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RESEARCH ARTICLE

MOSQUITOES (DIPTERA: CULICIDAE) AND THEIR CONTROL BY MEANS OF BIOLOGICAL AGENTS IN VILLA CLARA PROVINCE, CUBA

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ABSTRACT

The principal permanent breeding grounds in the 13 municipalities of the province of Villa Clara were sampled to examine the species of larvivorous fish existent in these reservoirs. The species of mosquitoes associated to these reservoirs were identified. An experiment was carried out to assess the predation capacity of the copepod *Mesocyclops aspericornis* on *Culex quinquefasciatus* larvae. Also, an economic evaluation was made to assess the expenses inverted in the Intensive Campaign in the year 2006 with the purpose decreasing mosquito populations. The proportions of positiveness for *Stegomyia aegypti were* compared between the different cycles of the campaign; this was achieved using a logistic model and a proportional hypothesis test. On the other hand, the results of the experiment of depredation were processed using simple ANOVA and polinomial regression. The results show that the species of mosquitoes and fish most widely distributed in the province corresponded to *Anopheles albimanus*, *Ochlerotatus mediovittata*, *Oc. taeniorhynchus*,*Psorophora confinnis*, *Culex quinquefasciatus* and *Cx. nigripalpus*, and to *Gambusia punctata*, *Girardinus metallicus,Poecilia reticulata*, *Limia vittata* and *Cichlasoma tetracanthus* respectively. Also it was demonstrated in *vitro*, that the copepod *Me. aspericornis* is able to depredate up to eight larvae of *Cx.quinquefasciatus* in 24 hours. We also concluded that the use of biological agents is a simpleand relatively economic alternative within the integrated programs of control of mosquito vectors of diseases in Villa Clara,unlike the utilization of insecticides which are highly expensive and noxious to the environment.

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INTRODUCTION

Millions of people suffer from infections transmitted by vectors arthropods. Among them, culicids are undoubtedly of the greatest hygienic-sanitary importance because they constitute one of the prioritizing health problems in almost tropical and subtropical regions. They are responsible of the keeping and transmission of pathogen agents causing Dengue, Yellow Fever, human Malaria, lymphatic Filariosis and other several mortal and weakening infections (Brenda *et al*., 2000; Chandra *et al*., 2008).

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Together with this problem, it is the planet heating and the increasing of extreme meteorological phenomena, which has brought changes in the behaviour of diseases and their transmitters, with settings in vector species in non ever registered places (Gore, 2007). Luciano *et al.*, 2007 state that of all vector transmitted diseases in the world, Dengue is of the highest incidence. This entity is currently considered the viral disease transmitted by most important arthropods affecting men; it is estimated that each year they are presented nearly 50-100 millions of Dengue casesand 250 000 to 500 000 Hemorrhagic Dengue cases depending on epidemicactivity (Guzmán *et al*., 1999; Guzmán and Kourí, 2002; CDC, 2002). However, the main health problem in vectors is undoubtedly, malaria; it is worldwide estimated 500 millions of reported cases and three millions of death each year (one million are

children younger than five years). It is considered the most spread disease, being endemic nearly about 100 developingcountries. In Africa it is reported 80 per cent of the cases and 90 per centof death (Dia *et al*., 2003; Chandra *et al.*, 2008). Cuba due to the geographical location and climatological characteristics possesses a wide fauna of culicids; most of them are important since the epidemiological point of view by the diseases, endemic and exotic, that can transmit to human and animal population (Guzmán and Kourí, 2002; González, 2006). Ghosh *et al.,* 2005 point out that the efforts to control such diseases have been stopped, because of the development of etiological agents resistant to drugs, mosquitoes resistant to insecticides, environmental pollution, remaining effect of chemical substances, high prices in markets and some other operational difficulties. Consequently, there is a necessity to develop other strategies of diseases control that can complementthe available methods (Brenda *et al*., 2000; Kay and Nam, 2005; Holynska, 2006).

