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# A comparison of a new centrifuge sugar flotation technique with the agar method for the extraction of immature *Culicoides* (Diptera: Ceratopogonidae) life stages from salt marsh soils[1]

# Сравнение нового метода центрифугирования и флотации в растворе сахара с агаровым методом для учёта преимагинальных стадий *Culicoides* (Diptera: Ceratopogonidae) из грунтов солёных маршей

**Daniel L. Kline**
**Дэниэл Л. Клайн**

Center for Medical, Agricultural and Veterinary Entomology, United States Department of Agriculture / Agricultural Research Service (USDA/ARS), 1600/1700 SW 23rd Drive, PO Box, Gainesville, Florida 32604, USA. E-mail: dan.kline@ars.usda.gov

KEY WORDS: *Culicoides*, immature stages, extraction techniques, agar, centrifuge, sugar flotation.

КЛЮЧЕВЫЕ СЛОВА: *Culicoides*, преимагинальные стадии, методы учёта, агар, центрифугирование, флотация в растворе сахара.

ABSTRACT. Two sampling techniques, agar extraction (AE) and centrifuge sugar flotation extraction (CSFE), were compared to determine their relative efficacy to recover immature stages of *Culicoides* from salt marsh substrates. Three types of samples (seeded with known numbers of larvae, homogenized field samples with approximately equal numbers of immatures, and regular field samples with an unknown number of immatures) were evaluated. Two substrate sample sizes were used (100 ml and 1000 ml). Agar extraction was used with both sample sizes, but CSFE was only used with the 100 ml size. The seeded samples were inoculated with 3rd–4th instar larvae at 5 levels (0, 25, 50, 100 and 200 larvae / sample). Mean percent recovery was similar at all inoculation rates within each extraction technique, but the CSFE recovered 95.7% compared to 35.3% for AE1000 and 16.7% for AE100. For the homogenized samples, on a per sample basis, the AE1000 method recovered about twice more immatures than the CSFE, but on a mean number of immatures recovered per ml basis, CSFE recovered 5.2 times more than AE1000. Similarly, for the regular field samples, on a per sample basis the AE1000 method recovered ca. 1.5 times more immature stages than samples processed by CSFE, but on the immatures per ml of substrate basis the CSFE method recovered ca. 6.6 times more than AE1000. Overall, on a per ml basis the AE100 and AE1000 recovered an approximately equal number of immatures. The CSFE technique proved to be advantageous because greater numbers of immatures were collected with less substrate. As a result, this technique allowed more samples to be taken over a larger area with less effort. Also larvae were recovered from the samples in hours instead of days. Thus, more information on the spatial dynamics of *Culicoides* immature populations may be obtained in less time using the CSFE technique. A major disadvantage of this method is that a fairly expensive centrifuge is required for processing samples.

РЕЗЮМЕ. Проведено сравнение двух методов учёта преимагинальных стадий *Culicoides* — экстракции с помощью агара (AE) и метода центрифугирования и флотации в растворе сахара (CSFE). Сравнение проводилось с целью оценки сравнительной эффективности этих методов для извлечения преимагинальных стадий *Culicoides* из субстратов солёных маршей. Для работы были использованы 3 типа проб: (1) очищенные от *Culicoides* субстраты, в которые затем было помещено точно известное число личинок 3–4-го возраста (0, 25, 50, 100 или 200 на каждую пробу); (2) гомогенизированные пробы, содержащие приблизительно равное число личинок и куколок; (3) обычные пробы, собранные в природе и содержащие различное неустановленное число личинок и куколок. Кроме того, использовались 2 типа проб по объёму субстрата (100 мл и 1000 мл). Экстракция агаром применялась для обработки проб обоих размерных групп, а метод CSFE — лишь для проб объёмом 100 мл. Для проб, содержащих точно известное число личинок, средняя доля особей, экстрагируемых с использованием каждого метода, не зависела от общего числа личинок в пробе. При этом метод

[1] This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

CSFE позволил учесть 95,7% от общего числа личинок, а метод AE — лишь 35,3% и 16,7% особей из проб объёмом 1000 и 100 мл, соответственно. Для гомогенизированных проб метод AE1000 позволяет учесть вдвое большее число особей из пробы, чем метод CSFE100, но в расчёте на единицу объема пробы эффективность второго метода в 5,2 раз выше. Аналогично этому, для обычных проб метод AE1000 позволяет учесть в полтора раза больше личинок из каждой пробы, чем метод CSFE100, но в расчёте на единицу объёма второй метод в 6,6 раз эффективнее.

