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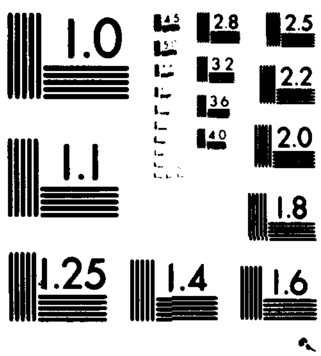

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A pilot field test of a genetically-induced sterile male was concluded, with the cooperation of Navy Environmental and Preventive Medicine Unit #2, Norfolk Naval Base. The experimental design involved low-level releases of males carrying double translocations with the aim of eliminating or, at least inhibiting rapid re-growth of pockets of cockroaches left after routine insecticide treatment. The results were positive in that the males were easily introduced into natural groups, within which they competed well against wild-type males. However, it also became apparent that the effective → next page

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use of these males would require a more thorough understanding of natural populations. The work conducted during the past year involved the initiation of some of these studies, along with preparation of data from the sterile-male experiment for publication. These studies are summarized below under each of the 3 objectives stated in the Contract for this year's research.

Objective 1 - To complete the sterile male experiment. The data have been analyzed and 3 manuscripts prepared for publication. That describing the development and properties of the sterile males has been published; that concerned with population estimation is in press; and that describing the results of the experiment has been submitted for publication.

Objective 2 - Behavior within harborages. Laboratory experiments have been conducted that show the distribution of mixed age groups within a harborage area. Density of the aggregation was affected by the reproductive state of the females. Adult females tended to stay within the harborage, whereas about 30% of the small nymphs were outside. At higher female density, a low frequency of non-egg case bearing females was found outside the harborage, although this was rarely the situation with egg-case bearing females. A feeding experiment that showed cyclic feeding of adult females provided a possible explanation of differences in the behavior of non-egg case bearing vs egg-case carrying females. It showed that females do not have to forage for food to any significant extent during embryogenesis.

Objective 3 - Population behavior. Preparations were made for lab and shipboard experiments on dispersion and mixing between groups in different harborages. Mutants to be used as markers in these studies were crossed to cockroaches from a shipboard-collected strain and a pure mutant strain re-isolated. Mutants from the re-isolated strain are being used in lab experiments on dispersion, and will likewise be available for shipboard experiments. Catch of jar type vs floor-entrance traps (Roatel) was compared in order to select the most effective trap for catching small nymphs. The floor-entrance type caught 4X more small nymphs than the mason jar type, although other age classes were caught with equal frequency.

OFFICE OF NAVAL RESEARCH

Contract #N00014-77C-0246

Task No. NR 205-028

ANNUAL REPORT NO. 3

The Use of Genetic Mechanisms and Behavioral Characteristics to Control Natural Populations of the German Cockroach

by

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March 1981

This research was supported in part by the Office of Naval Research,
Microbiology Program, Naval Biology Project under Contract N00014-77-C-0246.

The preferred environment of the German cockroach, _Blattella germanica_ (L.), is that favored by man. It thrives particularly well where there are large food preparation and serving areas. Given these conditions, as well as close to optimal levels of warmth and humidity, it is hardly surprising that this pest is difficult to deal with in a shipboard environment. The Navy has instituted well thought out control strategies combining cultural and mechanical management procedures with chemical control. Of necessity, it depends heavily on the latter. However; there are dangers inherent to the use of insecticides; specifically, the possibility of environmental contamination and the development of unmanageable levels of resistance. Thus, the search for new weapons that might help to reduce the frequency and/or amount of insecticide use is an important effort. ONR support for this type of research has led to the development and testing of a genetic mechanism as part of a cockroach control program. The results of a pilot experiment showed (1) the genetically-altered males joined and mated successfully within natural groups and (2) their effective utilization requires a refinement of the technique, based on a more thorough knowledge of natural populations. The work summarized here is the first year of a 3-year study designed to achieve the latter goal. We anticipate that the information gained through this effort will be useful to all approaches to cockroach control.

The work planned for the year covered by this Annual Report falls under three objectives, as stated in the Contract renewal proposal. Progress under each of these objectives is summarized below.