Such strategy is the application of biological methods to control larvalmosquito'spopulations. The main biological agents that have successfully applied are depredators, particularly fishes, copepods and entomopathogen agents, such as sporogenous bacteria: *Bacillus thuringiensis israelensis* (Bti) and*Bacillus sphaericus* (Bs), which attack immature stages of mosquitoes (Das y Amalraj, 1997; Hernández *et al*., 2005; CDC, 2006). Taking into consideration the above explained, the objective of this investigation is to: assess the potentialities of biological organisms as an alternative to decrease larval mosquito's populationsof hygienic-sanitary importance in Villa Clara province.

MATERIALS AND METHODS

The investigation was carried out in Villa Clara province, whose capital is Santa Clara municipality and comprised 13 municipalities (Figure 1).

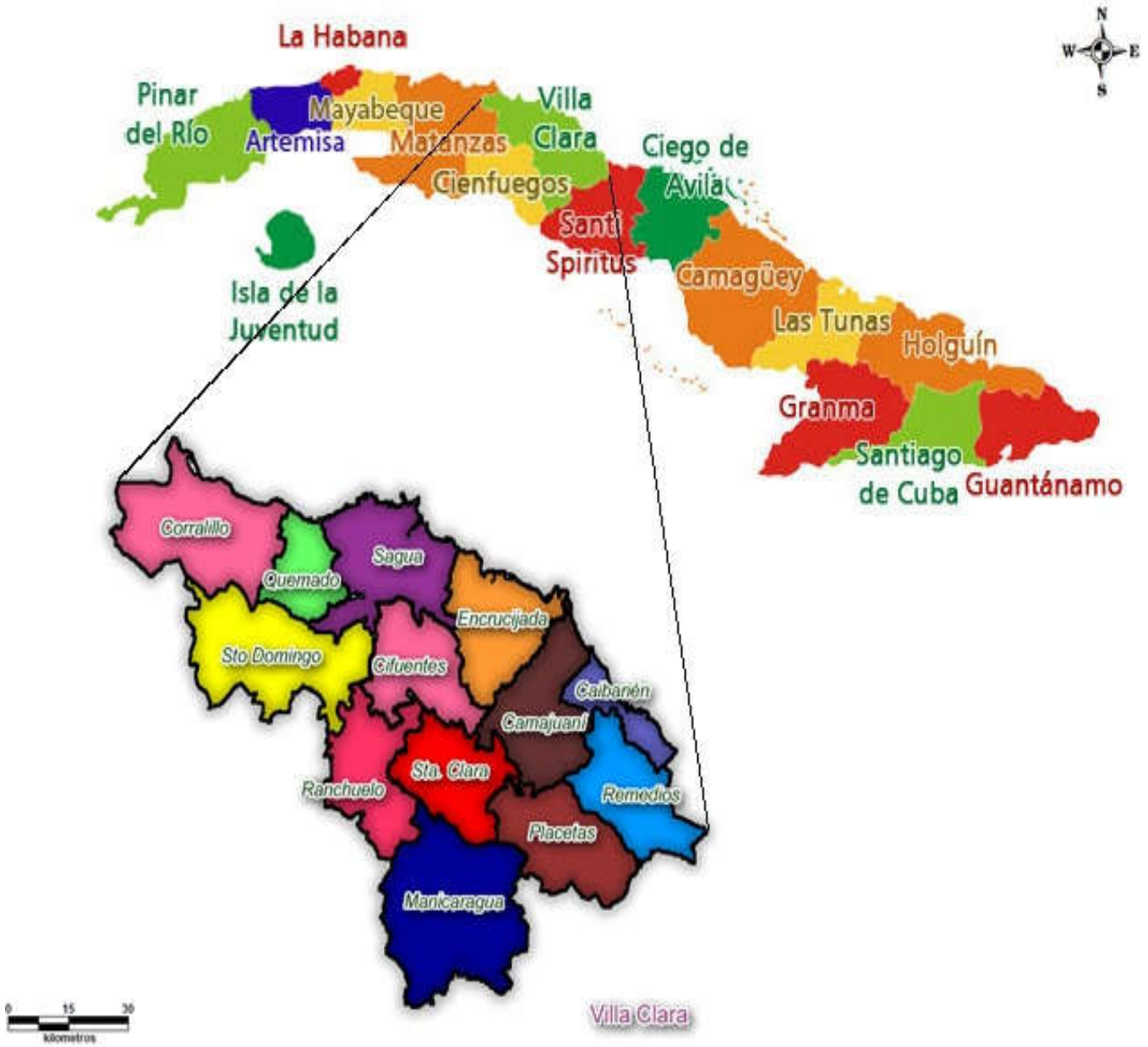

Figure 1.**Administrative Map of Cuba and Villa Clara province**

The sample

In Villa Clara province there are registered 304 permanent reservoirs with favorable conditions for breeding and spreading of culicids, distributed in the 13 municipalities; besides, there are identified nearly 218 temporal breeding grounds (active places in rain system), among themditches, swamps, depressions in low areas of the land, ponds and others. The permanent reservoirs (active during the whole year) are in rivers, lakes, streamsand ponds, etc. The main permanent breeding grounds of culicids were sampled, where there were identified species of larval fishes in existence; moreover, they were collected mosquitoes (larval stage), by ladle method (OMS, 1980) and adult stage (Method of Capture over humanbaitand Tramper) in 73 sampled reservoirs. In the case of mosquitoes species, they were identified by means of the stereo microscope using the following keys: The Culicids from Cuba (González, 2006), Pictorial Keyto Identifygenus of Cuban Mosquitoes in their Larval Stage (Méndez *et al*., 2005) and The Ixodides and Culicids from Cuba. Their Natural and Medical History (Pérez Vigueras, 1956). It was evaluated in laboratory conditions the depredating capacityof the copepod *Mesocyclops aspericornis* overmosquito larvae of the species*Culex quinquefasciatus*. In each flask 120 larvae of *Culex quinquefasciatus* were placed (glass flasks with 1 000 ml of dichloride water by distillation) and to each