В расчёте на единицу объёма субстрата, метод AE позволяет учесть примерно равное число личинок, вне зависимости от объёма пробы (100 или 1000 мл). Метод CSFE более эффективен, поскольку позволяет учесть большее число личинок из меньшего объёма субстрата. Этот метод позволяет с меньшими усилиями собрать большее число проб с большей территории, а также извлечь личинок в течение нескольких часов, а не дней, и, таким образом, получить более детальную информацию о популяциях мокрецов в более короткие сроки. Основной недостаток метода CSFE состоит в том, что для обработки проб необходима дорогостоящая центрифуга.

## Introduction

Biting midges of the genus *Culicoides* Latreille, 1809 are pests of man in coastal areas throughout the World because of their annoying attacks and high numbers. These attacks can make outdoor activities impossible in some areas [Blanton & Wirth, 1979]. The bites of *Culicoides* often may result in secondary infection (cellulitis) in sensitive individuals. Complaints and demands for control of these biting midges have increased with man's encroachment into areas adjacent to coastal salt marshes. Despite this considerable nuisance problem and the adverse effect that their presence has had on the quality of life, tourism, and land development in coastal areas of Florida, USA, and in many other areas [Linley & Davies, 1971], relatively few studies have been done on basic biology and ecology of *Culicoides* in most geographic areas. Thus, there is only limited knowledge from which to develop a rational pest management strategy.

While ecological studies on the populations of adult *Culicoides* are not numerous compared with other blood-sucking arthropods, such as mosquitoes and ticks, studies on their immature stages are even rarer. Part of the reason for this is that the immature stages of *Culicoides* occur in the substrate of semiaquatic habitats such as coastal salt marshes and mangrove swamps. As a rule, the presence of *Culicoides* immatures cannot be visibly observed, and their sampling is difficult.

Historically, larvae of biting midges have been recovered from salt marsh substrate samples by the following methods: (1) sieve-flotation [Kettle & Lawson, 1952; Wirth, 1952; Kettle et al., 1956; Jamnback, 1965; etc.]; (2) sand flotation [Bidlingmayer, 1957; Williams, 1960]; (3) direct flotation [Linley & Kettle, 1964; Linley & Adams, 1972]; and (4) Berlese funnels [Jamnback & Wirth, 1963; Jamnback, 1965]. Kline et al. [1975] found that all of these methods were tedious, time-consuming, and had various other disadvantages.

Sand flotation has been the most commonly used method, but is messy, and extracted larvae are often physically damaged and unfit for use in rearing, insecticide, or pathogen studies. Therefore, Kline et al. [1981] developed an agar extraction method (AE), which resulted in the recovery of undamaged larvae. While the AE method has been an improvement over other techniques, it requires at least 48 hours to obtain a number of larvae similar to that by sand flotation. For studies aimed at developing an integrated pest management program this time requirement can be a disadvantage. Another potential disadvantage of the AE method for developing such a program is that until now the AE's ability to quantify accurately the larvae in various habitats has not been tested. Although the information obtained by the AE method has been useful in establishing the relative abundance between sampling sites, absolute numbers need to be determined. In order to overcome these disadvantages a new method was developed known as the centrifuge sugar flotation extraction (CSFE) method. This paper reports the results of a study, in which the AE and CSFE methods were compared in terms of their speed and accuracy in recovering the immature stages of *Culicoides* from simulated and actual field samples obtained from salt marsh habitats with sand and mud substrates.

