CRP Title:
"Development of Standardized Mass Rearing Systems for Male Anopheles Arabiensis Mosquitoes"

Section/Division: Insect Pest Control / Joint FAO/IAEA Division (NAFA)

Project Officers: Jeremie Roger Lionel Gilles and Mac Vreysen

Period Covered: 2005 - 2010

CRP Participating Countries:

Country	Contracting Institute
Belgium	Ghent University
French Polynesia	Institut Louis Malarde(ILM)
France	Institut de Recherche pour le Développment (IRD)
Ghana	Entomology Section
Ghana	Biotechnology and Nuclear Agriculture Research Institute
Italy	Centro Agricoltura Ambiente "G. Nicoli"
Kenya	School of Biological Sciences
Pakistan	Pakistan Atomic Energy Commission (PAEC); Nuclear Institute for Food and Agriculture (NIFA)
Sudan	Epidemiology Department
United Kingdom	School of Biological Sciences
United Kingdom	Oxitec Ltd.
United Kingdom	University of Liverpool; School of Biological Sciences
United Republic of Tanzania	Ifakara Health Research and Development Centre
United Republic of Tanzania	Ifakara Health Research and Development Centre
United States	University of Georgia Research Foundation, Inc.
United States	Harvard School of Public Health; Department of Immunology and Infectious Diseases

CRP Overall Objective:

Any genetic control programme is a process, beginning with colonization and mass rearing of males and females, followed by shipping and finally release of the males in the target population. The overall objective of the CRP on Development of Standardized Mass Rearing System for male *Anopheles arabiensis* mosquitoes was developing colonization and rearing methods for this and other mosquito species, irradiation procedures and other processes prior

to field releases. The ultimate goal was to assist Member States in establishing mosquito colonies and assess their ability to study the use of area-wide SIT suppression programmes in their country.

Contribution Towards the Agency Project:

The Agency project overall objective is to assist Member States in developing technology and methods to put together a SIT package to suppress, as part of an integrated approach, mosquito populations that transmit diseases to human.

The CRP contributed significantly to this objective by developing mass-rearing tools for mosquitoes which were not available before the CRP started. Several of the developments have already been transferred to Member States or are in the process of being transferred. As a result of the CRP, several other Member States have expressed interest in developing and implementing the SIT for disease transmitting mosquitoes and they have requested assistance under the Agencies Technical Cooperation programme for the cycle 2012-2013. This assistance would not have been possible without the results obtained under this CRP.

Specific Research Objective(s):

1. EVALUATING COLONIZATION METHODS AND PROCEDURES.
2. DEVELOPING A MASS PRODUCTION SYSTEM
3. OPTIMIZING HOLDING, PACKAGING AND TRANSPORTATION

Assessment:

In reaching Specific Objective 1: Evaluate colonization methods and procedures

Significant progress has been made with developing methods and procedures to colonize several mosquito species and guidelines are now available for *An. arabiensis* and *Ae. albopictus*. The results of different experiments have shown a drastic loss of genetic diversity during mosquito colonization in the laboratory. Colonization effects and loss of genetic diversity can be reduced using a semi field system (SFS). In addition, detailed protocols were tested to assess the competitiveness of the produced mosquitoes (irradiated, CI-Wolbachia) in the laboratory and in semi-field settings.

The points 1.2. “Standardized procedures for detecting and avoiding the introduction of (intracellular) pathogens in new founding colonies (Output 2)” and 1.3. “Maintenance and holding procedures to minimize the possibility of immuno-compromising of brood stock colonies (Output 7)” were not addressed by CRP participants.

In reaching Specific Objective 2: Develop a mass production system.

Very significant progress was made towards developing a mass-rearing module for mosquito species. Different components of the mass-rearing module have been tested for Anopheline and Aedine species.

The adult mass-rearing cage prototype has been evaluated in several laboratories and feedback on its use has permitted the development of a final prototype for at least 2 species (*An. arabiensis* and *Ae. albopictus*). Detailed protocols were developed including the use of (i) an external blood feeding system (Hemotek), (ii) an internal sugar feeder dispenser, (iii) an oviposition system located at the bottom of the cage, and (iv) a system to provide adequate resting space.

The results demonstrated the effectiveness of the tray-rack system for the mass-production of mosquitoes. Indeed, one rack holding 50 trays can produce approximately 200 000 An. arabiensis and 1 000 000 *Ae. albopictus* (mixture larvae/pupae) using less than one square meter floor space.