pair they were added 5, 10,15, 20 and 25 copepods respectively, in a period of 24 hours they were counted the rests of larvae indicators of deaths by depredation. Chemical and biological treatments were compared; one with *Bacillus thuringiensis,* another one with fishes and chemical treatment. The results in depredation experiment of the copepod *Mesocyclops aspericornis* were processed by means of a simple variance analysis (ANOVA), a parametric test of SNK post-ANOVA and polynomialregression. They were compared the proportions of positiveness by *Stegomyia aegypti* among the different cycles of the Intensive Campaign in 2006 by means of a logistic model and a proportional hypothesis test, for which it was used the statistic computerized package STATGRAPHICS Plus version 5.0.

RESULTS

According to the samples in the reservoirs they were identified 11 species of larvivorous fishes, being the municipalities of Sagua (10), Quemado and Caibarién (8), followed by Corralillo and Encrucijada (7), of the highest diversity, richness of species and representation. In relation to species abundance by municipalities, resulted in *Gambusia punctata* (13), *Girardinus metallicus* (13), *Poecilia reticulata* (13), *Limia vittata* (12) and *Cichlasoma tetracanthus* (10), the species with the widest distribution and highest ecological plasticity (table 1). From 11 species of identified fishes in the province, five are the ones which have the best bio regulator potentialities over mosquitoes larvae, this was determined based on the criteria described by Koldenkova and García (1990), which resulted in: *Gambusia punctata*, *G. puncticulata*, *Limia vittata, Girardinus metallicus*and*Poecilia reticulata,* representing 45.5% of total of identified species. In figure 2 they are shown the proportions of these species in the 13 municipalities, having no significant differences ($p > 0.1$) among the proportions, probably due tosmall size of sample. In table 2 they are shown 43 species of culicids identified in Villa Clara province. The commonest and widest distributed in the province were *Anopheles albimanus, Ochlerotatus mediovittata, Oc. taeniorhynchus, Psorophora confinnis, Culex quinquefasciatus, Cx. nigripalpus,* being present in the 13 municipalities, followed by *Oc. scapularis, Ps. ciliata* and *Cx. corniger* (12 municipalities) and lastly, *Uranotaenia sapphirina* (9).

