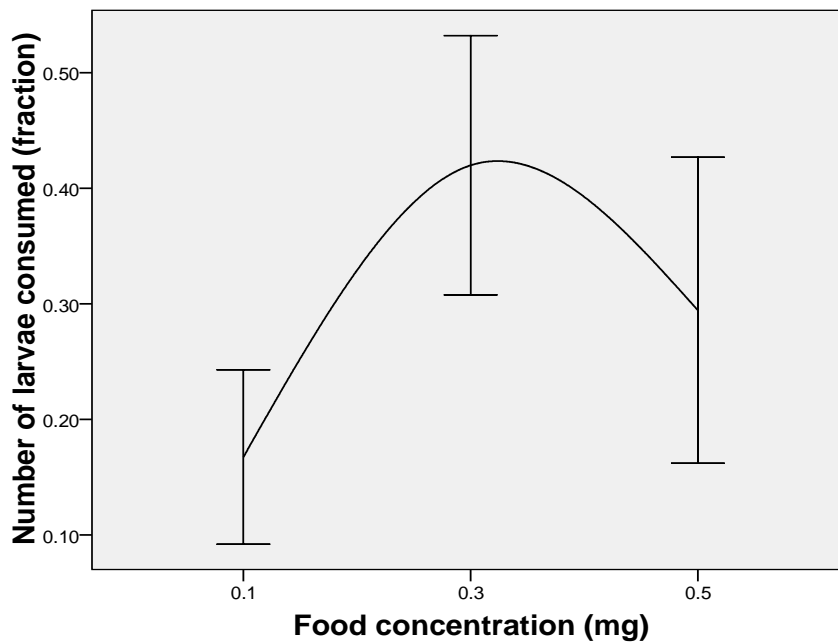


Fig. 21: The effect of nutrient concentration on cannibalism by *An. gambiae* fourth instars. Error bars: confidence interval of 95%.

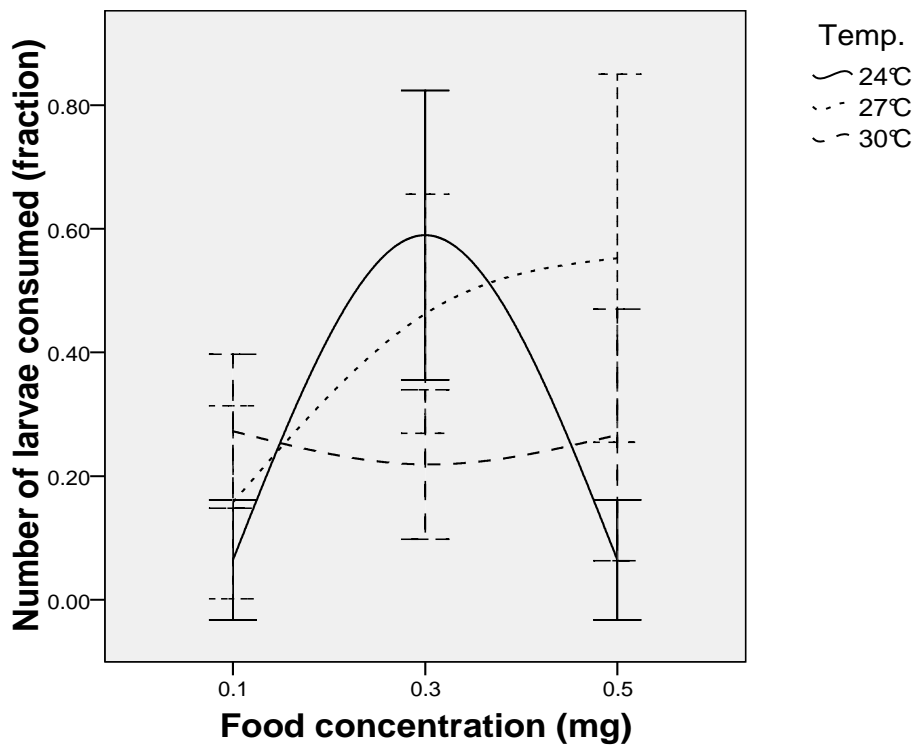


Fig. 22: The effect of the interaction between temperature and nutrient concentration, on cannibalism by *An. gambiae* fourth instars. Error bars: confidence interval of 95%.

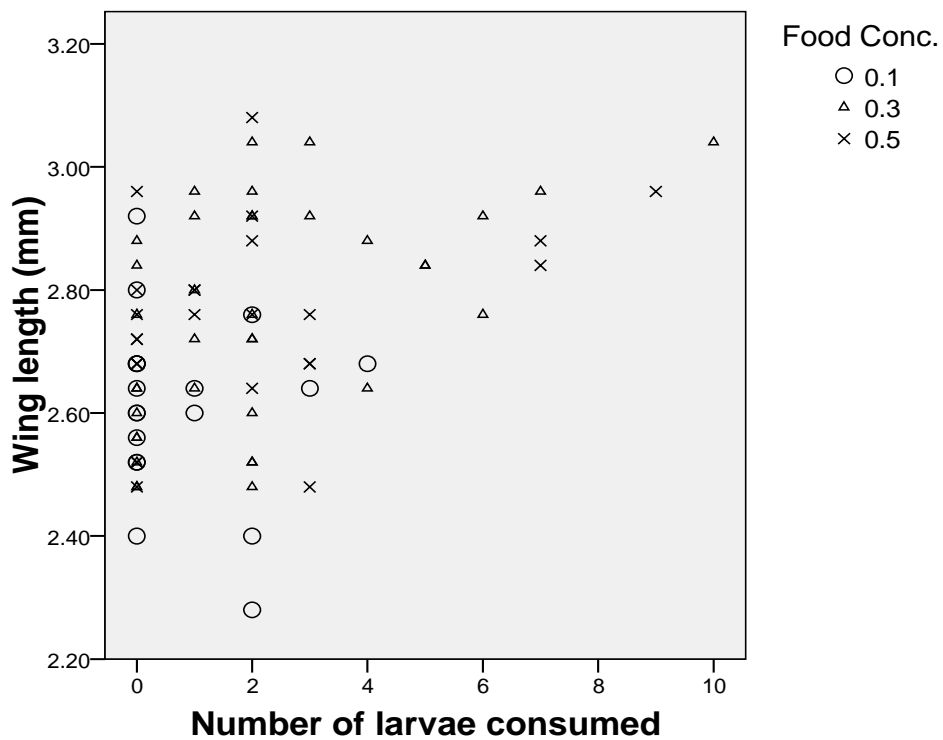


Fig. 23: The effect of number of larvae consumed on wing length of cannibalising *An. gambiae* larvae at different food concentrations. Error bars: confidence interval of 95%.