The development of the larvae-pupae separator for Anopheline species is one additional tool for the mosquito mass-rearing module. The results showed that a mixture of 30 000 larvae and pupae can be separated in 1.30 min with an efficacy superior to 99.99% for *An. arabiensis.*

In addition, considerable progress has been made in developing and testing a new and inexpensive larval diet composed of bovine liver powder, tuna meal and a vitamin mix. Larval food quality and quantity were also widely studied in the CRP and the results obtained for different species, demonstrated that larval development and survival were strongly impacted by these two parameters. Research showed the importance to adapt and refine protocols (development conditions, larval density & food amount) for each species.

In parallel, the use of Recirculating Aquaculture Systems (RAS) for mosquito mass-rearing has been investigated. The RAS was comparable to laboratory rearing at low larval density. Despite the interesting concept and the promising results, additional research is needed to assess the suitability of the RAS for mass production.

The sterilization at the pupal stage of male mosquitoes was assessed using different types of irradiator (60Cobalt, 137Caesium and X-ray Rad source irradiators). Detailed protocols have been produced to estimate the effect of the irradiation dose on the male sterility of *Ae. albopictus* and *An. arabiensis*. In addition, a new canister for the irradiation of 25 000 pupae in the X-ray Radsource irradiator was developed.

The point 2.4. “Develop total quality management (TQM) guidelines for mass rearing (Output 8)” was not addressed by CRP participants.

In reaching Specific Objective 3: Optimize holding, packaging and transportation.

It was demonstrated that *An. arabiensis* and *Ae.albopictus* could be transported in water from the production site to the field release sites as pupae with limited damage (5 to 10%). The transport of *An. arabiensis* at the adult stage was investigated and showed promising results even if it is logistically more difficult (space consuming).

The point 3.2. “Develop quarantine procedures to prevent accidental escape, and the economics of mass production will also be developed and assessed, respectively (Output 10)” was not addressed by CRP participants.

Activities

1.1. To determine how colonization (i.e. the transfer of wild insects to the laboratory), affects genetic diversity and whether changes in phenotypic expression are important life-history attributes and behaviours (Output 1)

1.2. Standardized procedures for detecting and avoiding the introduction of (intracellular) pathogens in new founding colonies (Output 2)

1.3. Maintenance and holding procedures to minimize the possibility of immuno-compromising of brood stock colonies (Output 7)

1.4. Laboratory-based bioassay procedures to evaluate competitiveness of mass-produced and sterilized males (Output 9)

2.1. To develop a strategy for the step-wise increase in colony size, in order to develop reliable (semi-) automated mass production systems (Output 3).

2.2. Analyses of the influence of various physico-chemical characteristics of the aquatic rearing environment (Output 4), types and quantities of larval nutrition therein (Output 5);
2.3. Maintenance and holding procedures for the adult nutrition brood stock colonies (Output 6)

2.4. Develop total quality management (TQM) guidelines for mass rearing (Output 8);
3.1. Evaluate a variety of packing and shipping procedures that would preserve the quality of mass-produced males during transportation from a mass-rearing facility to the site of release (Output 10)

3.2. Develop quarantine procedures to prevent accidental escape, and the economics of mass production will also be developed and assessed, respectively (Output 10)

Assessment:

Expected CRP Output:

1. EVALUATING COLONIZATION METHODS AND PROCEDURES.

1.1. Colonization methods and effect of colonization on genetic diversity (Output 1):
During the CRP, several mosquito colonies were established in different laboratories*: Aedes albopictus* in Italy, in La Reunion (France) and recently in Vienna (Austria); *Aedes polynesiensis* in French Polynesia; *Anopheles arabiensis* strains in Sudan, Tanzania, Ghana, Kenya and Austria; *Anopheles funestus* in Tanzania and *Anopheles stephensi* in Pakistan. The optimal rearing conditions (biotic and abiotic factors) for each species have been determined to facilitate the establishment of new colonies elsewhere and also to maintain mosquito strains for routine purposes. Guidelines and detailed protocols were produced for the rearing of *Ae. albopictus* and *An. arabiensis*.

The effect of colonization on genetic diversity of an *An. arabiensis* population in Tanzania has been assessed in a large semi-field system (SFS) (20 x 9 x 7.1 m) that simulated the mosquitoes' natural environment. Using microsatellite markers, the heterozygosity, number of alleles, degree of inbreeding (FIS), and genetic distance (FST) were estimated for mosquitoes in the SFS and compared with laboratory and field populations. The semi-field colony displayed more genetic diversity than laboratory colonies and was genetically closer to the field population. The SFS colony consistently showed higher genetic variability on loci located on the X chromosome. Laboratory colonies showed a higher degree of inbreeding as compared to SFS. The contained semi field system therefore seems a suitable testing ground for SIT and GM feasibility studies and may provide realistic rearing conditions of males destined for release in SIT and GM programmes. SFS may be useful for colonizing insect vectors for research as they may provide near realistic insights of their wild counterparts. This system could also be useful for colonization of insect vectors that are difficult to maintain under laboratory condition, e.g. *An. funestus* (one of the major malaria vectors in Africa).