Municipalities with greatest richness of species were: Remedios (31), Santa Clara (27), Placetas (26), Ranchuelo (20) and Camajuaní with (19), the rest had a similar distribution. From the total of culicids species available in the province, eight represent the greatest potential risk since epidemiological point of view: *Anopheles albimanus, Stegomyia aegypti, Ochlerotatus sollicitans, Oc. taeniorhynchus*, *Psorophora confinnis, Culex quinquefasciatus, Cx. nigripalpus* and *Uranotaenia sapphirina.* When comparing the proportions of these species in the municipalities of the province, there was no significant difference, for a trust level of 95 %. They could be also identified the proportions in high, with values higher than the mean (0.36) for Corralillo, Quemado, Sagua, Santo Domingo, Cifuentes, Encrucijada, Camajuaní, and Manicaragua and low, with values lower than the mean. As they can be observed, there were not significant differences ($p > 0.1$) among municipalities, due to small size of sample. Santa Clara, Ranchuelo and Placetas municipalities which have proportions 0. 259, 0, 35 and 0. 26 respectively have had focus for *St. aegypti* and in the case of the first, it keeps the establishedinfestation for such vector (Figure 3).

The use of copepods as a new alternative in the biological control has demonstrated to be an effective method that together with larvivorous fishes, sporogenous bacteria (*Bacillus thuringiensis israelensis* and *B. sphaericus*) and water insects put in our hands a whole arsenal in the fight againstmosquitos' larvae. In table 3 they are shown the results of depredation using ten copepods, against 40 and 60 larvae was effective, with 99. 4 and 99. 2 % respectively, even when they were added 80 larvae the per cent was high (99. 3 %), that is, for these three larval densities, the per cents do not differ from the per cents of depredation among them, and if there are differences among the percentages when increasing the number of larvae, so depredation is lower. At the same time, it was demonstrated that the quantity of ten copepods is not enough to depredate 150 larvae of *Culex quinquefasciatus,* havingno significant differences in the meanof depredation among densities of 100 and 150, but between this last one and the 80. The results indicate a tendency to the increasing of mortality of larvae by depredation when increasing the density of copepods, hence 20 and 25 copepods it was obtained 87 and 64. 16% oflarval mortality respectively, decreasingtill 22.16% against 5 copepods. ANOVA corroborates that these differences among the means of all treatments are significant, in ascendant order (figure 4). With all this analyzed it can be assumed that in a period of 24 hours, the depredating efficiency did not overcome the eight larvaeby copepods. In table 4 they are shown the results of comparing three treatments applied in the culicids control in Villa Clara. It can be observed that the price of Propoxur, which is higher than 11. 05 CUC per liter persists only during one or two days, because of that it has to be applied 12 to 16 times a year; besides, it has a danger of intoxication meanno for people and provokes some damages to natural fauna and companion. The *Bacillus thurigiensis* used to decrease mosquito populations has a costof 7.00 CUC per unitand persists during 7 to 60 days. Neither causes danger of intoxication nor damage to natural fauna.