## Material and methods

**Immature stages of *Culicoides***: The field-collected larvae of *Culicoides* were obtained from salt marsh areas at Yankeetown, Florida, known to produce almost exclusively large numbers of *Culicoides mississippiensis* Hoffman, 1926 larvae during winter (December through March) and early spring (April) [Wood & Kline, 1989]. Larvae of *C. mississippiensis* are about 1 mm long, transparent when newly hatched, and grow through 4 instars to reach a length of 6–7 mm when mature [Kline, personal observations]. First and second instar larvae (< 3 mm) can be separated from third and fourth instar (>3 mm) ones.

**Extraction techniques**: Two extraction techniques were compared to remove the *Culicoides* immature stages from three categories of substrate samples. The extraction techniques are described as follows:

*Agar extraction (AE)*. This technique is described in detail by Kline et al. [1981]. A 2% (weight / volume) non-nutrient agar solution was autoclaved for 15 min at a pressure of 15 pounds per square inch, allowed to cool to ca. 47°C, and then 300 ml of the solution poured on

top of standard substrate samples (1000 ml of substrate) that previously had been placed into 2-litre plastic containers (16 cm diameter × 11 cm deep). This resulted in a ca. 1 cm thick agar layer on top of each sample. After the agar gelled, container lids were replaced. After 48 hours, 200 ml of filtered estuarine water was poured onto each agar surface, swirled back and forth several times, and then decanted into a black photographic tray. Immature stages were removed with a pipette and counted. After removal of all visible larvae and pupae, the 200 ml of estuarine water was again poured into the container and the process repeated until 3 consecutive negatives were achieved. Even though all immatures were removed from the agar surface, some larvae remained embedded in the agar layer. These were removed with a dissecting needle with a slight bend at the distal end (i.e., probed from agar). While the standard size for comparing the extraction techniques was a 1000 ml, additional comparisons were made using 100 ml samples in 250 ml glass beakers painted black on the outside.

*Centrifuge sugar flotation extraction (CSFE)*. Samples consisted of 100 ml of substrate. Each sample was washed through a kitchen colander (15 cm diameter, 16 ×18 mesh [6.3 × 7.1 wires/cm]) with a water hose into a 2.3 litre plastic pitcher (model #3062; Rubbermaid, Fairlawn, Ohio, USA). Care was taken to wash all of the substrate from the sample container in order to ensure collection of all specimens. The pitcher contents were then poured through a U.S. Standard Testing sieve with 150 µm openings (100 mesh) (W.S. Tyler, Inc., Mentor, Ohio, USA) and rinsed with a steady stream of water from a hose until most of the mud/sand was washed through the sieve. Sieve contents (residue) from each sample were concentrated and rinsed with as little water as possible into a 250 ml plastic centrifuge tube. One hundred ml of saturated sugar solution was added to the tube and the mixture vigorously shaken. Then additional saturated sugar solution was added until the centrifuge tube was full. Tube contents were again vigorously shaken and then placed into a centrifuge (model Centra-4B; International Equipment Co., Needham, Massachusetts, USA) and spun at 2200 revolutions per minute for 3 minutes. This model was capable of processing 4 × 250 ml samples at the same time. The resultant supernatant was poured through a 100 mesh screen and the residue pellet discarded. Screen contents were gently rinsed with a water hose either into a 100 ml Quorpak™ jar (All-Pak, Bridgeville, Pennsylvania, USA) or a black photographic tray where the immature stages were removed with a pipette and counted. If the samples could not be processed within 48 hours the contents were placed in the jar and a preservative, such as 70–80% ethanol was added.

**Categories of substrate samples**: The three types of samples processed were classified as follows: seeded samples, homogenized samples, and regular field-collected samples. All substrate samples used in this study were collected from mid-March to mid-April.

*Seeded samples*. These samples consisted of reconstituted salt marsh substrate seeded with different levels of field-collected larvae. Salt marsh substrate was brought back to the laboratory, air-dried and then sieved through a screen with 850 µm openings (20 mesh) to remove plant roots and other large debris. The air-dried sieved substrate was then placed on trays and heated at 43.1°C in an oven for 2 hours to ensure that no viable *Culicoides* eggs or immature stages remained. The substrate was then cooled to room temperature. Distilled water was added to reconstitute the substrate to its pre-dried consistency. This reconstituted substrate was then used to perform a set of experiments in which the samples contained a known number of larvae. Samples to be processed by the AE technique were prepared by partitioning out either 100 ml or 1000 ml of reconstituted substrate into 2-litre plastic containers (16 cm diameter × 11 cm deep). The CSFE samples were prepared by partitioning out 100 ml of reconstituted substrate into 2-litre containers.