Objective 1 - <u>To complete the sterile male experiment</u>

This objective has been accomplished. The data from the experiment on the USS Papago have been analyzed. None were forthcoming from the USS Boulder since the cockroach collections were apparently lost in transit. As predicted, the genetic control experiment is giving rise to three publications:

1. Ross, M. H. and D. G. Cochran. Synthesis and properties of a double translocation heterozygote involving a stable ring-of-six interchange in the German cockroach. J. Hered. 72 (1):39-44. 1981.

This paper describes the properties and synthesis of the "sterile" double translocation-carrying males that were used in the genetic control experiment. It emphasizes properties such as male competitiveness, viability, and sterility, as they relate to the use of this mechanism for control purposes. The crossing systems used to develop and maintain a continuing supply of "sterile" males are also described. Approximately one sterile male/every 2 matings between parental translocation stocks was obtained from the first egg cases; this dropped to 1/every 3 females for 2nd egg cases. Saving females for 3rd egg cases did not seem worthwhile in view of a much lower productivity from hatch of these later egg cases.

Reprints of this paper were forwarded recently.

2. Keil, C. B. Structure and estimation of shipboard German cockroach (Blattella germanica L.) populations. Environ. Ent. (in press). 1981.

This paper presents data on age class distribution in collections from the USS Papago and the USS Boulder that were made prior to the sterile male releases. It is one of the few studies to incorporate observations on immature stages. Differences between populations are reported and discussed in light of carrying capacity of the environment and factors that may be instrumental in determining this capacity. The data show male nymphs to undergo 5 instars, in contrast to 6 in females. Assumptions basic to population estimation by a removal method of statistical analysis are discussed and recommendations made for meeting a better fidelity to these in the future. Proper acknowledgement is, of course, made for ONR support and the cooperation of Navy Preventive Medicine Unit #2, specifically that of the entomologists, CDR McDonald and Lt. Egan.

3. Ross, M. H., C. B. Keil, and D. G. Cochran. The release of sterile males into natural populations of the German cockroach. Submitted to Entomologia exp. & Appl. Feb., 1981.

A first draft was forwarded to ONR last spring. Alterations in the manuscript since then involve adding refences, a little additional data on oothecal size (compartments/ootheca), and polishing. One thought added to the discussion was that the high degree of mating success of the released males could reflect two characteristics: (1) full competitiveness, as demonstrated from the laboratory evaluation studies, and (2) an enhanced pheromone response due to the fact they were maintained separately from females up until their release. (Bell et. al., J. Chem. Ecol. 4:495-501). The results of this experiment were also summarized as part of a symposium paper at the International Congress of Entomology (Kyoto, August 1980). The symposium is to be published in book form through the auspices of Dr. Tozo Kanda, St. Mariannas University, Kawasaki, Japan.

Two aspects of the genetic control experiment that were of primary significance are emphasized in both the manuscript for publication and the preceding reports, i.e., (1) the ease with which genetically-altered, laboratory-bred males were introduced into the natural groups and (2) that sterility acted as a genetic marker, and it was this that gave insight into various aspects of group behavior and dynamics. The latter analyses led to the research planned under Objectives 2 & 3. It appeared that there was more reproductive isolation between groups within different harborages than anticipated, that egg-case bearing females tended to remain close to or within a given harborage, and that infestation of new harborages following insecticide treatment was largely by nymphs. These and other aspects of group behavior are under further investigation. A third aspect of the data from the experiment that we have not heretofore noted is its value for comparative studies that give added insight into group dynamics and behavior. For example, in other environmental situations where populations were at or near carrying capacity (Sherron, Wright, Ross, and Farrier, ms. subm.