Additional data confirmed the loss of genetic diversity when mosquitoes were colonized and kept under laboratory conditions. In Sudan, 58 field samples from Dongola area were compared to 51 samples from generation 13 from the *An. Arabiensis* colony maintained at the Tropical Medicine Research Institute (TMRI) in Khartoum. The mean number of alleles per locus and mean heterozygosity were dramatically reduced in the laboratory colony as compared to the field population. The reduction in mean number of alleles per locus was up to five fold in microsatellite loci at chromosome 3.

1.2. Standardized procedures for detecting and avoiding the introduction of (intracellular) pathogens in new founding colonies (Output 2):

Not addressed by CRP participants.

1.3. Maintenance and holding procedures to minimize the possibility of immuno-compromising of brood stock colonies (Output 7):

Not addressed by CRP participants.

1.4. Laboratory-based bioassay procedures to evaluate competitiveness of mass-produced and sterilized males (Output 9):

Several competitiveness studies were carried out under different settings for the different species. It is too pre-mature for a finalized common procedure to assess the sexual performance of mosquitoes, but the research conducted during the CRP demonstrated that competitiveness can be estimated using tents, semi-field cages or green-house.
In Italy, preliminary competitiveness tests conducted in 5 green-houses (8 x 5 x 3 m) showed that 40 Gy irradiated *Ae. albopictus* males were as competitive as wild males.

In French Polynesia, for *Ae. polynesiensis*, mating competitiveness was assessed in field population cage experiments using wild-collected mosquitoes. Propensity of mating was compared between wild and Wolbachia incompatible males under semi-natural conditions (large field cages).

In Northern Sudan, competitiveness studies for *An. arabiensis* were conducted in a large green-house consisting of a metal frame (18 x 8 x 2.75 m) covered with dark-green shade netting that permitted airflow and light entry to simulate ambient conditions. The system was divided into three equal sections (48 m2).

Advanced experiments and results are addressed in the on-going CRP on Biology of Male Mosquitoes in Relation to Genetic Control Programmes (G34002).

2. DEVELOPING A MASS PRODUCTION SYSTEM

2.1. Development of mass production equipment (Output 3):

Adult Mass rearing cage. During this CRP, the Mass Rearing Cage (MRC) developed at the Insect Pest Control Laboratory (IPCL) was tested for *An. arabiensis* and *An. gambiae* in Ghana and a short manual on its use was developed. The cage was designed to incorporate devices required for adult emergence, adult resting sites, sugar and blood feeding as well as egg collection. The MRC dimensions were 100 x 80 x 80 cm and the cage was designed to simulate an artificial horizon, which is important for swarming and mating of males Anopheles sp. The MRC was also tested for *Ae. albopictus* in Italy, and for *Ae. polynesiensis* in French Polynesia.

Based on the different tests performed on different species, the group provided feedback. Adult longevity, mating, and insemination rates were as expected in the MRC. While the sugar feeding device was well designed and easy to handle, the blood feeding system appeared to be complicated to use on a routine daily basis. The resting site made from dark fabric with a cylindrical shape had a different role/impact for the different species tested. While it appears essential for Anopheles species to be able to hide from sunlight during day time and to digest the blood, the Aedine species made use of the resting space during the day. Frequent flying and swarming were also observed in the proximity of this object.

The cubic shape of the MRC made handling and cleaning difficult. Moreover, space in a mass-rearing facility will not be optimally used with such a MRC. The oviposition site/plate needed important modifications and additional work to ensure high egg productivity based on key parameters for egg production determined by Balestrino et al. (2011).

The initial prototype did not meet all the requirements for adult mass-rearing and many of the modifications proposed by the participants found a solution at the workshop of the Agency's Laboratories in Seibersdorf before the last RCM meeting. For the new prototype, the frame of the Mediterranean fruit fly MRC developed at the IPCL was used and is still being tested and improved. The new prototype has been transferred to the CRP participants for further validation in the coming months.

Participants expressed the need to develop a common standard protocol for testing MRCs and a first SOP draft is being elaborated at the IPCL, and will be shared with the CRP participants.