Table 1. Species of identified fishes in fluvial ecosystems in Villa Clara province

	Species of Fishes											
Municipalities	1	2	3	4	5	6	7	8	9	10	11	Total
Corralillo	+	-		+	+	-	+	+	+	-	+	7
Quemado	+	-	+	+	+	-	+	+	+	-	+	8
Sagua	+	+	+	+	+	-	+	+	+	+	+	10
Encrucijada	+	-	+	+	-	-	+	+	+	-	+	7
Camajuaní	+	+	+	+	-	-	+	-	-	-	+	6
Remedios	+	-	+	+	-	+	+	-	-	-	+	6
Caibarién	+	-	+	+	+	-	+	+	+	+	-	8
Santa Clara	+	+	+	+	-	-	+	-	-	-	-	5
Placetas	+	+	+	+	-	-	+	-	-	-	+	6
Cifuentes	+	-	+	+	-	-	+	-	-	-	+	5
Santo Domingo	+	-	+	+	-	-	+	-	-	-	+	5
Ranchuelo	+	-	+	+	-	-	+	-	-	-	-	4
Manicaragua	+	+	+	+	-	-	+	-	-	-	+	6
Total	13	5	12	13	4	1	13	5	5	2	10	

Legend: +Presencia,-Ausencia, 1*Gambusia punctata*, 2*Gambusia puncticulata*, 3*Limia vittata*, 4: *Girardinus metallicus*,5*Girardinus falcatus*,6 *Girardinus microdactilus*, 7 *Poecilia reticulata*, 8 *Dormitator maculatus*,9*Cyprinodon variegatus*,10*Gambusia rizophorae*, 11 *Cichlasoma tetracanthus.*

Table 2. Species of identified mosquitoes in the 13 municipalities of Villa Clara

	Municipalities													
Genus and species	Corralillo	Quemado	Sagua	Encrucij.	Camaj.	Remedio	Caibarién	Sta Clara	Placetas	Cifuentes	St.Domin.	Ranchuelo	Manic.	Total
Anopheles albimanus	+	+	+	+	+	+	+	+	+	+	+	+	+	13
An. atropos					+	+								2
An. crucians						+		+				+		3
An. vestiti pennis			+		+	+	+	+	+		+			7
An. grabhamii						+	+					+		3
Stegomyia aegypti			+					+	+	+		+	+	6
Ochlerotatus mediovittata	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Oc. sollicitans	+		+	+	+	+	+			+	+			8
Oc.taeniorhynchus	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Oc.scapularis	+	+	+	+	+	+	+	+	+	+	+	+		12
Oc. totillis			+	+	+		+		+					5
Oc.condolescens	+					+		+						3
Psorophora ciliata	+	+	+	+		+	+	+	+	+	+	+	+	12
Ps. howardii						+	+	+	+	+		+	+	7
Ps. johnstonii						+								1
Ps. confinnis	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Ps. pygmaea	+			+	+	+	+		+	+			+	8
Ps. infinis								+						1
Ps.santamarinae						+								1
Ps.insularis						+								1
Culexquinquefasciatus	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Cx nigripalpus	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Cx bahamensis						+		+						2
Cx corniger	+	+	+	+	+	+	+	+	+	+		+	+	12
Cx sphinx						+								1
Cx secutor								+					+	2
Cx chidesteri	+	+				+		+	+		+	+		7
Cx. americanus						+			+					2
Cx atratus				+	+	+		+	+	+				6
Cx. erraticus				+	+	+	+	+	+	+	+	+	+	10
Cx. pilosus	+		+	+	+	+		+					+	7
Cx.*iolambdis*								+	+					2
Cx. ocossa								+				+		2
Cx. cancer	+				+	+								3
Uranotaenia sapphirina			+	+	+	+	+	+	+		+	+		9
Ortophodomyia signifera								+						1
Wyeomyia mitchelli								+	+					2
Wy. vanduzeei									+					1
Mansonia titillans			+			+		+	+	+	+	+		7
Coquilletidea nigricans									+		+			2
Toxorhynchites portoricensis					+	+								2
Aedeomyia scuamipennis									+			+		2
Limatus durhamii									+			+		2
Total	15	10	16	16	19	31	17	27	26	16	15	20	14	

