

The silver cyprinid *Rastrineobola argentea* as the main diet source for rearing *Anopheles arabiensis* mosquitoes

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Abstract *Background & objectives:* All organisms that are cultured for laboratory bioassays need food for sustenance and amplification of the colony to such numbers that would not compromise the progress of the research at hand. For effective turnover rate in the population generation, the diet should be such that it is readily available and provide adequate nutrients to the organisms. The aim of this study was to test and evaluate the efficacy of *Rastrineobola argentea* as a diet source for rearing *Anopheles arabiensis* Patton mosquitoes for research.

Methods: Crushed fish, *Rastrineobola argentea* and two other commercial larval feeds (Tetramin[®] Baby fish food and baker's yeast) were compared for their efficacy as diet sources for feeding *Anopheles arabiensis* mosquito larvae. The effects of these diet types were determined against fecundity, size, longevity and male mating competitiveness.

Results: Mosquitoes raised on Tetramin[®] Baby fish food laid larger egg batches (66.45 ± 5.03 mm) as compared to crushed fish (64.86 ± 4.93 mm) or bakers' yeast (50.49 ± 4.25 mm). However, the number of eggs laid by the mosquitoes irrespective of the diet type did not differ significantly ($p < 0.132$). Mosquitoes raised on Tetramin[®] Baby fish food were larger in size (3.06 ± 0.02 mm) as compared to those raised on crushed *R. argentea* (2.93 ± 0.01 mm) or baker's yeast (3.00 ± 0.02 mm). The choice of diet was found to influence both mosquito size ($p < 0.001$) and fecundity ($p < 0.013$).

Conclusion: This study found that crushed *R. argentea* is an effective mosquito larval diet and that it has the potential of being more effective than tetramin if refined further and supplements added.

Keywords *Rastrineobola argentea*; *Anopheles arabiensis*; Fecundity; Longevity; Eclosion

Background

Nutrition is paramount for all living things and has always determined among other things the rate of population growth as well as size of individuals in the population. Studies have been done to evaluate nutritional requirements of organisms including mosquitoes. For example; in one study using *Aedes aegypti*, demonstrated that nutrition was paramount for larval growth, pupation and adult emergence under sterile conditions (Akov, 1962). It has also been shown that both quantitative and qualitative aspects of larval nutrition are important to mosquito development and survival (Gullan and Cranston, 1999), and also to the emergence of adults (Tun-Lin et al., 2000). Other studies have shown that food availability not only

affects larval development (Yoshioka et al., 2012; Dominic and Das, 1996; Araújo et al., 2012), but also adult emergence, sexual maturation, fecundity, survival (Fellous and Lazzaro, 2010; Araújo et al., 2012), body size (Okech et al., 2007) nutrient reserves (Parker, 2005) and affects adult immunity (Grimstad and Walker, 1991). The importance of availability and quantity of food dominates the physiology of the female mosquito. It has been shown that a female mosquito does not simply choose randomly on where to lay her eggs as has been demonstrated using *Culex quinquefasciatus* (Chaves et al., 2009) and *Aedes aegypti* (Zahiri and Rau, 1998) but rather the choice of an oviposition site must demonstrate conditions more

suitable to offspring development (Yoshioka et al., 2012) as well.

Important food substances necessary for optimal development of mosquitoes include carbohydrates, fats, minerals, proteins, lipids, vitamin B complex and yeast nucleic acid and these have been classified into: i) carbohydrates and its products, ii) animal proteins, iii) artificial diets, iv) yeast, v) infusions and others including guinea pig faeces etc (Asahina, 1964).

Rastrineobola argentea is a small, zooplanktivorous cyprinid fish with four rows of pharyngeal teeth (Ahnelt et al., 2006) that is not only abundant in Lake Victoria and Lake Kioga, East Africa but is also commercially valuable (Zahiri and Rau, 1998; Wanink and Witte, 2000) in East Africa. Being abundant means it is an alternative cheaper source and easily available in Kenya compared to other food substances used in mosquito culture. In addition, *Rastrineobola argentea* contains more than protein as a food source that influence various parameters of organism's growth. This study was therefore undertaken to evaluate the efficacy of *Rastrineobola argentea* as a diet source for rearing anopheles mosquitoes in a laboratory set up.

1 Results

1.1 Effects of diet on pupae eclosion success

This experiment was conducted for twenty days. Over 90% of the pupae used in the experiment eclosed to adults (Table 1). Eclosion success did not differ significantly between early and late pupae when releases were made on damp and wet surfaces irrespective of the diet type ($p > 0.05$). The highest number of emerging females was recorded for Baker's yeast for both early (69%) and (58%) and late (65%) and (61) when the pupae were released on damp and wet surfaces respectively. Less than 50% females emerged from pupae arising from larvae raised on

crushed fish *R. argentea* and Tetramin® Baby fish food (Figure 1).

