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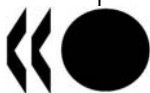
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No. 136

VALIDATION REPORT OF THE CHIRONOMID FULL LIFE-CYCLE TOXICITY TEST

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Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
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FOREWORD

This document presents the validation report of the Chironomid full life-cycle toxicity test. The report includes experiments demonstrating 1) the relevance of the test using various chemicals in a small number of participating laboratories, and 2) the reproducibility of the test and especially the ability of a large number of laboratories to meet the proposed validity criteria. This validation report is intended to support the Test Guideline on Chironomid Life-Cycle Test, which was approved by the Working Group of the National Coordinators of the Test Guidelines Programme in April 2010, and then published as TG 233.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to the declassification of this document on 15 September 2010.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

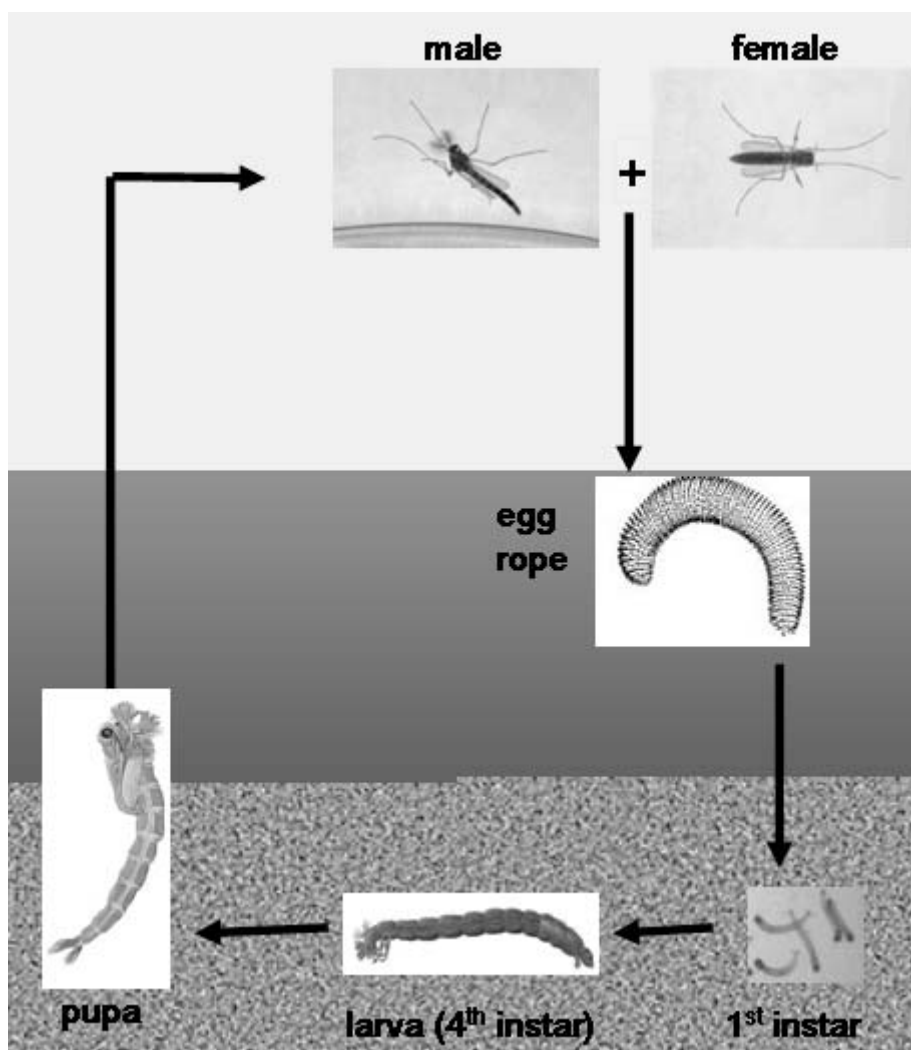
The Chironomid full life-cycle test

Validation report

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February 2010

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In the past years many people contributed to the development of the draft Guideline, by providing comments, reviewing papers, discussing and last but not least conducting studies.

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In no particular order we would like to mention: Verena Taenzler, Marcus Ebeling, Michael Dorgerloh, Gabriele Silke, Dominik Reinhard, Verena Pfeifle, Kate Benyon, James Wheeler, Sabine Zok, Peter Dohmen, Daniela Belz, Annika Pupp, Philipp Egeler, Rebecca Pierstorf, Tania Jarosz, Daniela Schroth, Ribana Seliger, Lucas Jagodzinski, Christian Vogt, Matthias Oetken, Jörg Oehlmann, Candida Shinn, Torsten Hahn, Koffi Tassou and Ralf Schulz for their contributions.

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Last but not least we owe debt to our ringtest participants, who contributed valuable data reported here.