Legend: Encrucij. Encrucijada, Camaj. Camajuaní, Sta Clara. Santa Clara, St.Domin. Santo Domingo and Manic. Manicaragua

Table 3. Results of the counting of exoskeletons of mosquitoes with different densities, at the end of 24 hoursof depredation with 10 copepods

Number of Larvae	Percentages of Predation	Means of Predation	Standard Error
40	99.4 a	39.7 d	0.88
60	99.2 a	59.5 c	0.88
80	96.3 a	77.0 b	0.88
100	80.0 b	81.2 b	0.93
150	53.8 c	80.7 a	0.98

(a, b, c, d): Mean Values with uncommon letters differ by Bonferroni a (P< 0. 05).

Table 4. Analysis of cost/risk of three treatments used in the control of mosquitoes larval populations in Villa Clara

Parameters to compare	Propoxur	*Bacillus thuringiensis*	Larvivorous Fishes
Cost per Unit (CUC)	11.05 Lt	7.00 Lt	0
Persistence	48 – 72 hours	7 to 60 days	Permanent
No applications per year	12 - 16	3 - 6	1
Danger of intoxication	Meanno	None	None
Damage to natural fauna and companion	Yes	None	Minimum

Natural Fauna: it refers to useful insects like bees and butterflies.
Companion Fauna: it refers to organisms with bio regulator potentials like nematodes, water insects and Copepods.

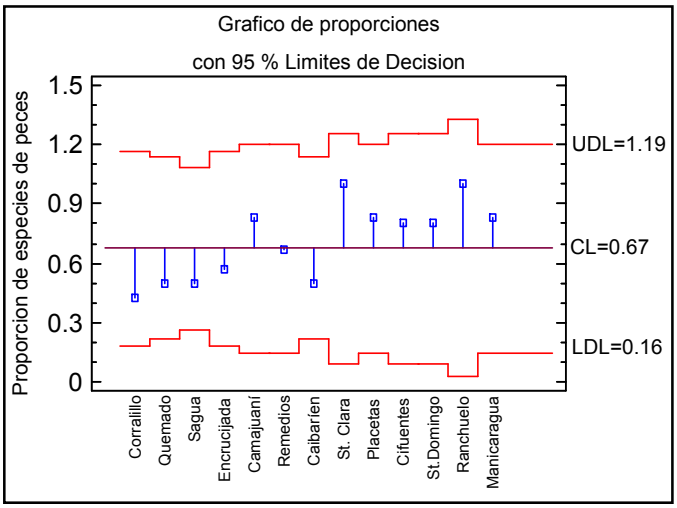

Figure 2. Proportions of bio regulator fishes species in Villa Clara municipalities.

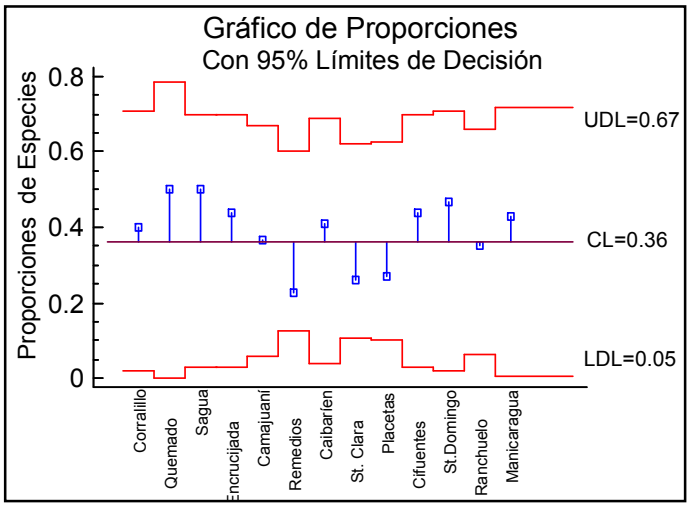

Figure3. Proportions of main species of mosquitoes in Villa Clara municipalities (p >0.1) for a trust level of 95%.