* *An. quadriannulatus* predation:

Food concentration significantly affects the predation rate of fourth instar *An. quadriannulatus* larva on first instar *An. gambiae* larvae ($P < 0.001$) (Table 26). Food concentration is negatively correlated with larvae consumed (Fig. 24). Although temperature has no significant effect on larval consumption ($P = 0.274$), both variables do have an interaction ($P < 0.001$); the 30°C line runs dissimilar with both other temperatures lines (Fig. 25). The 24°C and 27°C line run parallel, expressing per food concentration the same effect on larval consumption. Even if the wing lengths of *An. quadriannulatus* fourth instars, that did not predate a single larvae, are compared to the wing lengths of individuals that did predate one or more *An. gambiae* first instars, larval consumption remains insignificant to affect adult wing length ($P = 0.613$).

* *An. quadriannulatus* cannibalism:

Food concentration is the only variable that significantly affects larval consumption ($P = 0.021$) for cannibalising *An. quadriannulatus* fourth instars (Table 27). Figure 26 shows a hyperbole curve between food concentration and larval consumption with a higher larval consumption at 0.1mg and 0.5mg than at 0.3mg Tetramin®. The P values for temperature and for the interaction between both variables are 0.743 and 0.906, respectively. Consumption of conspecific larvae by cannibalising fourth stage larvae of *An. quadriannulatus* does not result in a larger body size. Even when the wing lengths of *An. quadriannulatus* fourth instars, that did not cannibalise a single larvae, are compared to individuals that did consume one or more *An. quadriannulatus* first instars, larval consumption did not affect adult size ($P = 0.904$).

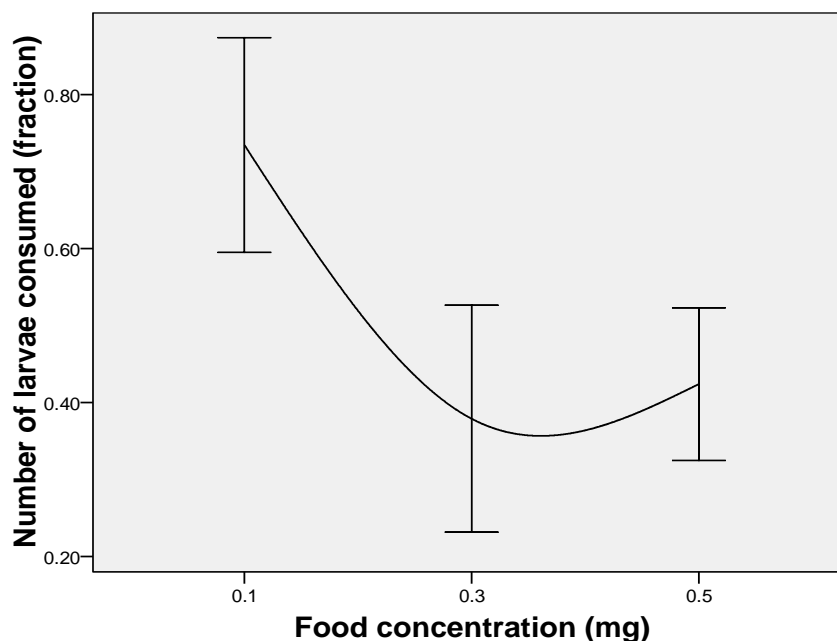


Fig. 24: The effect of nutrient concentration on predation by *An. quadriannulatus* fourth instars. Error bars: confidence interval of 95%.