Tray-Rack system. A new larval tray system and a rack to hold the trays were developed at the IPCL to mass rear mosquito larvae have been assessed. The larval rearing unit is based on a stainless steel rack that supports and operates 50 thermoformed plastic trays, each holding 4000 *An. arabiensis* larvae and that can successfully bring to completion the larval development of approximately 200 000 *An. arabiensis* adult mosquitoes. The rearing unit is mechanically simple to handle, maintains minimal water temperature variation and negligible water evaporation and allows normal larval development. The mosquito mass-rearing tray was designed to provide a large surface area of shallow water that would closely mimic natural breeding sites and allow larval diet to be easily accessible. The trays stack into a dedicated rack structure and filling, draining and administration of diet were all easily performed. The close stacking of the trays in the rack and the possibility to tightly line up several racks makes this rearing unit a valid solution for maximal use of the space thus reducing construction, heating and cooling costs. The low amount of labour required to operate the system also reduces labour costs which represent one of the main expenditures in any mass-rearing facility operation.

The Tray-Rack system has also been tested for *Ae. albopictus* at the CAA by Romeo Bellini in Italy. The preliminary results showed a production of larvae and pupae mixture up to 20 000 per tray which would mean around 1 000 000 larvae and pupae mixture per rack at the optimal production capacity.

The Tray-Rack system has also been transferred to the TMRI (Sudan) for additional testing on *An. arabiensis*, especially on the genetic sexing strain (Ano IPCL1).

Larvae-pupae separator (LPS). A system for continuous separation of *An. arabiensis* larvae from pupae was assessed for *An. arabiensis*. The LPS was developed at the IPCL and is based on the natural difference in buoyant density and difference in behaviour between the two developmental stages. Temperatures between 4-15 °C caused no mortality or reduction in likelihood of pupation or emergence. Separation improved as temperatures decreased down to 4 °C. In the context of mass-rearing processes of mosquitoes, the results showed that a 15 °C water vortex separator can process a mixture of approximately one million larvae and pupae per hour with a pupal contamination rate of less than 0.3 %. The LPS has been transferred to the TMRI (Sudan) for additional tests on *An. arabiensis*, in view of plans to upscale the production.
The LPS was also tested for *Ae. Albopictus*, but results were not satisfactory as there is no difference in buoyant density between the larvae and pupae. Additional efforts need to be made to develop efficient separation methods for Aedine mosquitoes.

Irradiation procedures. The effect of the irradiation dose on male sterility has been determined for several mosquito species and for different types of irradiators. For example, 100% sterility of male *Ae. albopictus* was achieved with a dose of 40 Gy. The optimal irradiation dose was determined using 20-24 hour-old pupae concentrated in small containers (Petri dishes or small trays customized by the IPCL workshop) with a fine layer of water. The effect of the irradiation dose of 40 Gy was independent from the type of irradiator used i.e. 60Cobalt or 137Caesium. Similar work done on *An. arabiensis* gave 100% sterility with a dose of 100 and 80 Gy given to 20 hour-old pupae of the Dongola and the Ano IPCL 1 strain, respectively.

The X-ray irradiator (Radsource 2400) has also been tested at the IPCL for both *Ae. albopictus* and *An. arabiensis* and the results were comparable to 60Cobalt and 137Caesium irradiators. A new canister was developed specifically for irradiation of mosquito pupae and it showed a uniform dose distribution (with less than 10 % deviation). The central cubic structure than contained 12 Plexiglas layers allow to irradiate more than 25 000 pupae in few minutes per canister.

2.2. Analyses of the influence of various physico-chemical characteristics of the aquatic rearing environment (Output 4), types and quantities of larval nutrition therein (Output 5):

Significant work was carried out regarding these aspects for different mosquito species. Larval food quality and quantity were shown to be definitively crucial parameters for mosquito larval development and adult performance.

Larval food quality. Around 20 single diet components were tested for *Ae. albopictus* and *An. arabiensis*. For both species, larval development parameters such as pupation/emergence time and pupation/emergence rates were significantly influenced by the different components tested. These differences were clearly due to the composition (protein, carbohydrates, lipids, polyunsaturated fatty acids) of each of the components. Both species were clearly affected, but *An. arabiensis* was much more sensitive to food diversity as good emergence rates were only obtained for few (5-6) components tested, whereas *Ae. albopictus* accomplished its development for all components. For both species, the bovine liver powder, the tuna meal and the squid liver powder were the best candidates and these were combined to obtain an optimal mixture. The final diet, originally developed for *An. arabiensis*, was composed of 50% tuna meal and 50% bovine liver powder with some vitamin mix as additive. The combination of those ingredients had a synergetic effect and significantly reduced the time to pupation and increased the larval survival. The diet as it is described here is called the "IAEA diet" hereafter.