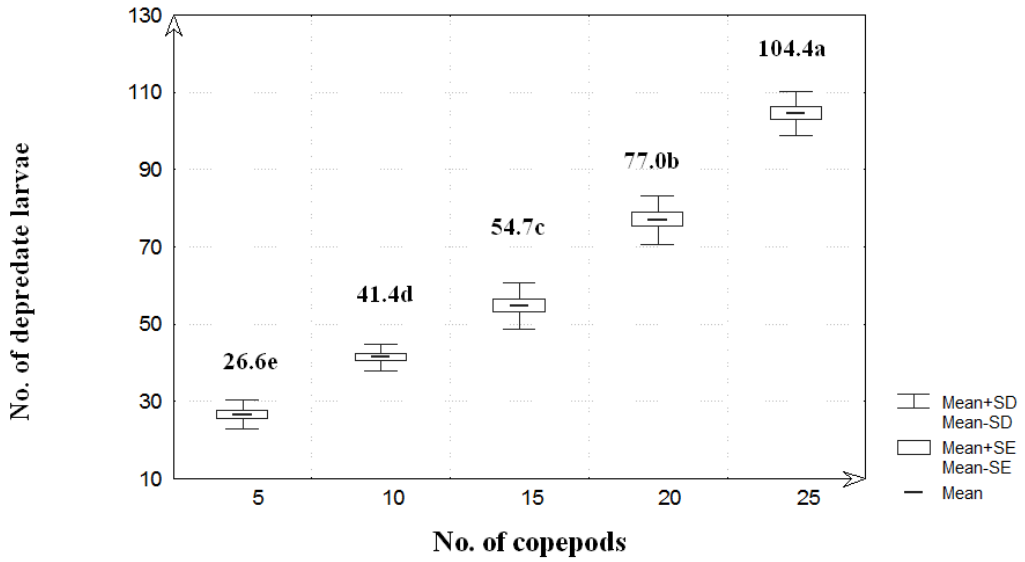

Figure 4. Variation of mosquitos' larvae quantity depredated according to density of copepods in the experiment

It was confirmed that larvivorous fishes constitute the cheapest treatment, present a permanent persistence, and then it has to be applied once a year. They neither cause any danger of intoxication to people nordamage to biodiversity, if endemic species are used.

DISCUSSION

In relation to larvivorous fishes species collected in the province, constitute the poecilids of highest representation and even most diverse and with greatest richness of species among the fluvial biocontrollers, these results obtained by Hernández (1999). However, in this investigation it was confirmed the decreasing of population densities of the endemic poecilid *Gambusia puncticulata,* this has been pointing out several years ago in other provinces of the country (Sancti Spiritus and Camagüey) (Fimia *et al.*, 2003), all seem to point out to a multicause in the effect previously said; where the introductions of alloctonous fishes play a fundamental role done by the aquiculture with alimentary purposes, especiallyTilapias, Tenca and the Claria (Quesada,2000). About the species of fluvial fishes collected, it has to be highlighted that our province has an active ictiological potential, but it is necessary to implement all the scientific-technological knowledge for a consequent and optimumfluvial ictiocontrol mosquitoes larvaewith greatest entomoepidemiological implication, prioritizingalways endemic species(OMS, 1982). Because of the quantity of species with best bio regulator potentialities, all municipalities have conditions to face such epidemic situation of vectorial transmission by mosquitoes since they have an acceptable coveringand representation. According to this investigation, Villa Clara province has 63.2 % of culicids species reported in Cubaby Rodríguez *et al*. (2005). Their distribution is similar to the one reported by Marquetti *et al*. (1999) and Aguilera *et al.* (2000) for Havana city and with Rodríguez *et al*. (2006) for Villa Clara province. This investigation has great importance, because it allows to know the mosquitoes species in existence, and relate them with the rest of the zoological groups in the province like birds, equines, bats, etc. the knowledge is enriched to elaborate control programs directed topopulation decreasing of such vectorsand; hence the diseases they transmit (Acha y Szyfres, 2003; Charrel *et al*., 2007).