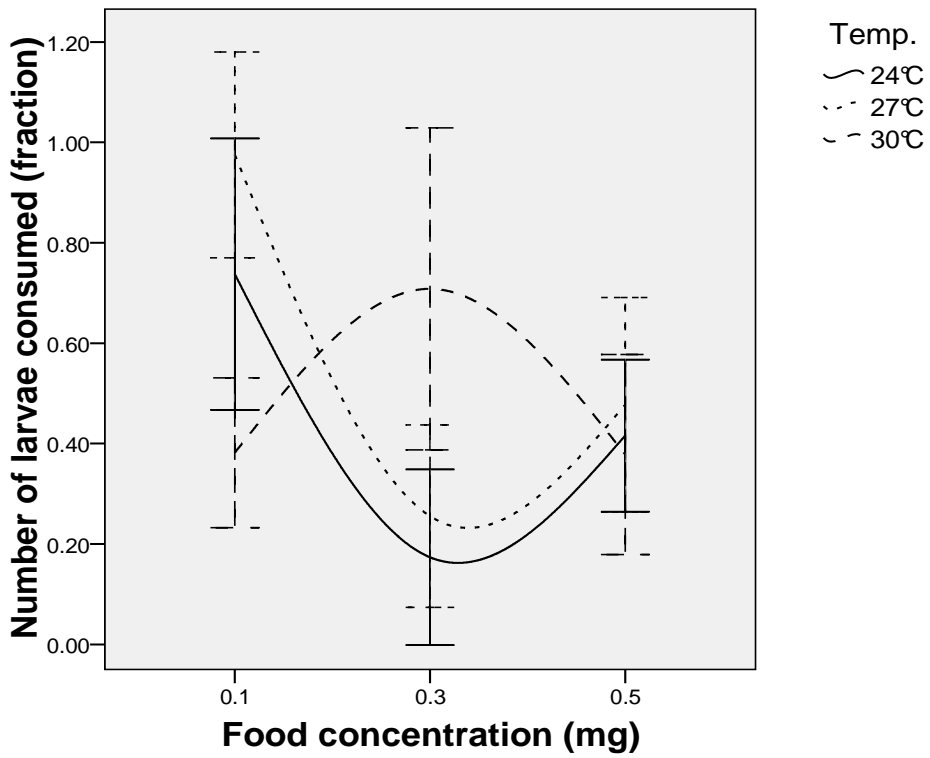


Fig. 25: The effect of the interaction between temperature and nutrient concentration, on predation by *An. quadriannulatus* fourth instars. Error bars: confidence interval of 95%.

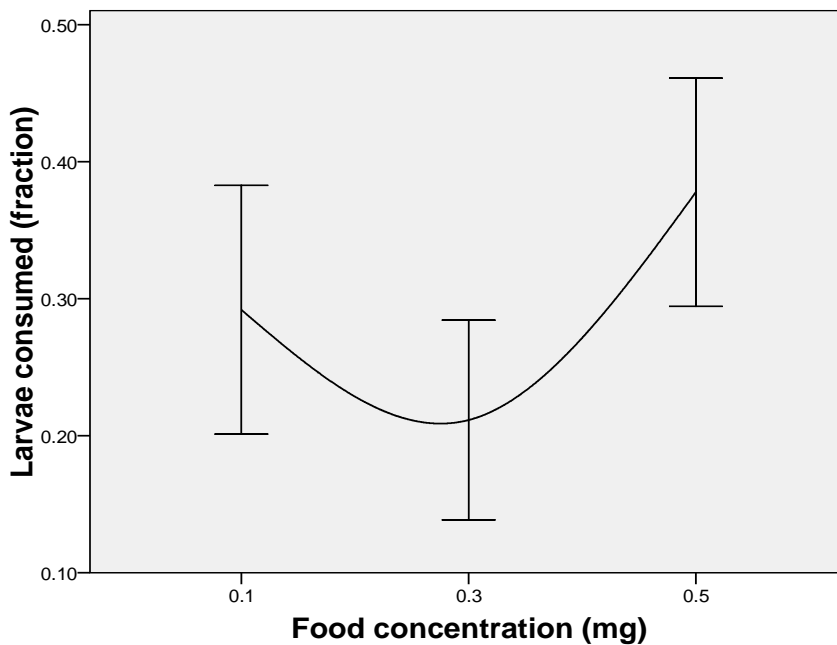


Fig. 26: The effect of nutrient concentration on cannibalism by *An. quadriannulatus* fourth instars. Error bars: confidence interval of 95%.

7.2.6 Does pupation time affect adult wing length?

The wing length of cannibalising *An. gambiae* and *An. quadriannulatus* fourth instars, are significantly affected by pupation time ($P = 0.039$ & $P = 0.001$, respectively). The cannibalising larvae grow into larger adult mosquitoes, when they needed more time to develop. The wing length of *An. gambiae* and *An. quadriannulatus* that fed on another anopheline species, are not significantly affected by pupation time ($P = 0.395$ & $P = 0.657$ respectively).

Pupation time was not significantly affected by temperature ($P = 0.063$) but food concentration did significantly affect the pupation time of the larvae (Table 28). Lower food quantities caused larvae to develop significantly more slowly than higher food concentrations ($P = 0.001$).

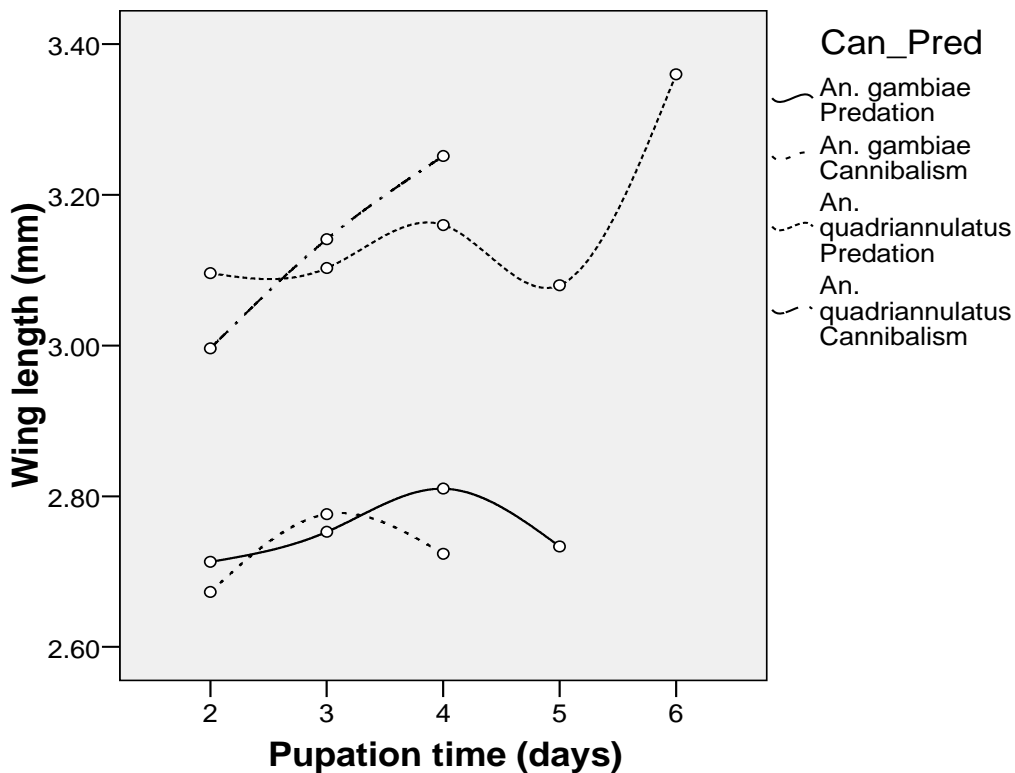


Fig. 27: The effect of pupation time on adult wing length of predating and cannibalising *An. gambiae* and *An. quadriannulatus* fourth instars.