1.2 Effects of larval diet type on adult fecundity

Majority of the female gravid mosquitoes raised on crushed *R. argentea* (63%) laid eggs as compared to Tetramin® Baby fish food (52%) or baker's yeast (45%) (Table 2). Bigger egg batches were laid by mosquitoes raised on Tetramin® Baby fish food (66.45 ± 5.03), as compared to crushed *R. argentea* (64.86 ± 4.93) or bakers' yeast (50.49 ± 4.25). However, the number of eggs laid by the mosquitoes irrespective of the diet type did not differ significantly ($p < 0.132$). Gravid female mosquitoes raised on Tetramin® Baby fish food were larger in size (3.06 ± 0.02 mm) as compared to those raised on crushed *R. argentea* (2.93 ± 0.01 mm) or baker's yeast (3.00 ± 0.02 mm). Mosquito size significantly influenced the number of eggs laid by the female mosquitoes raised on crushed *R. argentea* ($p < 0.001$), Tetramin® Baby fish food ($p < 0.001$) and baker's yeast ($p < 0.001$). Each of the diet type given to the gravid mosquitoes significantly influenced their body size ($p < 0.001$) and the number of eggs laid ($p < 0.013$).

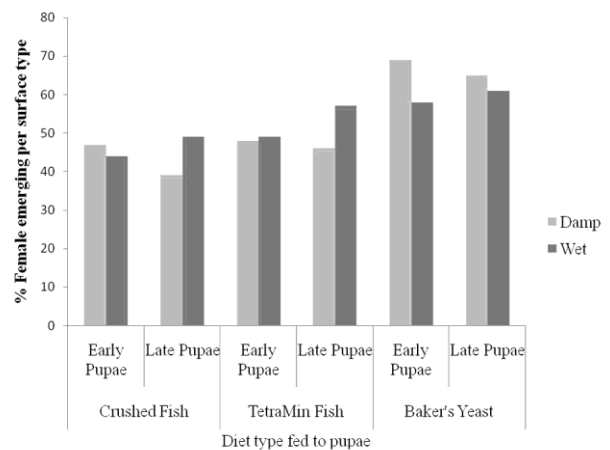


Figure 1 Percentage number of female mosquitoes emerging per diet type per release surface

Table 1 Percentage early and late *An. arabiensis* pupal eclosion with respect to diet (crushed fish *R. argentea*, Tetramin® Baby fish and baker's yeast) and release surface types (damp, wet)

Surface	Crushed Fish <i>R. argentea</i>			Tetramin® Baby fish Food			Baker's yeast		
	Early Pupae	Late pupae	p	Early Pupae	Late pupae	p	Early Pupae	Late pupae	p
Damp	93 ^b	98 ^b	0.723	91 ^b	94 ^b	0.702	93 ^b	95 ^b	0.574
Wet	96 ^b	96 ^b	0.267	96 ^b	96 ^b	0.427	95 ^b	95 ^b	0.748

Notes: 1. Numbers of female mosquitoes pupating as early and late pupae followed with superscripts of different letters differ significantly. 2. P depicts statistical differences in eclosion success of early and late pupae per diet and release surface types. 3. Damp surfaces involved laying single sheets of filter papers in Petri dishes containing single layers of cotton wool soaked in distilled water, and wet surfaces involved placing 30 ml of distilled water in clean Petri dishes

Table 2 Fecundity of adult *An. Arabiensis* mosquitoes raised on different larval diets. The percentages of ovipositing females per diet type are shown

Diet type	N	Percentage of gravid mosquitoes that laid eggs	Mean number of eggs laid per gravid mosquito	Mean Wing size (mm)	p
Crushed fish <i>R. argentea</i>	180	63	64.86 ± 4.92	2.93 ± 0.01 ^a	0.027
Tetramin® fish food	220	52	66.45 ± 5.03	3.06 ± 0.02 ^a	0.001
Baker's yeast	240	45	50.49 ± 4.25	3.00 ± 0.02 ^a	0.001

Note: 1. Mean wing size followed by superscript of the same letter indicates a significant influence of mean mosquito size on the number of eggs oviposited. 2. N is the number of adult female gravid mosquitoes used per diet type, and P is the value for levels of significance between mosquito size and the number of eggs by each mosquito

1.3 Effect of diet type on larval turnover rate and emerged adults longevity

Larvae provided with maize pollen and crushed *R. argentea* pupated within 5 to 6 days (Table 3). Mosquitoes raised on crushed *R. argentea* lived for an average period of between 12 & 13 days, a

time that was almost similar to that lived by mosquitoes raised on Tetramin® Baby fish food (11 & 13 days). However, they were relatively smaller in size (3.22 ± 0.03 mm) than their counter parts raised on those raised on Tetramin® Baby fish food (3.36 ± 0.03 mm).