Field-collected larvae (late 3rd – early 4th instars) at five inoculation levels (0, 25, 50, 100 and 200) were placed into randomly selected containers. Larvae were extracted from the substrate by AE [Kline et al., 1981] and separated according to size. First and second instar larvae (< 3 mm) were separated from third and fourth instar (> 3 mm) ones. Only third and fourth instar larvae were used in the seeded experiments.

For the main comparisons, six containers were set up at each inoculation level and substrate amount, except the 200 inoculation level, which had three replicates. Containers with 1000 ml of substrate were processed by AE, and the 100 ml containers were processed by CSFE.

As a side study, five 100 ml reconstituted samples with three replicates of 50 larvae per sample and two control replicates of zero larvae per sample were placed into 250 ml beakers to be processed using AE. These were compared with a number of samples processed by the AE1000 and CSFE methods.

*Homogenized samples*. Homogenized samples were based on field-collected samples of substrate from the salt marsh areas mentioned above. All samples were obtained in the last two weeks of March when the immature populations consist almost exclusively of *Culicoides mississippiensis* [Wood & Kline, 1989]. Substrate up to a depth of ca. 3 cm was collected into large plastic buckets (19 litres). Based on visual determination, samples were taken from areas that had either a very sandy or silty mud substrate. In the laboratory, the bucket contents were thoroughly mixed (homogenized) by hand for several minutes using rubber-gloved hands. The hypothesis was that homogenizing these field samples would evenly distribute the immature stages and produce samples with approximately similar numbers of immatures. The sand and mud samples were mixed in different containers and processed separately. After homogenizing, the contents were quickly and randomly partitioned out into plastic containers (1000 ml per 16 cm diameter × 11 cm deep cup) with lids, beakers (100 ml

per 250 ml beaker), and glass jars (100 ml per 150 ml jar) with lids.

Two variants of comparison were used. In the first, 10 samples with 1000 ml of sand substrate and 5 samples with 1000 ml of mud substrate in the plastic containers were processed by AE, and 10 samples with 100 ml of sand substrate and 5 samples with 100 ml of mud placed in the glass jars were processed by CSFE. In the second variant, 5 replicate samples of sand substrate were processed. The 1000 ml plastic cups and 100 ml beaker samples were processed using AE while the 100 ml glass jar samples were processed by CSFE.

*Regular field-collected samples*. This group of samples refers to those which were processed as they came directly from field locations mentioned above. Plastic 2-litre containers (16 cm diameter × 11 cm deep), beakers (250 ml) and glass jars (150 ml) were used to collect 1000 ml (ca. 300 $cm^2$), 100 ml (ca. 30 $cm^2$) and 100 ml (30 $cm^2$), respectively, of salt marsh surface soil (ca. 3 cm deep).

Two variants of comparison were used. In the first, only the AE1000 and CSFE methods were compared. Replicate samples were taken at 54 sample locations with mud substrate and 10 locations with sand substrate in close proximity ($\leq 1$ $m^2$) of each other. In the second variant, three replicate samples of each type were retrieved from 9 similar marsh locations with the replicate number of each type again being collected in close proximity of each other. The 1000 ml plastic cups and 100 ml beaker samples were processed using the AE technique, and the 100 ml glass jar samples were processed using the CSFE technique.

**Statistical Analyses**: Where appropriate, data were subjected to analysis of variance (SAS Proc GLM) and t-tests, and means were separated by Duncan's Multiple Range test [SAS Institute, 2003]. Percentage data were transformed by arc sine of the square root of the count data prior to analysis. Unless otherwise stated, the alpha level of significance was 0.05.