Proc. Entomol. Soc. Wash.), the proportion of non-egg case bearing females and mean size and variability of the egg cases (eggs/egg case) differed from that of the experimental groups on shipboard. In Sherron's data, mean oothecal size ranged from 37.0-39.7, essentially identical to egg cases classes as 1st & 2nd in the shipboard study (Fig. 1). It appears that under stresses which severly limit growth, the females only produced one or two egg cases, in contrast to many that lived to produce 3 or 4 in the more favorable environment on shipboard (below carrying capacity). This would account for both the smaller mean size and greater variability of egg cases from the shipboard collections. This type of comparison, together with data from the feeding experiment (Obj. 2), and data from studies planned under Obj. 3 are needed to reach an understanding of factors that regulate population growth. In the German cockroach, it appears that primary regulation is at the level of egg case formation, with nymphal mortality being of secondary importance (Sherron, Wright, Ross and Farrier, unpubl.). Another value to collating these several types of data is that it may be possible to devise a standard for determining whether a population is growing or whether it is stabilized at the observed level.

Objective 2 - Behavior within harborages

Two experiments have been conducted under this Objective. In one, the behavior of mixed age groups within a harborage area was examined; in the other, feeding habits of gravid females were investigated. Both experiments furnish information relevant to studies of shipboard populations planned for the summers of 1981 & 1982.

The within-harborage laboratory experiments used a harborage area consisting of 4 side-by-side shelters made from black construction paper and placed sub-centrally in a 22.5L (5 gal.) glass aquarium. All experiments used 4 adult males, 16 medium-sized nymphs (N), and 16 small nymphs (n). Adult females were varied as to density (4, 8 or 16) and reproductive stage (no ootheca; new ootheca, Stage I; and mature ootheca, Stage IX-XII). Groups within each shelter and those outside were censused after the cockroaches had had time to settle in their new environment (4 days). Shelters were never re-used. Each experiment was replicated 6-8 times. On advice of a consultant statistician, the results are being analyzed after arranging the data according to number/shelter, i.e., shelter I-that contain the largest number; II-the next largest, etc. This reveals whether there is one primary aggregation, which is not apparent if a large group occurs in one shelter in 1 replicate but in different shelters in other replicates.

Work yet to be completed on these experiments includes (1) the statistical analyses and (2) sufficient data from a shipboard-collected strain for comparison to that using the laboratory wild-type strain. Nevertheless, there are effects evident in the data thus far summarized that are worthy of note. Data from the experiment using non-oothecal-bearing females (Fig. 2) and females with immature oothecae (Fig. 3) serve to illustrate some of these findings. For example, certain general characteristics were evident in all experiments:

1. Outside the shelters (Figs. 2 and 3, V) the largest age class was small nymphs (n); least frequent and/or absent were the adult females.

2. Groups with adult females always contained representatives of all other age classes, including adult males.

3. Within shelters, a few small groups were entirely nymphal, but adult males were found only in shelters with adult females.

4. The only occurrence of adult males where no adult females were present was outside the shelters (V).

Characteristics related to the reproductive state of the females, female density, or a joint effect of both, are as follow:

1. Lowest density clumps, i.e., greatest spread through the 4 shelters (I-IV), occurred at the higher densities of non-egg case bearing females (Fig. 1, 8 & 16♀).

2. A single, high density cluster occurred in all experiments with oothecal-bearing females (Fig. 2 I) and also where there were only a few non-oothecal bearing females (Fig. 1 I - 4).

3. At the higher densities (8 & 16), a few non-oothecal bearing females were found outside the shelters, but oothecal-bearing females were rarely outside.

It appears that female behavior varies with respect to reproductive stage and to female relative density. This, in turn, may affect the behavior and distribution of other members of the harborage group. On the basis of these preliminary data, it appears that adult females may play a much larger role in governing cockroach populations than was previously suspected.

A second study was conducted on the feeding habits of gravid females of the German cockroach. The idea was to gain an insight into the food requirements of such females over the course of their reproductive lives. This kind of information could have a bearing on female foraging activities which may in turn relate to the opportunity for exposure to insecticide treatments.

A series of mated adult females were set up in individual cages provided with food, water and a small harborage. The food was weighed on a daily basis over the entire reproductive life of the females. Observations were also made daily on reproductive status. It was found that much individual variation occurs with respect to food consumption. However, a clear pattern emerged in spite of this variability. Adult females consume at a high rate for several days just prior to production of an egg case. Thereafter they consume food at a very low rate or not at all until the egg case hatches.