Since its initial development, the IAEA diet has already been further improved. For example, to reduce the costs, but keeping good insect quality, the proportion of bovine liver powder was reduced in favour of brewer's yeast and some squid liver powder. The current diet for *Ae. albopictus* contains 50% of tuna meal, 25% of bovine liver powder, 12.5% brewer's yeast and 12.5% of squid liver powder with some vitamin mix as additive.

The cost for producing one million sterile male mosquitoes per day using this diet is estimated at around USD 50-60 against USD 120 when the Tetramin fish food is used. Even though the Tetramin fish food remains a good diet for mosquito larval culture, the IAEA diet is a good and much cheaper alternative to mass produce mosquitoes.
Studies with *An. stephensi* in Pakistan demonstrated that mosquito larval stages could achieve their development if fed with a mixture of bovine liver powder, bean, corn, wheat, rice, and chickpea giving new perspectives for the development of a new and inexpensive diet.

Larval food quantity. The CRP participants carried out extensive work on the effects of larval food quantity and larval density on the development of *Ae. albopictus*, *Ae. aegypti*, *Ae. polynesiensis*, *An. arabiensis* and *An. stephensii*.

It was shown that the amount of larval food given to larvae strongly affected their development. A clear distinction between Aedine and Anopheline species was observed. While Aedeine species were able to complete their larval cycle with 0.5-8% of food concentration (optimum 4%), the Anopheline mosquito larvae could only complete development within a 0.5-2% food concentration range (optimum 1%). According to the diet, we estimated that *An. arabiensis* needs 200-260 µg of diet/larva/day to reach the pupal stage

over 6-7 days. Those results confirmed that Anopheline mosquitoes are “clean water” mosquitoes and do not thrive in breeding sites with high water turbidity. Conversely, it confirmed the aptitude of the Aedine mosquitoes to extend their distribution area by colonizing a broader range of breeding sites.

The optimal larval density for rearing was determined and appeared to differ for Anopheline and Aedine mosquitoes, but also between species within the Aedine group. For *An. arabiensis* and *An. stephensii*, the optimal larval density was 1 larva per ml of water, whereas it was 2 larvae per ml for *Ae. aegypti*, and *Ae. polynesiensis* and 4 larvae per ml for *Ae. albopictus*.

Additional studies demonstrated that splitting (morning & evening) the daily amount of larval food required for their development resulted in better survival of *An. arabiensis*.

Recirculating Aquaculture Systems (RAS). Mosquito larval development studies were conducted in different RAS for *An. arabiensis*, *An. gambiae* and *Ae. aegypti*. Feeding schedule and density effects on survival were assessed in the different systems and compared to larval development using “classic’ static water trays. Anopheles larvae were able to survive and grow in turbulent (aerated) water conditions. Performance of the larvae was even better as compared to the static tray system under the same environmental and feeding conditions. Moreover, culture of Anopheles larvae in proper recirculation systems was successful. Growth and pupation parameters compared favourable when compared to static systems.

Also *Aedes aegypti* larvae were successfully reared in recirculation systems. Densities of up to 500 larvae/L had no negative effects on growth or survival. It was also observed that pupation in the recirculation systems was more synchronized as compared to the static trays.

2.3. Maintenance and holding procedures for the adult nutrition brood stock colonies (Output 6):

Sugar meal. Effects of several energetic sources have been tested on adult longevity of several mosquito species (*An. stephensi*, *An. arabiensis* and *Ae. albopictus*). Adult diets have included various sugar and honey solutions, fruits (either whole or their juices), raisins, and dates. It was demonstrated that various additives to a sucrose meal increased longevity and possibly fecundity. These consisted of an antimicrobial preservative (methylparaben) and ascorbic acid. This additive has been integrated in the diet for *Ae. albopictus* at the IPCL and at the CAA laboratory in Italy. Longevity of *Ae. albopictus* fed on a 10% w/v sucrose solution with 0.2% methylparaben could reach 60-70 days in the laboratory.

Blood meal. A new blood feeding system was designed based on the feedback received from researchers that tested the first MRC with the internal blood feeding system. The blood feeding system is external and is composed of a Hemotek® machine with a customized aluminium plate covered by a collagen membrane. The blood is replenished using disposable luer-lock syringes (50 ml capacity). The new system has been tested on mass-rearing cages

with *An. arabiensis* and *Ae. albopictus* containing more than 10 000 females. It appeared to be attractive with several hundred females taking a blood meal in a short time. The results also showed that while *An. arabiensis* could be fed on defrosted blood, *Ae. albopictus* only accepted fresh blood for feeding.