8. Discussion:

To assess the impact of predation and cannibalism on the fitness of *An. gambiae* and *An. quadriannulatus*, two parameters were examined: wing length and the number of eggs produced by the blood fed female mosquitoes, in the first gonotrophic cycle. The latter parameter, however, could not be assessed because pupae and newly emerged mosquitoes died, which made it impossible to collect sufficient data. At first the pupae were placed in a plastic cup (coffee cup) with a 5mm thick layer of water. But for both *Anopheles* species, the majority of pupae did not emerge and the ones that did, drowned. A rectangle shaped filter-paper was placed in the cups, but this did not prevent the adult mosquitoes from drowning. Secondly we tried to place the pupae just in a drop of water in the cups, but again fewer than half of the pupae emerged. Finally the pupae were placed in a glass tube containing some water and sealed off with a wad of cotton wool with 6 % glucose water. The tubes were placed at an angle of 45 degrees so the emerged mosquitoes were able to rest. Now almost all pupae emerged successfully and all adult mosquitoes survived. For the female mosquitoes to produce eggs, they were offered a blood meal. The opening of the tube was placed for ten minutes on a human arm for the female mosquitoes to blood feed. This was done during the mosquito's night-time. But less than one out of every ten mosquitoes took a blood meal. To solve this, the female mosquitoes were placed individually in 16 cubic cm cages and were offered an arm, which was placed on top of the cages. Nevertheless the mosquitoes were not triggered to blood feed and therefore the data of the number of produced eggs is lacking. Hence, the present study examined only the effect of predation/cannibalism on adult size as a proxy for fitness (Lyimo et al. 1992; Takken et al 1998).

Previously Koenraadt (2003) showed that predatory and cannibalistic behaviour is a common phenomenon among larvae of the *An. gambiae* complex and may have an evolutionary origin. Larval consumption could deliver extra nutrition to allow larvae to grow better and to develop into a larger mosquito with more metabolic reserves. Consequently these extra metabolic reserves may provide the mosquito with a longer period to search for plant sugars and a suitable host for a blood meal, resulting in a higher fecundity for larger females. Secondly, for evolutionary reasons, in nature it could be advantageous to engage more in predation than in cannibalism, giving your proper species more chance to develop and finally to reproduce. In the present experiments, the fourth instars did not get the choice to cannibalise or to act as predator. But the L4 were offered the possibility to feed on non-conspecific anopheline larvae or to consume conspecifics, to feed on the L1's of the other species or L1's of their own species, respectively.

In these settings *An. quadriannulatus* consumed significantly more *An. gambiae* first instars than it cannibalised *An. quadriannulatus* first instars, and also more than *An. gambiae* predated on *An. quadriannulatus* first instars (Tables 21 & 22). Thus, *An. quadriannulatus* is a better predator than *An. gambiae*, while both species exhibit the same intensity of cannibalism. Despite the predatory and cannibalistic behaviour, larval consumption did not confer a fitness advantage. In all four distinctive treatments, larval consumption did not significantly affect the adult wing sizes, demonstrated by the fact that it did not give rise to females with a larger wing length.

Since the fourth instars did not have the option to choose between predation and cannibalism, the evolutionary advantage to predate more than to cannibalise is less relative in these settings. Still it is the case for *An. quadriannulatus*, on average 4th stage larvae of *An. quadriannulatus* consumed 3.02 first instars of *An. gambiae* and only 1.27 first instars of its

own species. This distinction was not seen in *An. gambiae* where no significant difference was found in larval consumption between the treatments. Regardless of the fact that larval consumption did not result in larger adult mosquitoes, the rate of predation and cannibalism did differ. This might be the consequence of stronger and fitter *An. quadriannulatus* first instars, making the *An. quadriannulatus* L1 more difficult to catch than the first instars of *An. gambiae*. Because of this I had expected cannibalising *An. gambiae* to consume larvae at a higher rate than larvae of *An. quadriannulatus*. However, this was not the case. Therefore *An. gambiae* fourth instars are considered less dominant in larval consumption than the *An. quadriannulatus* fourth instars. Unless fourth instars are able to distinguish a first instar of their own species from a different one and consequently suppress cannibalism.

Table 29: Mean wing length and mean number of larvae consumed by predation or cannibalism by fourth instars of *An. gambiae* and *An. quadriannulatus*.

	Can / Pred	Mean wing length (mm) \pm S.E.	Mean No of larvae consumed	Std. Error
Larvae consumed	<i>An. gambiae</i> (Predation)	2.74 \pm 0.018	1.33	0.211
	<i>An. gambiae</i> (Cannibalism)	2.72 \pm 0.019	1.54	0.208
	<i>An. quadriannulatus</i> (Predation)	3.11 \pm 0.022	3.02	0.287
	<i>An. quadriannulatus</i> (Cannibalism)	3.10 \pm 0.025	1.27	0.143

The rates of emergence of *An. gambiae* (Table 5), reared under 27°C and 30°C with only 0.1mg Tetramin®, confirmed that *An. gambiae* larvae are less vital when raised under low food concentrations. *An. gambiae* L1's are easily consumed and less than 50% of *An. gambiae* fourth instars reared with 0.1mg of Tetramin® develop into pupae, except when reared at 24°C. By contrast, the emergence rate of *An. quadriannulatus* never drops below 72.7%.