Table 3 Mean number of days taken by the larvae to develop from first instar (L1) to pupae and adult mosquitoes to live till death

Treatments	L1s at start	Emerging Adults	Mean pupation time	Mean adult longevity
Crushed fish <i>R. argentea</i>	50	45	5.86 ± 0.11 ^a	12.80 ± 0.78
TetraMin® Baby fish food	50	42	5.39 ± 0.07 ^a	12.29 ± 0.97
No food supplements given	50	0	0	0

Note: Mean pupation time followed by superscript of the same letter indicates a significant influence of mean pupation time on mean adult mosquito longevity

2 Discussions

Rapid and massive progeny generation is important for laboratory procedure that requires large numbers of organisms for bioassays. Diet on the other hand is the single most important component of rearing and constitutes the main cost of a production process. A diet source that ensures rapid production of large egg batches even with few female mosquitoes will reasonably be cheap in terms of production cost. If such a diet is of high quality, then this will also lead to production of quality mosquitoes (Wanink and Witte, 2000). The end results in this case will be a lot of quality mosquitoes produced over a shorter period of time.

Crushed fish, as mosquito larvae diet performed same as the commercially available larval feeds that were used in the rearing of the larvae of *An. arabiensis* mosquitoes. In many insects, the nutrient reserves acquired during the larval stage and carried through the pupae stage affect the longevity of the adult. The longevity/stress test that is often related to pupae size measures the percentage of the adults that survive for

a set time period depending on species without food or water (Wanink, 1999). Nutritious mosquito larval diets will therefore give rise to bigger pupae that will translate to larger insects with a lot of nutrient reserves and hence a longer life span. In this study mosquitoes raised on crushed fish lived slightly longer than those raised on Tetramin® Baby fish food, indicating that it leads to accumulation of vital nutrients that are suitable for adult longevity. This study concludes that crushed *R. argentea* is efficacious as a mosquito larval diet and can be used in the culture of mosquitoes for any type of laboratory exercise or bioassay. However, improvement need be done on crushed *R. argentea* if it is to be produced commercially as a mosquito larval diet.

3 Materials and Methods

This study was carried out at the laboratories and insectaries of the School of Biological Sciences, University of Nairobi. *Anopheles arabiensis* Patton mosquitoes obtained from the International Atomic Energy Agency (I.A.E.A., Seibersdorf laboratories) in Vienna, Austria, but originally from Dongola in

northern Sudan, were used. The mosquitoes used in the studies were from the F5 generation onwards. The conditions within the insectaries were maintained at temperatures of 27 – 30 °C, relative humidity of 70~80% and photoperiod of 12 hours of light (06.30 – 18.30 hours) alternated with 12 hours of darkness (18.30 – 06.30 hours).

3.1 Mosquito culture

The mosquitoes were reared following standard techniques (Dominic et al., 2005). The adults were offered 10% sucrose solution soaked in cotton pads daily and placed on top of the cages, as a source of energy. Two days after emergence, the females however were offered animal blood collected from an abattoir and mixed with EDTA to prevent coagulation. The blood was provided via Hemotek® membrane feeding apparatus and on the second day after the blood meal, an oviposition dish was placed in the adult-holding cages for the collection of eggs.

The collected eggs were dispensed in three sets of larval rearing trays, each measuring 21 × 15 × 8 cm. Each tray contained one liter of distilled water. The eggs were left to hatch into first instar larvae that were later redistributed in three groups of one hundred larvae per tray. The first group was fed Tetramin® Baby fish food, the second crushed fish *Rastrineobola argentea* (Pellegrin, 1904) and the third bakers' active yeast. The larvae in each tray were fed thrice daily, at 09.00, 13.00 and 17.00 hours. Each tray received an approximate of 3 milligrams of the respective larval diet type each day.

Larval rearing water was changed every day by sieving the larval instars 1 (L1s) using a piece of clean fine cloth and transferring them into a one liter of fresh sterile distilled water in clean trays. Second instar (L2) and older larvae were sieved using a plastic tea sieve. This process was continued until pupation. Pupae were collected using plastic pipettes and placed in separate clean holding cups measuring 7.5 cm at the mouth 5.0 cm at the base and 8 cm height and then transferred into separate 30 x 30 x 30 cm adult mosquito holding cages covered with mosquito netting. Each cage was labeled with the diet type on which emerged adults had been fed on while in the larval stage.

3.2 Larval diets

One kilogram of *R. argentea* also known as sardine in English was bought from a local market, oven dried to a constant temperature, crushed into powder using a food blender and then put in a glass vial. Tetramin® Baby fish food was obtained from I.A.E.A., Seibersdorf laboratories in The Netherlands while Bakers' active yeast was bought from the local supermarket. All the food types were kept at 4°C.