Immediately after hatch, the females again consume food at a high rate. This cyclic feeding behavior continues until the female dies.

On the basis of these results, one could suggest that adult females eat at a high rate of consumption to support egg case production and at a very low rate for body maintenance. This conclusion indicates that the female does not have to forage for food to any significant extent during embryogenesis. If water consumption follows this pattern, then it appears that gravid females could remain deep within harborages throughout much of the egg-case incubation period.

Objective 3 - Population behavior

Work under this objective was planned for the next 2 years of this Contract. It will involve studies of dispersion and mixing between groups in separate harborages and will require the use of mutant markers. During this past Contract year, we have prepared for these studies by crossing laboratory-reared mutants to cockroaches from shipboard strains and re-isolating the pure mutant strains from the F_2 generation. Mutants from the re-isolated strains will be used in shipboard studies planned for the summers of 1981 & 1982. This guards against genetically-based differences in behavior that might evolve through long-time rearing in the lab, although the existence of such has not been documented. Rather, the ease with which genetically altered, lab-reared males were introduced into the shipboard populations argues against genetic differences, at least those that might affect mating behavior. In order to make the laboratory studies on dispersion as comparable as possible to those planned for the summer, we are utilizing mutants from the re-isolated strains here too. Dispersion experiments have been initiated recently by Mr. Bret, the graduate student supported on this Contract. A second tool for use in the dispersion experiments is being developed by Mr. Bret, with the help of Dr. John Eaton. This is a type of actograph that will record the number of individuals leaving and entering a particular area.

The studies of natural populations planned for the summer will require a trap that will effectively catch small nymphs. A trap with floor-type entrances (Raotel®) was tested against a mason jar trap in order to select the better of the two types (Ross 1981). The Roatel caught about 4X as many 1st & 2nd instars as the jar type, although no difference was apparent in the other age classes (Table 1). A second experiment showed water is an effective bait in situations where this resource is limited, but this is hardly useful information for most shipboard situations.

PUBLICATIONS/MANUSCRIPTS PREPARED DURING THIS CONTRACT YEAR[a]

Keil, C. B. Structure and estimation of shipboard German cockroach (Blattella germanica L.) populations. Environ. Entomol. (in press). 1981.

Ross, M. H. Genetics and Cytogenetics of the German Cockroach. Abstracts XVIth Internat. Congr. Entomol., Kyoto, Japan. p 468. 1980.

Ross, M. H. Trapping experiments with the German cockroach. Blattella germanica (L) (Dictyoptera:Blattellidae), showing differential effects from the type of trap and the environmental resources. Proc. Entomol. Soc. Wash. 83:160-163. 1981.

Ross, M. H. and D. G. Cochran. Synthesis and properties of a double translocation heterozygote involving a stable ring-of-six interchange in the German cockroach. J. Hered. 72(1):39-44. 1981.

Ross, M. H. and D. G. Cochran. Differential effects of duplication-deficiency gametes from T(8:12) on embryonic development in the German cockroach. J. Hered. 72:57-59. 1981.

Ross, M. H., C. B. Keil, and D. G. Cochran. The release of sterile males into natural populations of the German cockroach. Subm. Entomologia Exp. & Appl. Feb., 1981.