## Results

*Seeded samples*. Mean percent recovery was very similar at all inoculation rates from the 100 ml substrate samples processed by CSFE (Table 1). A similar trend was obtained for 1000 ml samples processed by AE, although the mean values varied over a wider range (Table 1). However, mean recovery was about 2.7 times as great for the CSFE method. The overall recovery rate was 95.7% for the CSFE method compared with 35.3% for the AE method. When the AE method was used to recover 50 larvae previously inoculated into either a 1000 ml or a 100 ml substrate sample, the recovery rate was about twice as great from the larger sample (Table 2). In this second set of experiments recovery rates for the CSFE 100 ml and AE 1000 ml samples were similar to those obtained in the first experiment (Table 1). In all cases, the mean recovery rate between extraction methods was highly significantly different ($p < 0.001$).

Table 1. Mean percent (standard error value in parentheses) of 3rd and 4th instar larvae of *Culicoides* recovered from salt marsh substrate seeded at four levels, using agar extraction (AE) or centrifuge-sugar flotation extraction (CSFE).
Таблица 1. Средняя доля личинок *Culicoides* 3-го и 4-го возрастов (%; в скобках — среднеквадратичная ошибка), извлечённых методом агаровой экстракции (AE) или методом центрифугирования и флотации в растворе сахара (CSFE) из субстратов солёного марша, в которые было помещено точно известное число личинок, от 25 до 200.

| Seed level | n | Extraction method | |
|---|---|---|---|
| | | AE1000 | CSFE100 |
| 25 | 6 | 38.0 (4.9) | 97.3 (2.0) |
| 50 | 6 | 28.7 (4.6) | 96.7 (1.4) |
| 100 | 6 | 44.2 (4.2) | 93.6 (0.9) |
| 200 | 3 | 25.3 (6.4) | 95.3 (2.2) |
| Total | 21 | 35.3 (2.8) | 95.9 (0.8) |

*Homogenized samples*. Substrate from field samples was visually classified as either predominantly sand or mud. Results comparing the relative recovery of immature stages by the AE1000 and CSFE100 methods were similar for the two substrate types (Table 3). A comparison of raw means showed that 1.9 and 1.8 times more immature stages were obtained from samples processed by the AE1000 method compared to the CSFE method for sand and mud substrate samples, respectively. These differences were statistically significant. When compared on the basis of immatures recovered per ml of substrate processed, 5.2 and 5.6 times more immatures were obtained by the CSFE method than the AE1000 method for sand and mud substrate samples, respectively. These differences were highly significant ($p < 0.001$).

In the special tests (Table 4) in which different amounts of the same type of substrate were used (standard 1000 ml versus 100 ml sample size), 16.6 and 124.0 immature stages per sample were recovered for AE100 and AE1000, respectively. Based on total immatures per sample, the AE1000 method recovered very significantly ($p < 0.001$) more immatures than both the CSFE (2.7 times) and AE100 (7.4 times) methods. However, on a total of immatures recovered per ml of substrate basis, the CSFE method caught significantly more (3.7 times) than the AE1000 method. The CSFE method caught significantly more (2.8 times) than the AE100 method both ways.

*Regular field-collected samples*. Similar results were obtained from the first set of regular field samples as were obtained for the homogenized randomly collected field samples (Table 5). A comparison of raw means showed that 1.4 and 1.5 times more immature stages were obtained for samples processed by the AE1000 method compared with the CSFE method for sand and mud substrate samples, respectively. These differences were significant ($p = 0.02$). However, the reverse was true when sampling efficacy was determined based on

the total number of immatures recovered per ml of substrate processed. The CSFE method recovered very significantly ($p < 0.001$) more (6.9 times) immatures per ml of substrate than the AE1000 method for both sand and mud samples.

In the second set of field samples (Table 6), the AE1000 method recovered 1.7 times more immatures per sample than the CSFE method, but the difference was not significant. Both the AE1000 (15.8 times) and CSFE (9.5 times) methods caught significantly more immatures than the AE100 method. On immatures per ml of substrate basis, the CSFE method recovered significantly more (6 times) immatures than the AE1000 method.