During the CRP, the researchers showed that mosquitoes can be fed on different blood sources (cheep, human, cattle, rabbit…) with satisfactory results, although more caution is required in terms of quality control for insecticide or drug residues in the blood.

2.4. Develop total quality management (TQM) guidelines for mass rearing (Output 8):
Not addressed by CRP participants

3. OPTIMIZING HOLDING, PACKAGING AND TRANSPORTATION

3.1. Evaluate a variety of packing and shipping procedures that would preserve the quality of mass-produced males during transportation from a mass-rearing facility to the site of release (Output 10):

Strategic and technical solutions for irradiation, transportation and release of mosquitoes were studied during the CRP. For example, an aluminium canister prototype holding 15 metallic plates was designed for the irradiation, the transport and the releases of *An. arabiensis* pupae. Pupae survival after irradiation remained high for several hours when the environment in the canister stayed moist. In addition, field trials in Sudan demonstrated that pupae kept at low density in cups containing water can also be transported for 6-7 hours with an adult emergence of 90%. Transport of adult male mosquitoes in cages was also assessed. The mortality of irradiated and un-irradiated males was 1.1 and 1.3% respectively.

The transport of irradiated pupae of *Ae. albopictus* in the field during pilot release tests in Bologna was carried out in water (ca. 40 litres) using large thermally insulated portable containers (80 x 40 x 40 cm). Pupae transported for 2-4 hours by car had a mortality consistently below 5%. The pupae were distributed in water in field release stations (black plastic vase; Ø 20cm, h 20 cm) provided with additional sugar source for the emerged adult males.

Field trials demonstrated that males engorged with a sucrose solution prior to their release had a better dispersal than those released directly as pupae. Sugar-soaked sponges are now used in all release containers in the field pilot campaign in Italy.

3.2. Develop quarantine procedures to prevent accidental escape, and the economics of mass production will also be developed and assessed, respectively (Output 10):

Not addressed by CRP participants

Impact of the CRP:

The development of cost-effective mass rearing equipment and diet has been highly desirable. The technology transfer and the implementation of such mass rearing tools are a prerequisite for effective SIT programmes for mosquito species, leading to reduced chemical applications to the environment, and protecting human populations to virus and parasite exposures. Knowledge gained and practical procedures developed during this CRP have already been transferred to counterparts and are transferable, at least in part, to other mosquito species with control programmes that include an SIT component.

Relevance of the CRP:

The CRP was extremely relevant to advance towards mosquito SIT implementation. Focusing research on the development of a mass-rearing module was crucial for the implementation of future control programmes in Member States and highly relevant to the mosquito project 2.1.4.4, now part of the Insect Pest Control Sub-programme activities. The CRP succeeded in creating a research group focusing on the importance of mass-producing good quality mosquitoes. Effectively the CRP brought the participants together, resulting in goal-oriented applied research that produced tangible results. Dissemination of these results, either as protocols, guidelines, or in the peer reviewed scientific literature, provides universal access to the advances made. In addition, the technology developed has been now transferred to several counterparts and will be tested and refined according to the biology of the target mosquito species. The progress obtained in the CRP highly contributes to the feasibility studies for the use of SIT in an AW-IPM to control mosquito population in Sudan, in Italy and in La Reunion and in French Polynesia.

Recommendation:

1) Experience shows that placing a project administratively in one division, but making simultaneously first one and later another division responsible for the day to day implementation is not effective and results in poor coordination and follow-up.

2) At least some degree of institutional memory is important, in particular for implementing CRPs that have a life of 6-8 years when taking into account planning, execution and final publication.

3) In view of the above, there is a need to minimize the on-going staff turn-over within the mosquito group at the Entomology Laboratory at Seibersdorf, which has been largely supported through students, interns and consultants.

4) Carry out additional research to refine and improve the mass-rearing equipment developed at the IPCL.

5) Support further studies on developing the post production phase: mosquito packing, transport and release.

6) Start to investigate new tools (traps, for example) to monitor the efficacy of mosquito releases and develop strategy and guidelines.

7) Support more work on the development of new markers for the creation of new genetic sexing strains for the different mosquito programmes.

14. Resulting Publications:

AGEEP, T.B., J. COX, M.M. HASSAN, B.G.J.KNOLS, M.Q. BENEDICT, C.A. MALCOLM, A. BABIKER and B.B. EL SAYED (2009). Spatial and temporal distribution of the malaria mosquito *Anopheles arabiensis* in northern Sudan: influence of environmental factors and implications for vector control. Malaria Journal 8:123.