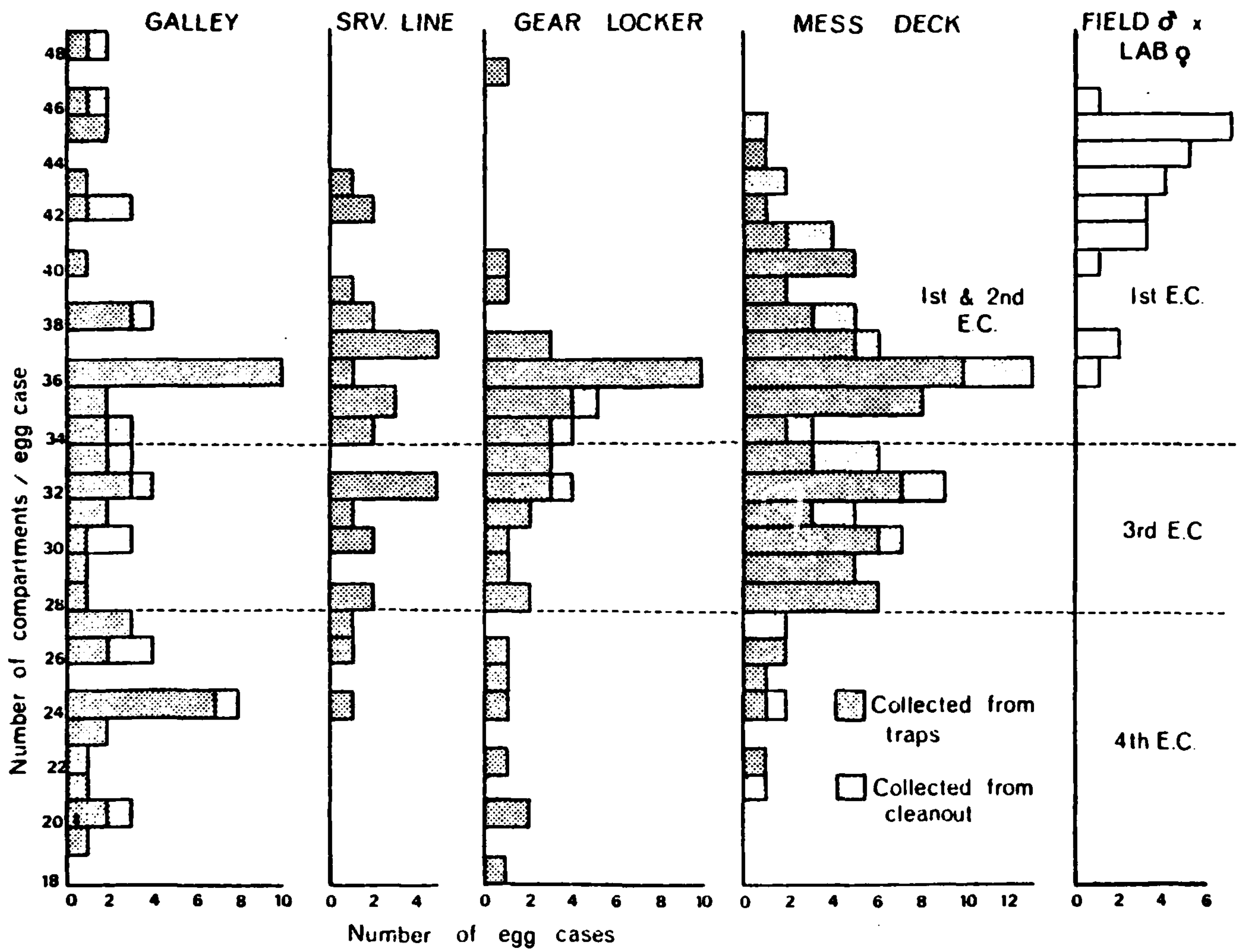
[a]Eight other publications of earlier research on this Contract have been listed in preceding Annual Reports.

Table 1. Collections of German cockroaches from roatel vs jar-type traps.

Type of trap	Collection period	Number of cockroaches: Adults	Nymphs[a]: L	M	Sm	Total
Roatel	2 wks	29	33	36	127	235
	2 wks	34	16	24	55	129
Jar	2 wks	30	44	45	29	148
	2 wks	25	36	37	15	113

[a]Nymphs grouped as large (L), medium (M) or small (Sm).

Figure 1. Egg cases grouped as to approximate age, i.e., 1st and 2nd, 3rd or 4th egg cases, on the basis of the number of compartments (eggs)/egg case. The first 4 columns are from shipboard-mated females; the last column shows 1st egg case of laboratory wild-type females mated to field-collected males.