Table 2. Mean percent (n=3; standard error value in parentheses) of 50 *Culicoides* 3rd and 4th instar larvae seeded into either 100 or 1000 ml of substrate and recovered by agar extraction (AE100, AE1000) or by centrifuge-sugar flotation extraction (CSFE100).
Таблица 2. Средняя доля от 50 личинок *Culicoides* 3-го и 4-го возрастов (%; n=3; в скобках — среднеквадратичная ошибка), помещённых в 100 или 1000 мл субстрата и извлечённых оттуда методом агаровой экстракции (AE100, AE1000) или методом центрифугирования и флотации в растворе сахара (CSFE100).

| Extraction method | | |
|---|---|---|
| AE100 | AE1000 | CSFE100 |
| 16.7 (7.1) | 34.8 (3.9) | 95.3 (0.8) |

Table 3. Mean number (standard error value in parentheses) of immature stages of *Culicoides* recovered by agar extraction (AE1000) or by centrifuge-sugar flotation extraction (CSFE100) from homogenized field samples collected from two substrate types in salt marsh habitats at Yankeetown, Florida.
Таблица 3. Среднее число преимагинальных стадий *Culicoides* (в скобках — среднеквадратичная ошибка), извлечённых из гомогенизированных проб, которые были собраны на двух типах субстратов в пределах солёных маршей у г. Янкитаун, Флорида.

| Substrate | Extraction method | n | 1st/2nd instars | 3rd/4th instars | Pupae | Probed [1] from agar | Total |
|---|---|---|---|---|---|---|---|
| Sand | AE1000 | 10 | 13.0 (4.3) | 51.0 (12.8) | 0.0 (0.0) | 12.4 (1.8) | 76.4 (17.5) |
| | CSFE100 | 10 | 5.3 (1.2) | 33.8 (2.3) | 0.2 (0.1) | — | 39.3 (2.9) |
| Mud | AE1000 | 5 | 5.6 (0.9) | 13.0 (2.7) | 1.0 (0.6) | 9.2 (1.5) | 28.8 (4.4) |
| | CSFE100 | 5 | 5.8 (0.7) | 9.0 (2.0) | 1.4 (0.5) | — | 16.2 (2.2) |

[1] Late instar (3rd and 4th) larvae physically removed with a slightly bent dissecting needle from agar layer.
[1] Личинки старших возрастов (3-го и 4-го), извлечённые из слоя агара с помощью слегка изогнутой препаровальной иглы.

Table 4. Mean number (standard error value in parentheses) of immature stages of *Culicoides* recovered by agar extraction (AE100, AE1000) or by centrifuge-sugar flotation extraction (CSFE100) from homogenized field samples collected from sand substrate in salt marsh habitats at Yankeetown, Florida.
Таблица 4. Среднее число преимагинальных стадий *Culicoides* (в скобках – среднеквадратичная ошибка), извлечённых из гомогенизированных проб, которые были собраны на песчаном субстрате в пределах солёных маршей у г. Янкитаун, Флорида.

| Extraction method | n | 1st/2nd instars | 3rd/4th instars | Pupae | Probed [1] from agar | Total |
|---|---|---|---|---|---|---|
| AE100 | 5 | 3.4 (1.2) | 10.4 (2.5) | 0.0 (0.0) | 2.8 (0.3) | 16.6 (2.7) |
| AE1000 | 5 | 24.6 (3.9) | 85.8 (9.5) | 0.0 (0.0) | 15.6 (2.4) | 124.0 (13.3) |
| CSFE100 | 5 | 8.0 (1.3) | 37.6 (2.1) | 0.4 (0.2) | — | 46.0 (2.5) |

Table 5. Mean number (standard error value in parentheses) of immature stages of *Culicoides* recovered by agar extraction (AE1000) or by centrifuge-sugar flotation extraction (CSFE100) from regular field samples collected from two substrate types in salt marsh habitats at Yankeetown, Florida.
Таблица 5. Среднее число преимагинальных стадий *Culicoides* (в скобках – среднеквадратичная ошибка), извлечённых из обычных проб, которые были собраны на двух типах субстратов в пределах солёных маршей у г. Янкитаун, Флорида.