BALESTRINO, F., A. MEDICI, G. CANDINI, M. CARRIERE, B. MACCAGNANI, M. CALVITTI, S. MAINI and R. BELLINI (2010). Gamma ray dosimetry and mating capacity studies in the laboratory on *Aedes albopictus* males. Journal of Medical Entomology 47(4):581-591.

BALESTRINO, F., S.M. SOLIBAN, J. GILLES, C. OLIVA and M.Q. BENEDICT (2010). Ovipositional behaviour, in the context of mass rearing of *Anopheles arabiensis*. Journal of the American Mosquito Control Association 26:365-372.

BELLINI, R., A. ALBIERI, F. BALESTRINO, M. CARRIERI, D. PORRETTA, S. URBANELLI, M. CALVITTI, R. MORETTI and S. MAINI (2010). Dispersal and survival of *Aedes albopictus* (Diptera: Culicidae) males in Italian urban areas and significance for sterile insect technique application. Journal of Medical Entomology 47(6): 1082-1091.

BELLINI, R., M. CALVITTI, A. MEDICI, M. CARRIERI, G. CELLI, and S. MAINI (2007). Use of the Sterile Insect Technique against *Aedes albopictus* in Italy: First Results of a Pilot Trial, pp. 505-515. In Vreysen, M.J.B., A.S. Robinson, and J. Hendrichs (Eds.), Area-Wide Control of Insect Pests: From Research to Field Implementation. Springer, Dordrecht, Netherlands.

BENEDICT, M.Q., R.C. HOOD-NOWOTNY, P.I. HOWELL and E.E. WILKINS (2009). Methylparaben in Anopheles gambiae s.l. sugar meals increases longevity and malaria oocyst abundance but is not a preferred diet. Journal of Insect Physiology 55:197-204.

BENEDICT, M.Q., B.G.J. KNOLS, H.C. BOSSIN, P.I. HOWELL, E. MIALHE, C. CÁCERES and A.S. ROBINSON (2009). Colonization and mass rearing: learning from others. Malaria Journal 8(Suppl 2):S4.

BOYER, S., J. GILLES, D. MERANCIENNE, G. LEMPERIERE, and D. FONTENILLE. Sexual performance of male mosquito *Aedes albopictus*. Medical and Veterinary Entomology (in press).

CALVITTI M., R. MORETTI, D. PORRETTA, R. BELLINI, and S. URBANELLI (2009). Effects on male fitness of removing Wolbachia infections from the mosquito *Aedes albopictus*. Medical and Veterinary Entomology 23: 132140

CHAMBERS E.W., L. HAPAIRAI, B.A. PEEL, H. BOSSIN and S. DOBSON (2011). Male mating competitiveness of a wolbachia-introgressed Aedes polynesiensis strain under semi-field conditions. PLoS Negl Trop Dis 5(8): e1271. doi:10.1371/journal.pntd.0001271.

DAME, D.A., C.F. CURTIS, M.Q. BENEDICT, A.S. ROBINSON and B.G.J. KNOLS (2009). Historical applications of induced sterilisation in field populations of mosquitoes. Malaria Journal. 8(Suppl 2):S2.

EL SAYED, B.B., C.A. MALCOLM, A. BABIKER, E.M. MALIK, M.A.H. EL TAYEB, N.S. SAEED, A.H.D. NUGUD and B.G.J. KNOLS (2009). Stakeholders first: ethical, legal and social aspects of the approach in Sudan. Malaria Journal 8(Suppl 2):S3.

GILLES, J., R.S. LEES, S.M. SOLIBAN and M.Q. BENEDICT (2011). Density dependence effects in experimental larval populations of *Anopheles arabiensis* (Diptera: Culicidae) can be negative, neutral or over compensatory depending on density and diet levels. Journal of Medical Entomology 48:296-304.

GOUAGNA, LC. JS. DEHECQ, R. GIROD, S. BOYER, G. LEMPERIERE and D. FONTENILLE (2011). Spatial and temporal distribution patterns of *Anopheles arabiensis* breeding sites in La Reunion Island - multi-year trend analysis of historical records from 1996-2009. Parasites & Vectors. 4:121.

HELINSKI, M.E.H., A.G. PARKER and B.G.J. KNOLS (2006). Radiation-induced sterility for pupal and adult stages of the malaria mosquito *Anopheles arabiensis*. Malaria Journal 5: 41.

HELINSKI, M.E.H., B. EL-SAYED and B.G.J. KNOLS (2006). The Sterile Insect Technique: can established technology beat malaria? Entomologische Berichten 66: 13-20.