Figure 28 clearly shows that the rate of predation by *An. quadriannulatus* is significantly higher than of the other 3 groups, especially when only given 0.1mg. Also the rate of cannibalising *An. quadriannulatus* and *An. gambiae* are significantly affected by food concentration (Tables 23, 25 & 26). When 0.1mg of Tetramin® as larval food is left out of the analysis, larval consumption is not affected by food concentration any more (P=0.462).

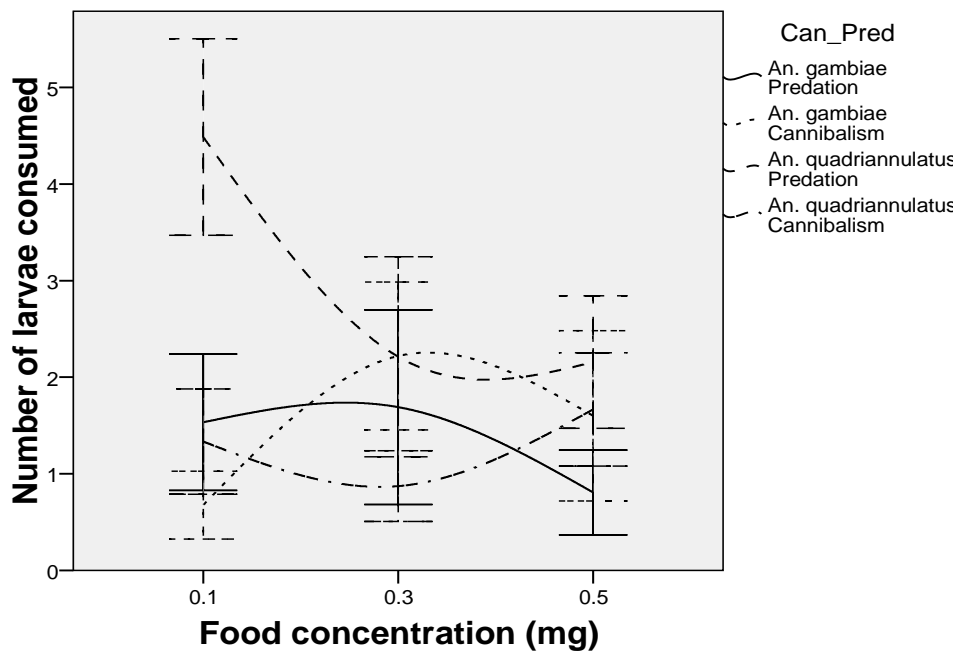


Fig. 28: The effect of food concentration on larval consumption by *An. gambiae* and *An. quadriannulatus* fourth instars. Error bars: confidence interval of 95%.

Although larval consumption remains significantly different between the four distinctive groups ($P=0.023$), the rates of predation and cannibalism occur differently under the same food quantities.

Besides larval consumption also food concentration, temperature and pupation time have an influence on wing size. The number of cannibalised first instars by *An. gambiae* and *An. quadriannulatus* fourth instars is significantly affected by food concentration (Fig. 9 & 13). For cannibalising *An. gambiae* significantly more larvae are eaten at 0.3 and 0.5mg fish food and similarly significant larger adults emerge with 0.3 and 0.5 mg Tetramin® food (Fig. 9 & 21). Especially in the experiments with cannibalising *An. gambiae*, larvae with a greater fitness develop under higher nutritional quantities, and these are able to consume significantly more first instars and consequently emerge as larger adults. Nonetheless when the results are analysed, including all variables, cannibalism had no significant effect on wing length. Food concentration overpowers the positive influence of cannibalism on wing size. In the *An. gambiae* predation experiments no single variable significantly influenced the adult wing size. Although the rate of predation of *An. quadriannulatus* was significantly higher than that of the other 3 treatments, predation of *An. gambiae* by *An. quadriannulatus* did not result in larger mosquitoes. Even with 0.1mg fish food, predation did not compensate for the obvious lack of food, 4th instars of *An. quadriannulatus* ate on average 4.49 first instars of *An. gambiae*, still predation did not result in larger adult mosquitoes. Fourth instar larva that consumed 7 or more first instars were not significantly larger than the ones that consumed fewer than 3 larvae.

Temperature had a significant effect on wing size of cannibalising *An. gambiae* (Fig. 8), *An. quadriannulatus* that engaged in predation of *An. gambiae* (Fig. 11) and cannibalizing *An. quadriannulatus* (Fig 12). In all three cases, temperature was negatively correlated with wing length, larger wing sizes were recorded with 24°C than with 27°C and 30°C. *An. quadriannulatus* is known to breed in more temperate conditions (Gimnig et al. 2001), and

therefore *An. quadriannulatus* larvae may develop better at temperatures lower than 27°C. For *An. gambiae* a mean temperature of 27°C is assumed to be optimal (Paaijmans et al. 2008) and this is in agreement with our findings (Fig. 8), also when *An. gambiae* is feeding on *An. quadriannulatus* larvae.

Pupation time, influenced by temperature and food concentration, also had a significantly positive effect on wing length for both cannibalistic experiments. Logically the more time a larva takes or needs to develop, the more time it can eat and consequently stock more nutrients to grow larger. However, this is ecologically not very sound, as long development times increase the risk of predation. In nature more predators, pathogens and even drought can affect larval development in a negative way.

In all four treatments, consumption of larvae of either species did not significantly affect the adult wing sizes. Neither predation nor cannibalism resulted in fitness advantages. As this predatory behaviour freely occurs in nature it still may give the species an advantage. Schneider (2000) proved that *An. arabiensis* had a competitive disadvantage in mixed populations with *An. gambiae*. Koenraadt (2004) recorded that smaller environments did affect the occurrence of predation and cannibalism, but not for reasons of food shortage. These experiments showed that the nutrients from larval consumption did replace the uptake of other food particles to a certain extent, yet did not result in larger mosquitoes similarly as in the present study.