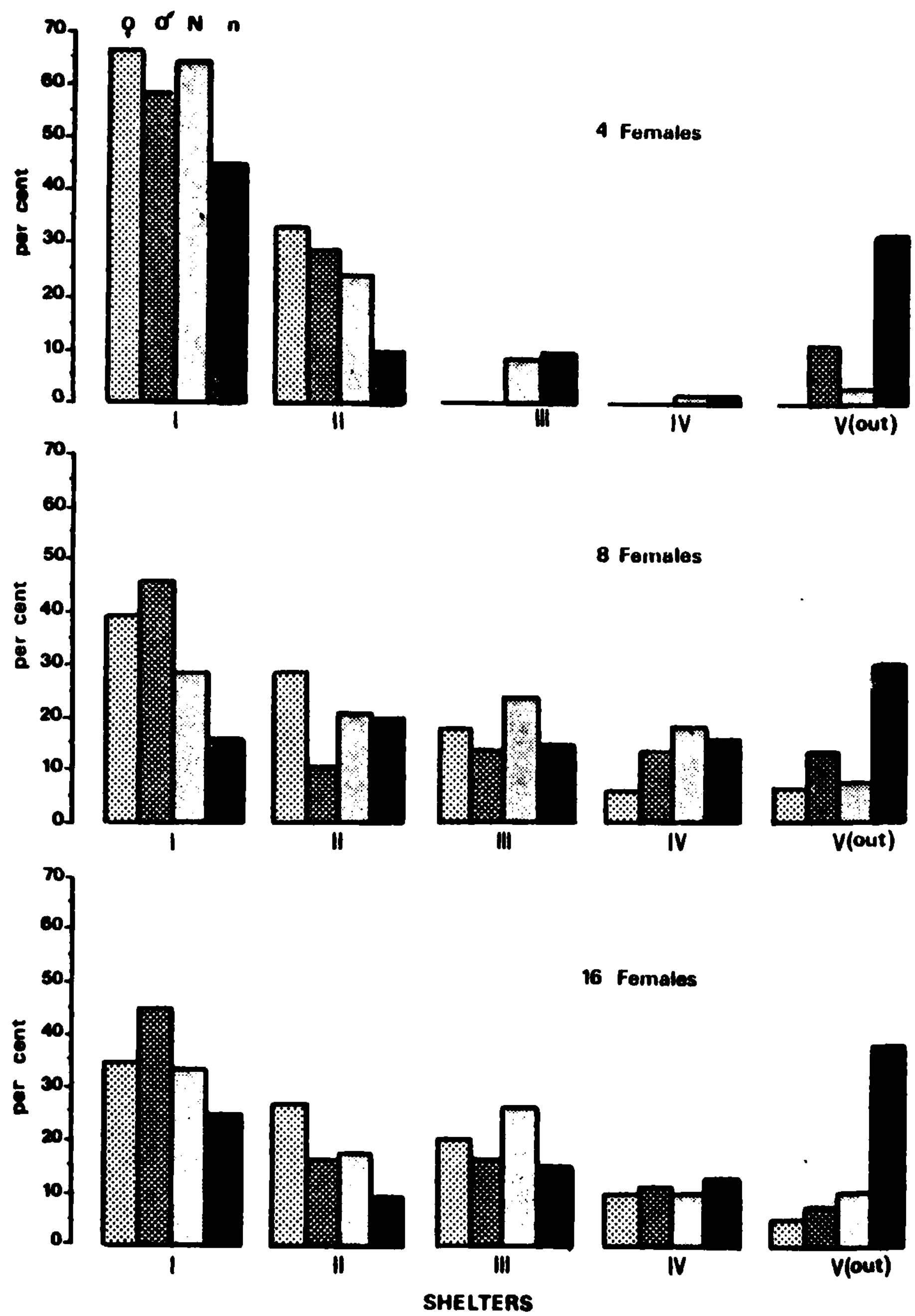


Figure 2. Distribution of a mixed age group containing non-egg case bearing females within a harborage area consisting of four side-by-side shelters (I-IV) and outside the shelters (V). Age classes within shelters from left to right are as follow: adult females (♀); adult males (♂); medium-sized nymphs (N) and small nymphs (n).