HELINSKI, M.E. and B.G.J. KNOLS (2008) Mating competitiveness of male *Anopheles arabiensis* mosquitoes irradiated with a partially- or fully-sterilizing dose in small and large laboratory cages. Journal of Medical Entomology 45, 698-705.

HELINSKI, M.E.; M.M. HASSAN, W.M. EL MOTASIM, C.A. MALCOLM, B.G.J. KNOLS and B. EL SAYED (2008). Towards a sterile insect technique field release of *Anopheles arabiensis* mosquitoes in Sudan: Irradiation, transportation, and field cage experimentation. Malaria Journal 7, 65.

HELINSKI, M.E.H., A.G. PARKER and B.G.J. KNOLS (2009). Radiation biology of mosquitoes. Malaria Journal 8(Suppl 2):S6.

HELINSKI, M.E., and B.G. KNOLS (2009). Sperm quantity and size variation in un-irradiated and irradiated males of the malaria mosquito *Anopheles arabiensis* Patton. Acta Tropica 109:64-69.

HELINSKI, M.E.H., and B.G.J. KNOLS (2009). The influence of late-stage pupal irradiation and increased irradiated: un-irradiated male ratio on mating competitiveness of the malaria mosquito *Anopheles arabiensis* Patton. Bulletin of Entomological Research 99:317-322.

HOWELL, P.I., and M.Q. BENEDICT (2009). Mating competitiveness of *Anopheles arabiensis* males as a function of transgenic state and genetic similarity to females. Journal of Insect Behavior 22:477-491.

HOWELL, P., and B.G.J. KNOLS (2009). Male mating biology. Malaria Journal 8(Suppl 2):S8.

KHAN, I., A. FARID and ALAMZEB (2011). Development of inexpensive and globally available larval and adult diets for rearing *Anopheles stephensi* mosquitoes. Entomologia Experimentalis Applicata (submitted)

KHAN, I., S. SOLIBAN, and J. GILLES. Effect of eggs drying, storage and irradiation on hatch ability, development and flight performance of *Anopheles arabiensis* (in preparation for Malaria Journal)

KNOLS, B.G., BOSSIN, H.C., MUKABANA, W.R., and A.S., ROBINSON (2007). Transgenic mosquitoes and the fight against malaria: managing technology push in a turbulent GMO world, American Journal of Tropical Medicine and Hygiene 77 (6): 232-42.

MALCOLM, C.A., D.A. WELSBY and B.B. EL SAYED (2007) "SIT for the malaria vector *Anopheles arabiensis* in Northern State, Sudan: an historical review of the field site", Area-Wide Control of Insect Pests. From Research to Field Implementation (VREYSEN, M.J.B., ROBINSON, A.S., HENDRICHS, J., Eds.), Springer, Dordrecht, The Netherlands 361-372.

MALCOLM, C.A., B.B. EL SAYED, A. BABIKER, R. GIROD, D. FONTENILLE, B.G.J. KNOLS, A.H. NUGUD and M.Q. BENEDICT (2009). Field site selection: getting it right first time round. Malaria Journal 8(Suppl 2):S9.

MEDICI, A., M. CARRIERI, E.-J. SCHOLTE, B. MACCAGNANI, M.L. DINDO, R.BELLINI (2011). Studies on Aedes albopictus larval mass-rearing optimization. Journal of Economic Entomology 104(1): 266-273; DOI: 10.1603/EC10108

NG'HABI, K.R., B. JOHN, G. NKWENGULILA, B.G.J. KNOLS, G.F. KILLEEN, and H.M. FERGUSON (2005) Effect of larval crowding on mating competitiveness *of Anopheles gambiae* mosquitoes. Malaria Journal 4: 49.

NG'HABI, K.R., D. MWASHESHI, B.G.J KNOLS, AND H.M. FERGUSON (2010). Establishment of a self-propagating population of the African malaria vector *Anopheles arabiensis* under semi-field conditions. Malaria Journal 9:356.

OLIVA C. F., M. Q BENEDICT, G. LEMPÉRIÈRE and J. GILLES (2011). Laboratory selection for an accelerated mosquito sexual development rate. Malaria Journal 10:135.

PAPATHANOS, P.A., H.C. BOSSIN, M.Q. BENEDICT, F. CATTERUCCIA, C.A. MALCOLM, L. ALPHEY and A. CRISANTI (2009). Sex separation strategies: past experience and new approaches. Malaria Journal 8(Suppl 2):S5.

ROBINSON, A.S., B.G.J. KNOLS, G. VOIGT and J. HENDRICHS (2009). Conceptual framework and rationale. Malaria Journal 8(Suppl 2):S1.