Church (1996) investigated the benefits of larval cannibalism in the Neotropical mosquito *Trichoprosopon digitatum*. No measurable advantage to cannibalism in terms of survival rate, emergence time or adult size was found but larvae were significantly more cannibalistic when food availability was lower. Nevertheless, under low food concentrations an important fitness benefit of cannibalism was revealed: individuals who consumed first instar larvae, survived significantly longer as larvae than those which did not. This increased longevity is likely to provide an important advantage to mosquito larvae when they depend on the input of unpredictable food sources. However, one can also argue the enhanced larval longevity puts them at great risk of death by predation. Hence, predation would only be advantageous to the species, if it results in higher fitness of adult mosquitoes.

As both predatory and cannibalistic behaviour are also enhanced as a result of more frequent interactions between individuals (Koenraadt et al. 2004), consequently the value of larval consumption increases as larval density increases. If densities in a breeding site become higher as the habitat shrinks through drying out, predation and cannibalism would eliminate competition for food and space. This behaviour favours the individual larva and might influence adult population densities.

Cannibalism and predation are widespread phenomenon in nature; both can reduce competitors and introduce a high-quality food into the cannibal's and predator's diet. Snyder (2000) investigated the dietary benefits of cannibalism for larvae of the multicoloured Asian lady beetle, *Harmonia axyridis* (Pallas). Similar to this study, cannibalism did not directly affect fitness but increased survivorship, to help the larvae to develop.

In general further experiments on the inter-related effects of larval density, larval consumption, development time, temperature and food concentration on fecundity will help explore the population dynamics of the two sibling species. Is the *An. quadriannulatus* predation rate always this prominent? What are the effects of all these variables in mix populations?

Acknowledgments:

Many thanks go to Willem Takken for the supervision of the experiments and analysis, Jeroen Spitzen and Sander Koenraadt for their advice on material and methods and help in the statistical analysis. I am also grateful to Leo Koopman, Frans van Aggelen and André Gidding for maintenance of the mosquito colonies and many more people at the Laboratory of Entomology in Wageningen for their help and advice.

Appendix:

All values typed in bold are significant.

Table 3: Preliminary experiments; the effect of temperature, food concentration and it's interaction, on the number of An. gambiae larvae to pupate. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: larvae to pupate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3672.963(a)	8	459.120	7.279	.000
Intercept	25330.704	1	25330.704	401.602	.000
Food_Conc	2826.741	2	1413.370	22.408	.000
Temperature	374.296	2	187.148	2.967	.077
Food_Conc * Temperature	471.926	4	117.981	1.871	.159
Error	1135.333	18	63.074		
Total	30139.000	27			
Corrected Total	4808.296	26			

R Squared = .764 (Adjusted R Squared = .659)

Table 4: Preliminary experiments; the effect of temperature, food concentration and it's interaction, on the number of An. quadriannulatus larvae to pupate. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: larvae to pupate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6252.667(a)	8	781.583	8.738	.000
Intercept	23408.333	1	23408.333	261.708	.000
Food_Conc	5643.556	2	2821.778	31.548	.000
Temperature	66.889	2	33.444	.374	.693
Food_Conc * Temperature	542.222	4	135.556	1.516	.240
Error	1610.000	18	89.444		
Total	31271.000	27			
Corrected Total	7862.667	26			

R Squared = .795 (Adjusted R Squared = .704)

Table 6: Mann-Whitney test compared the wing lengths of *An. gambiae* and *An. quadriannulatus* mosquitoes.

Ranks

	Species	N	Mean Rank	Sum of Ranks
millimeter	<i>An. gambiae</i>	149	92.11	13725.00
	<i>An. quadriannulatus</i>	187	229.36	42891.00
	Total	336		

Test Statistics^a

	millimeter
Mann-Whitney U	2550.000
Wilcoxon W	13725.000
Z	-12.883
Asymp. Sig. (2-tailed)	.000

a. Grouping Variable: Species

Table 7: Mann-Whitney test compared the wing lengths of fourth instar larvae of *An. gambiae* that did not consume first instars with fourth instar larvae of *An. gambiae* that did. Not taking predation and cannibalism into account.

Ranks

	Larval consumption	N	Mean Rank	Sum of Ranks
millimeter	<i>An. gamb.</i> No larval consumption	70	63.34	4433.50
	<i>An. gamb.</i> larval consumption	79	85.34	6741.50
	Total	149		

Test Statistics^a

	millimeter
Mann-Whitney U	1948.500
Wilcoxon W	4433.500
Z	-3.116
Asymp. Sig. (2-tailed)	.002

a. Grouping Variable: Larval_cons

Table 8: Mann-Whitney test compared the wing lengths of fourth instar larvae of *An. quadriannulatus* that did not consume first instars with fourth instar larvae of *An. quadriannulatus* that did. Not taking predation and cannibalism into account.

Ranks

	Larval consumption	N	Mean Rank	Sum of Ranks
millimeter	<i>An. quadr.</i> No larval consumption	53	91.61	4855.50
	<i>An. quadr.</i> larval consumption	134	94.94	12722.50
	Total	187		

Test Statistics^a

	millimeter
Mann-Whitney U	3424.500
Wilcoxon W	4855.500
Z	-.380
Asymp. Sig. (2-tailed)	.704

a. Grouping Variable: Larval_cons

Table 9: Mann-Whitney test, to compare the wing lengths of *An. gambiae* female and male mosquitoes.

Ranks

	Sex	N	Mean Rank	Sum of Ranks
millimeter	Female	88	83.66	7362.00
	Male	61	62.51	3813.00
	Total	149		

Test Statistics^a

Statistics	millimeter
Mann-Whitney U	1922.000
Wilcoxon W	3813.000
Z	-2.951
Asymp. Sig. (2-tailed)	.003

a. Grouping Variable: Sex

Table 10: Mann-Whitney test, to compare the wing lengths of *An. quadriannulatus* female and male mosquitoes.