The eight species of culicids present in the province which represent the highest potential risk since epidemiological point of view (*Anopheles albimanus, Stegomyia aegypti, Ochlerotatus sollicitans, Oc.Taeniorhynchus, Psorophora confinnis, Culex quinquefasciatus, Cx. nigripalpus* and *Uranotaenia sapphirina*) are involved in transmitted infectious diseases such as Malaria, Dengue, Fever of theEastern Nile, yellow Fever, Filariosis and EquineEncephalitis. In 2007, they were reported in Villa Clara eight cases of Malaria by *Plasmodium falciparum*: six in Ranchuelo municipality, one in Santo Domingo and one in Santa Clara.Moreover, in Cuba they are reported three positive cases of infection in humans by Eastern Nile Virus, two in Jatibonico (Sancti Spiritus municipality) and onein Caibarién, municipalityfrom Villa Clara (Cruz and Cabrera, 2006). Another classic example is the re infestation of *Stegomyia aegypti* in 2006, that transmitted serotypes of Dengue virus that affected the whole island of Cuba. According to the results in this investigation, it was confirmed that *Me. aspericornis* resulted in an active depredator of first stage larvae of *Cx. quinquefasciatus*, the depredation was manifested dense-dependentin relation to depredator *Me. aspericornis*, the increment in densityof the depredator was proportional to the number of consumed larvae. The 87% of larval mortality in a period of 24 hours is higher than the obtained by Brown *et al*. (1991) where mortality of 25 first stage larvaeof*Cx. quinquefasciatus* per liter against 25 adult copepods of the specie *Me. aspericornis* during 72 hours was of 81 % under laboratory conditions.

Kay *et al*., 1992 refer a mortality of 70% of 200 larvae of *Cx. quinquefasciatus* against 25 specimens of *Me aspericornis*, although with thespecie *Me. longisetus* was obtained 86% of mortalityfor equal larval density, explaining that can be due to the size of depredator, *Me aspericornis*has nearly smallersize (1.5mm) compared to*Me. longisetus* (1. 62mm). The depredator activity of 10 copepods over different larval densities increased proportionally up to the quantity of 80 larvae of *Cx. quinquefasciatus* per copepod in 24 hours it is found in the reported range by other authors (Kay and Nam, 2005; Holynska, 2006; Marten and Reid, 2007) that consider an average of 2 to 30 consumed larvaeby day depending on the copepod specie. The results of the evaluation of cost and risk of chemical and biological treatments confirmed the results obtained by Mathur in the year 2003 and Boyce and Brown (2003), that biological control with fishes is superior, more effective and economical than chemical controls. The use of endemic fishes does not need capital to acquire raw material to be used, which differs from the other treatments applied; this is mainly due to these fishes species are breaded in a wild wayin natural sources and then are captured and sown in artificial sources that most of timesenable the spreading of mosquitoes. Together with this are the sporogenous bacteria, the micro crustaceans' copepods, water insects and nematodes; as they put in our handsthe whole arsenal in the fight againstmosquito's larvae. It is concluded that the species of larvivorous fishes with highest bio regulators potentialities are *Gambusia punctata*, *Girardinus metallicus* and *Limia vittata,* being Sagua, Quemado, Caibarién, Corralillo and Encrucijada the municipalities with greatest availability, in fishes species, as well as in abundance and distribution. It is confirmed that the species *Cyprinodon variegates* and *G. puncticulata* are the best bioregulators qualities possessed about the coast larval populations of mosquitoes, which coincides with the results obtained by Menéndez *et al*. (2007). It was evidenced the use of biological organisms in the regulation of mosquitoes larval populations is relativelyeasy, of low cost and does not cause damage to the environment.

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