3.3 Estimating mosquito size

Mosquito size was estimated by measuring the length of one of the mosquito wing (Zahiri and Rau, 1998). The right wing was pulled off from each experimental mosquito using a pair of fine tip forceps and placed over a drop of water on a clean microscope slide. The wing was covered with a cover slip and then placed on a compound microscope stage and its length measured under a magnification of × 40 from the distal end of alula to the tip, excluding the fringe scales.

3.4 Effects of diet on pupae eclosion success

Two types of pupae release surfaces (damp and wet) were prepared and used every day for twenty days. Damp surfaces were prepared by laying single sheets of filter paper in Petri dishes containing single layers of cotton wool soaked in distilled water, and wet by placing 30 ml of distilled water in clean Petri dishes. Ten of each surface type was prepared per day, five in which were placed early and the other five late stage pupae. Early stage pupae were those that metamorphosed from L4 larvae within a two hour window. Ten of these pupae were used per experiment. Late stage pupae consisted of one-day-old pupae (including those that were not used in the 'early stage pupae' category the previous day and were thus one day old). Ten of these pupae were used per experiment. In all cases, L4 larvae were selected and placed in trays two hours before starting experiments. A total of 1000 pupae were used where 20 early pupae and 20 late pupae were used each day. The experiments were started at 16.00 hours and the set ups left to stand in the insectaries until 16.00 hours the next day. The time taken for the pupae to eclose and the number of adult mosquitoes emerging from the pupae per treatment were recorded and the sex and size of the emerged mosquitoes determined.

3.5 Effects of larval diet type on adult fecundity

Three sets of adult mosquito holding cups consisting of twenty cups each measuring $7.5 \times 5.0 \times 8$ cm were used. In each cup 30 ml of distilled water was put, and the mouth covered with a piece of mosquito netting secured safely with a rubber band. Into each of these cups was put fully gravid adult female mosquitoes raised on the test diet types. Twenty fully gravid female mosquitoes raised on crushed *R. argentea* in their larval stages were each put in the first twenty cups, the next twenty fully gravid females raised on Tetramin® Baby fish food in their larval stages in the next twenty cups and lastly twenty fully gravid females raised on Baker's yeast in their larval stages were placed in the last set of twenty cups. This was done by releasing a small part of the netting material at the mouth of the cup, introducing the gravid mosquito into the cup then re-securing the netting. The experiments were conducted for a period of thirteen days with a total of 420 fully gravid female *An. arabiensis* mosquitoes being used. Set experiments were left to stand in the insectaries from 16.00 hrs until 11.00 hrs the next day and thereafter all the mosquitoes retrieved from the cups, the number of eggs laid counted and the size of the mosquito determined.

3.6 Effect of diet type on larval turnover rate and emerged adults longevity

The experiments were conducted for a period of five days. Each day 20 first larval instars (L1s) hatched from eggs laid by mosquitoes raised on crushed fish *R. argentea* were randomly selected and were placed individually in twenty larval holding cups containing 100 ml of distilled water each. The larvae in the first set of ten cups were fed on crushed fish *R. argentea* while the other ten were left unfed. This arrangement was repeated for 20 L1s hatched from eggs laid by mosquitoes raised on Tetramin® Baby fish food. The larvae were provided with an approximate of 0.03 mg of each larval diet type per larva per day. On pupation, the mouth of each larval holding cup was covered with mosquito netting secured with a rubber band at the base of the mouth to prevent the emerged adult from escaping. The time it took the individual larva to develop from L1 to pupa was noted and recorded as a function of turnover rate. The sex of the emerged adult was also determined.

Emerged adult mosquitoes were fed on 10% sugar solution via cotton wool soaked in the sugar solution and placed on the netting material covering the cup. The adult mosquitoes were observed on a daily basis and the time it took each individual to die noted. The experiment was stopped after the last mosquito died and mosquito size was determined by measuring the right wing.

3.7 Statistical analysis

The effects of larval diets on the eclosion success of pupae, the fecundity of adult female, the efficacy of diet types and the proportion of male and female *An. arabiensis* mosquitoes emerging from feeding arrangements were analyzed as a function of mosquito size (based on wing length) and all possible interaction thereof as predictors. A backward likelihood ratio stepwise logistic regression procedure and Pearson's Correlation statistics were used. All analysis was done using the Statistical Package for Social Scientists (SPSS for windows version 11.5).

Authors' contribution

YJO did the experiments, collected, analyzed and interpreted the data, wrote, corrected and edited the manuscript. ZNO-A participated in mosquito culture, read and corrected the manuscript, HO provided supervisory and guidance during experimentation, read and corrected the manuscript. WRM provided supervisory and guidance during experimentation, sourced for the funds, read and corrected the manuscript.

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