Ranks					Test Statistics ^a	
	Sex	N	Mean Rank	Sum of Ranks		millimeter
millimeter	Female	94	112.57	10582.00	Mann-Whitney U	2625.000
	Male	93	75.23	6996.00	Wilcoxon W	6996.000
	Total	187			Z	-4.728
					Asymp. Sig. (2-tailed)	.000

a. Grouping Variable: Sex

Table 11: Predating fourth instars larvae of *An. gambiae*; the effect of larval predation, temperature, food concentration and it's interactions on adult wing length. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: millimeter

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.909(a)	27	.034	2.316	.008
Intercept	187.801	1	187.801	12916.568	.000
Temperature	.035	2	.018	1.205	.311
Food_Concentration	.062	2	.031	2.123	.133
Larvae_consumed	.121	9	.013	.927	.512
Temperature *					
Food_Concentration	.096	4	.024	1.651	.181
Temperature *					
Larvae_consumed	.007	2	.003	.240	.788
Food_Concentration *					
Larvae_consumed	.199	5	.040	2.735	.033
Temperature *					
Food_Concentration *					
Larvae_consumed	.001	2	.001	.043	.958
Error	.567	39	.015		
Total	503.938	67			
Corrected Total	1.476	66			

a R Squared = .616 (Adjusted R Squared = .350)

Table 12: Cannibalising fourth instars larvae of *An. gambiae*; the effect of larval cannibalism, temperature, food concentration and it's interactions on adult wing length. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: millimeter

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.786(a)	35	.051	3.884	.000
Intercept	229.968	1	229.968	17505.13	.000
Temperature	.136	2	.068	5.166	.009
Food_Concentration	.121	2	.060	4.605	.015
Larvae_consumed	.057	9	.006	.481	.880
Temperature * Food_Concentration	.139	4	.035	2.646	.045
Temperature *	.067	8	.008	.634	.745
Larvae_consumed *	.140	7	.020	1.526	.182
Food_Concentration *	.020	2	.010	.760	.473
Larvae_consumed *	.604	46	.013		
Error	.604	46	.013		
Total	609.059	82			
Corrected Total	2.390	81			

R Squared = .747 (Adjusted R Squared = .555)

Table 13: Predating fourth instars larvae of *An. quadriannulatus*; the effect of larval predation, temperature, food concentration and it's interactions on adult wing length. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: millimeter

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.279(a)	51	.045	1.042	.452
Intercept	527.647	1	527.647	12298.965	.000
Temperature	.292	2	.146	3.397	.044
Food_Concentration	.031	2	.015	.359	.701
Larvae_consumed	.334	10	.033	.779	.648
Temperature *	.301	4	.075	1.757	.158
Food_Concentration *	.534	12	.044	1.037	.437
Larvae_consumed *	.090	10	.009	.209	.994
Temperature *	.504	8	.063	1.467	.202
Food_Concentration *	.504	8	.063	1.467	.202
Larvae_consumed *					
Error	1.630	38	.043		
Total	875.021	90			
Corrected Total	3.910	89			

R Squared = .583 (Adjusted R Squared = .023)

Table 14: Canibalising fourth instars larvae of *An. quadriannulatus*; the effect of larval predation, temperature, food concentration and it's interactions on adult wing length. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: millimeter

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.074(a)	36	.085	1.969	.010
Intercept	334.198	1	334.198	7705.856	.000
Temperature	.349	2	.175	4.024	.023
Food_Concentration	.333	2	.167	3.839	.027
Larvae_consumed	.394	7	.056	1.297	.267
Temperature *	.204	4	.051	1.174	.331
Food_Concentration *	.290	7	.041	.955	.472
Larvae_consumed *	.152	7	.022	.500	.831
Temperature *	.092	6	.015	.353	.906
Food_Concentration *					
Larvae_consumed *					
Error	2.602	60	.043		
Total	935.269	97			
Corrected Total	5.677	96			

R Squared = .542 (Adjusted R Squared = .267)

Table 16: The number of larvae predated and cannibalised by *An. gambiae* fourth instars, at 24°C and 0.1mg food. Mann-Whitney U test.

Test Statistics(b)

	Larvae consumed
Mann-Whitney U	9.000
Wilcoxon W	64.000
Z	-3.279
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	.001(a)

Table 17: The number of larvae predated and cannibalised by *An. gambiae* fourth instars, at 27°C and 0.3mg food. Mann-Whitney U test.

Test Statistics(b)

	Larvae consumed
Mann-Whitney U	16.000
Wilcoxon W	71.000
Z	-2.916
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.005(a)

Table 18: The number of larvae predated and cannibalised by *An. quadriannulatus* fourth instars, at 24°C and 0.1mg food. Mann-Whitney U test.

Test Statistics(b)

	Larvae consumed
Mann-Whitney U	21.000
Wilcoxon W	87.000
Z	-2.435
Asymp. Sig. (2-tailed)	.015
Exact Sig. [2*(1-tailed Sig.)]	.016(a)

Table 19: The number of larvae predated and cannibalised by *An. quadriannulatus* fourth instars, at 27°C and 0.1mg food. Mann-Whitney U test.

Test Statistics(b)

	Larvae consumed
Mann-Whitney U	8.000
Wilcoxon W	74.000
Z	-3.990
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000(a)

Table 20: The number of larvae predated and cannibalised by *An. quadriannulatus* fourth instars, at 30°C and 0.3mg food. Mann-Whitney U test.

Test Statistics(b)

	Larvae consumed
Mann-Whitney U	24.000
Wilcoxon W	90.000
Z	-2.464
Asymp. Sig. (2-tailed)	.014
Exact Sig. [2*(1-tailed Sig.)]	.016(a)

Table 21: Number of larvae predated by fourth instars of *An. gambiae* and *An. quadriannulatus*, Mann-Whitney U test.