| Substrate | Extraction method | N | 1st/2nd instars | 3rd/4th instars | Pupae | Probed [1] from agar | Total |
|---|---|---|---|---|---|---|---|
| Sand | AE1000 | 10 | 19.9 (3.1) | 101.1 (18.9) | 0.2 (0.1) | 27.7 (7.0) | 148.9 (20.3) |
| | CSFE100 | 10 | 12.7 (2.1) | 90.0 (18.7) | 1.0 (0.4) | — | 103.7 (18.8) |
| Mud | AE1000 | 54 | 13.6 (2.7) | 30.9 (4.6) | 12.4 (2.0) | 0.5 (0.2) | 57.4 (8.0) |
| | CSFE100 | 54 | 10.1 (1.5) | 26.1 (2.7) | 3.1 (0.8) | — | 39.3 (4.2) |

[1] See footnote to Table 2.
[1] См. сноску к Таблице 2.

Table 6. Mean number (standard error value in parentheses) of immature stages of *Culicoides* recovered by agar extraction (AE100, AE1000) or by centrifuge-sugar flotation extraction (CSFE100) from regular field samples collected from sand substrate in salt marsh habitats at Yankeetown, Florida.
Таблица 6. Среднее число преимагинальных стадий *Culicoides* (в скобках — среднеквадратичная ошибка), извлечённых из обычных проб, которые были собраны на песчаном субстрате в пределах солёных маршей у г. Янкитаун, Флорида.

| Extraction method | n | 1st/2nd instars | 3rd/4th instars | Pupae | Probed [1] from agar | Total |
|---|---|---|---|---|---|---|
| AE100 | 9 | 3.0 (1.4) | 1.4 (0.7) | 0.3 (0.3) | 0.0 (0.0) | 4.8 (2.1) |
| AE1000 | 9 | 12.0 (3.9) | 45.8 (14.5) | 0.1 (0.1) | 17.8 (5.3) | 75.8 (16.4) |
| CSFE100 | 9 | 8.2 (1.5) | 35.0 (7.4) | 2.6 (0.8) | — | 45.8 (8.5) |

[1] See footnote to Table 2.
[1] См. сноску к Таблице 2.

## Discussion

Based upon these limited observations, it would appear that AE and CSFE methods were useful in recovery of immature *Culicoides* from salt marsh substrate. Both techniques produced viable larvae that could be used in further studies, such as pesticide screening. This said, one of the stated objectives of this study was to determine if the CSFE method would be useful in determining the spatial and temporal presence of the immature *Culicoides*. Although AE is currently our best sampling technology for obtaining immature stages from salt marsh habitats [Kline et al., 1981], the process requires 48 hours to recover sufficient numbers of immatures. Indeed, greater numbers can be recovered if the samples are held for 72 or 96 hours before the sample is processed [Kline et al., 1981]. The data reported herein demonstrate that the CSFE technique was very efficient (95%) in the recovery of *Culicoides* immatures associated with salt marshes.

An advantage of the CSFE technique was the relatively rapid determination of the presence or absence of immatures and their abundance. Once the sample is returned to the laboratory an answer may be obtained within an hour. Another advantage is that much less substrate is required to obtain the same answer. In the seeded 100 ml samples processed by CSFE yielded a mean recovery rate of 95% compared to 35% for AE1000. In the field-collected samples, the number of immature stages recovered per ml was 8.6 vs. 1.4 for sand substrate, and 3.9 vs. 0.5 for mud substrate samples for the CSFE and AE1000 methods, respectively (Table 5). Thus, when the data are compared on a ml of substrate basis, the CSFE method recovered 6–8 times more immatures than the AE1000 method in contrast to 1.5 times greater number of immatures recovered by AE1000 on a per sample basis. More 100 ml samples can be physically carried from the salt marsh during a single trip. Thus, more areas can be sampled during a single trip. The greatest disadvantage of this method is that a good centrifuge is needed. Not every research project has one and a good centrifuge may be expensive.

Further work needs to be conducted to determine the usefulness of the CSFE technique.

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