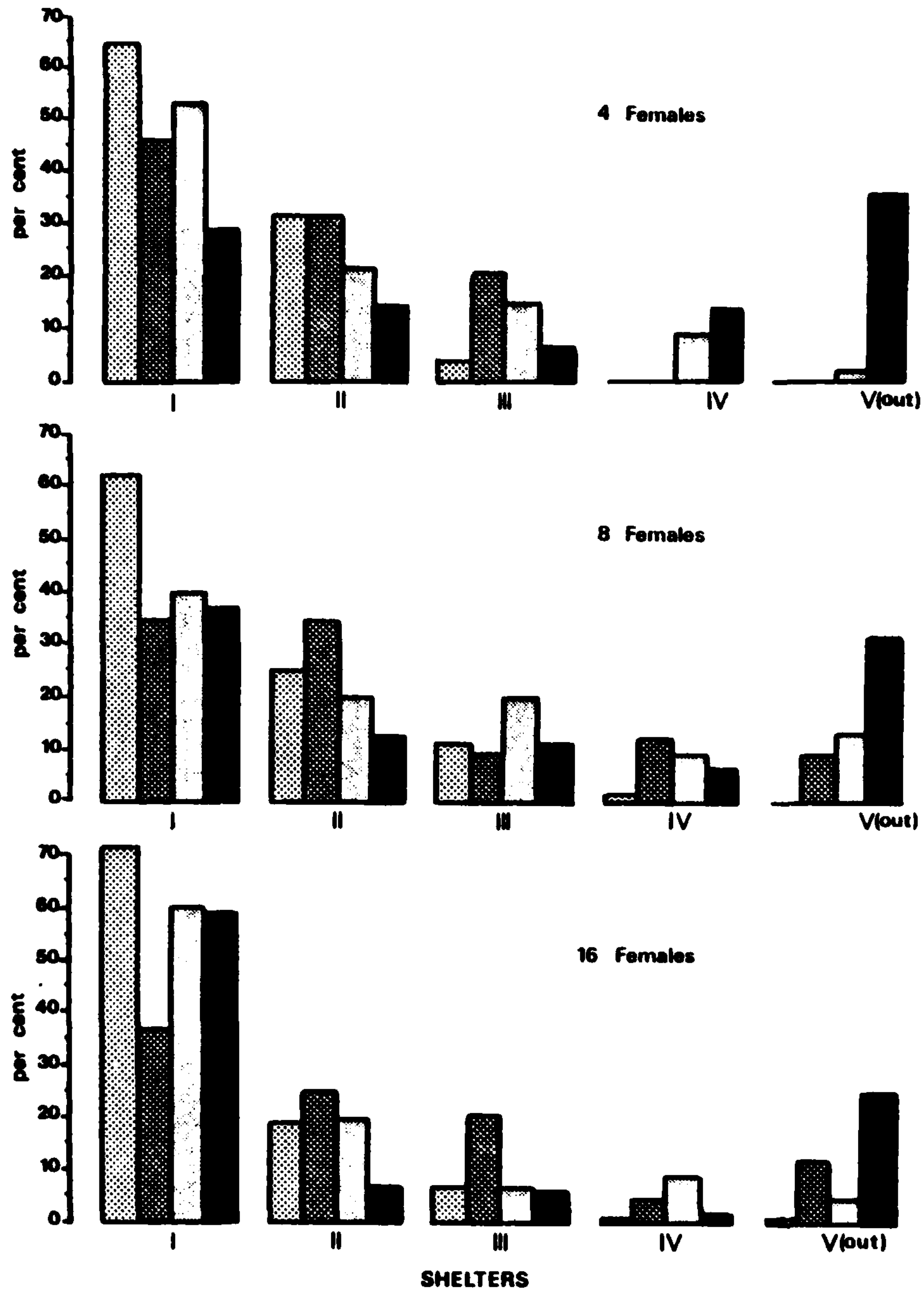


Figure 3. Distribution of a mixed age group containing females with immature egg cases (Stage I) within a harborage area consisting of four side-by-side shelters (V). Age classes within shelters as in Figure 2.