Ranks				
	Predation	N	Mean Rank	Sum of Ranks
Larvae consumed	<i>Pr. An. gambiae</i>	90	77.07	6936.00
	<i>Pr. An. quadriannulatus</i>	102	113.65	11592.00
	Total	192		

Test Statistics(a)	
	Larvae consumed
Mann-Whitney U	2841.000
Wilcoxon W	6936.000
Z	-4.681
Asymp. Sig. (2-tailed)	.000

a Grouping Variable: Predation

Predation			Statistic	Std. Error
Larvae consumed	<i>Pr. An. gambiae</i>	Mean	1.33	.211
		95% Confidence Interval for Mean		
		Lower Bound	.91	
		Upper Bound	1.75	
		Std. Deviation	2.000	
		Minimum	0	
		Maximum	9	
	<i>Pr. An. quadriannulatus</i>	Mean	3.02	.287
		95% Confidence Interval for Mean		
		Lower Bound	2.45	
		Upper Bound	3.59	
		Std. Deviation	2.894	
		Minimum	0	
		Maximum	10	
Range	10			
Interquartile Range	4			
Skewness	.892	.239		
Kurtosis	-.138	.474		

Table 22: Number of larvae cannibalized by fourth instars of *An. gambiae* and *An. quadriannulatus*, Mann-Whitney U test.

Ranks				
	Canibalism	N	Mean Rank	Sum of Ranks
Larvae consumed	<i>Can. An. gambiae</i>	105	104.96	11021.00
	<i>Can An. quadriannulatus</i>	105	106.04	11134.00
	Total	210		

Test Statistics(a)	
	Larvea consumed
Mann-Whitney U	5456.000
Wilcoxon W	11021.000
Z	-.134
Asymp. Sig. (2-tailed)	.893

a Grouping Variable: Canibalism

Canibalism			Statistic	Std. Error
Larvae consumed	<i>Can. An. gambiae</i>	Mean	1.54	.208
		95% Confidence Interval for Mean		
		Lower Bound	1.13	
		Upper Bound	1.96	
		Minimum	0	
		Maximum	10	
		Range	10	
	<i>Can An. quadriannulatus</i>	Mean	1.27	.143
		95% Confidence Interval for Mean		
		Lower Bound	.98	
		Upper Bound	1.55	
		Std. Deviation	1.463	
		Minimum	0	
		Maximum	9	
Range	9			
Interquartile Range	2			
Skewness	2.136	.236		
Kurtosis	7.313	.467		

Table 23: The effect of nutrient concentration and temperature on the amount of larval predation, by fourth instars larvae of *An. gambiae*. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: L_consumed_2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.509(a)	8	.439	6.990	.000
Intercept	6.063	1	6.063	96.633	.000
Food_Concentration	.240	2	.120	1.915	.154
Temperature	.926	2	.463	7.383	.001
Food_Concentration * Temperature	2.361	4	.590	9.406	.000
Error	5.082	81	.063		
Total	14.813	90			
Corrected Total	8.591	89			

a R Squared = .408 (Adjusted R Squared = .350)

Table 24: Effect of nutrient concentration and temperature on the amount of larval cannibalism by fourth instars larvae of *An. gambiae*. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: L_consumed_2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.677(a)	8	.460	5.689	.000
Intercept	8.859	1	8.859	109.647	.000
Food_Concentration	1.227	2	.614	7.594	.001
Temperature	.475	2	.237	2.938	.058
Food_Concentration * Temperature	2.091	4	.523	6.471	.000
Error	7.757	96	.081		
Total	21.029	105			
Corrected Total	11.434	104			

R Squared = .322 (Adjusted R Squared = .265)

Table 25: Cannibalising *An. gambiae* fourth instars larvae; the effect of larval cannibalism and food concentration and it's interactions, on their adult wing length. Selected data: *An. gambiae* larvae cannibalising four or more larvae.

Coefficients(a)

Model	Unstandardized Coefficients		Standardized Coefficients	t		Sig.
	B	Std. Error	Beta	B	Std. Error	
1 (Constant)	2.660	.105		25.246		.000
Larvae consumed	.047	.014	.839	3.332		.010
Food Conc.	-.256	.290	-.222	-.883		.403

a Dependent Variable: millimeter

Table 26: The effect of nutrient concentration and temperature on the amount of larval predation, by fourth instars larvae of *An. quadriannulatus*. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: L_consumed_2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.828(a)	8	.854	7.955	.000
Intercept	25.095	1	25.095	233.872	.000
Food_Concentration	2.060	2	1.030	9.598	.000
Temperature	.282	2	.141	1.314	.274
Food_Concentration * Temperature	3.732	4	.933	8.695	.000
Error	9.979	93	.107		
Total	44.612	102			
Corrected Total	16.808	101			

a R Squared = .406 (Adjusted R Squared = .355)

Table 27: The effect of nutrient concentration and temperature on the amount of larval cannibalism, by fourth instars larvae of *An. quadriannulatus*. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: L_consumed_2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.595(a)	8	.074	1.215	.299
Intercept	8.971	1	8.971	146.460	.000
Food_Concentration	.490	2	.245	4.003	.021
Temperature	.036	2	.018	.298	.743
Food_Concentration * Temperature	.062	4	.016	.255	.906
Error	5.880	96	.061		
Total	15.243	105			
Corrected Total	6.475	104			

a R Squared = .092 (Adjusted R Squared = .016)

Table 28: The effect of temperature and nutrient concentration on pupation time of fourth instars larvae of *An. gambiae* and *An. quadriannulatus*. Univariate Analysis of variance.

Tests of Between-Subjects Effects

Dependent Variable: # days L4 - Pupae

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.620(a)	8	2.078	3.340	.001
Intercept	3044.608	1	3044.608	4894.704	.000
Temperature	3.457	2	1.729	2.779	.063
Food_Concentration	9.571	2	4.786	7.694	.001
Temperature * Food_Concentration	3.481	4	.870	1.399	.234
Error	244.454	393	.622		
Total	3326.000	402			
Corrected Total	261.075	401			

a R Squared = .064 (Adjusted R Squared = .045)

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