Lennart Weltje and Eric Bruns

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1. INTRODUCTION

A higher tier full life-cycle test using chironomids (Arthropoda, Insecta, Diptera, Chironomidae) has been developed. The increasing interest in potential adverse effects due to endocrine modes of action made the development of an appropriate invertebrate assay a primary goal of national and international testing programs, such as the one under development at the OECD. Thereby it should be considered that the various invertebrate assays (including the presented chironomid test) are not mechanistic in nature, i.e. they do not reveal if chemicals act on the endocrine system. Instead the assays are designed to study effects of chemicals on reproduction and development by covering the complete life cycle of the organisms and thus would pick up any adverse effect, regardless of the mode-of-action.

The presented chironomid full life cycle test completes the existing acute and chronic test cascade for these widely used test organisms. The acute and the chronic chironomid tests are part of the standard test procedures for registration of agrochemicals under the European plant protection products directive 91/414/EEC and also for biocides. Therefore, the chironomid life cycle test yields results, which directly fit into the general risk assessment. The life cycle test is an extension of the existing OECD guidelines 218 and 219 for the testing of chemicals "Sediment water chironomid toxicity test using spiked sediment" (OECD, 2004a) and "Sediment water chironomid toxicity test using spiked water" (OECD, 2004b), respectively. The development of the presented method also took the development and validation of the long term toxicity test with chironomids (edited by Streløkke and Köpp, 1995) and the existing guidelines OPPTS 850.1735 (US-EPA, 1996a), OPPTS 850.1790 (US-EPA, 1996b) and EPA 600/R-99/1064 (US-EPA, 2000) into consideration.

Chironomids have been chosen for the development of a freshwater invertebrate life cycle test due to numerous reasons. As freshwater insects, chironomids belong to one of the most abundant, species-rich and ecologically relevant taxonomic groups.

As the endocrine system of insects is the best described among the invertebrates, potential endocrine related effects can be investigated in this sexually reproducing organism. Specific third generation insecticides, juvenile hormone (ant)agonists and ecdysone (ant)agonists, have been developed to target the insect endocrine system and can be used as reference chemicals for potential endocrine effects. As holometabolic insects, chironomids undergo a full hormonally-governed metamorphosis during a chronic test and after emergence the separate sexes are easily identified. The life cycle of *Chironomus riparius* at 20°C is completed in about 25 days. This time period is long enough to represent a chronic exposure in the environment and at the same time is feasible from a laboratory testing perspective.

Chemicals can enter the aquatic environment by different routes, such as run-off, drainage and spray drift. The bioavailability of a chemical in the aquatic compartment is related to its physico-chemical properties, but also depends on the organism under investigation. The exposure of sediment dwelling organisms, such as chironomid larvae, may occur via the overlying water, the pore water and the sediment. Different exposure scenarios can be investigated by the use of chronic chironomid tests. However, regardless of the selected exposure scenario, the inserted first instar chironomid larvae will be exposed to all phases simultaneously: the overlying water, the pore water and the sediment (potentially containing its food). Depending on the chemical's entry route in aquatic systems (i.e. the chosen spiking scenario) concentrations between the various phases may differ. The exposure situation to be investigated can be adapted according to the exposure scenario of interest. Both, spiked water and spiked sediment tests can be performed using the chironomid life cycle test protocol.

The results obtained in the chironomid life cycle tests can be compared to historical data obtained in chironomid studies conducted according to the OECD guidelines 218 and 219. Chironomids are standard test organisms which are easy to handle and to culture. The knowledge which is needed to perform life-cycle tests on chironomids is already existing in a vast number of academic, industry and contracting laboratories, that perform ecotoxicological studies.

In comparison with the other aquatic arthropod species for which there are currently life-cycle tests under discussion, initiated or being validated at the OECD, the chironomid test has some distinct features:

- Chironomids are insects, while all the other species (daphnids, mysids, copepods) belong to the crustaceans. Crustaceans are already represented in the aquatic testing suite by *Daphnia magna* with an acute and a reproduction test, TGs 202 and 211 (OECD, 2004c and 2008), while aquatic insects are not, although the insects are representing a higher number of species (in freshwaters).
- Chironomids, like daphnids, are freshwater species, while copepods and mysids are saltwater species (some requiring filtered seawater as test and/or culture medium). For most chemicals, freshwaters are more important than marine waters in terms of exposure concentrations.
- Chironomids reproduce sexually like mysids and copepods, but unlike daphnids, which reproduce parthenogenetically (unless a stress factor induces male production and thereafter sexual reproduction).
- Exposure of chironomids via both water and sediment is relevant and possible, like in the copepod test, but this is not a relevant route in the daphnid and in the mysid test.
- Chironomids can easily be fed with TetraPhyll/TetraMin, but daphnids and copepods feed on algae, which need to be cultured additionally. Mysids feed on neonates of the crustacean *Artemia salina*, which also require to be cultured in parallel.
- Whilst chironomids and daphnids are established test species in many laboratories all over the world, mysids are only established in a few laboratories in the US, which are based on the coast. Copepods present a relatively new test system, which is not established yet for regulatory testing.
- Finally, chironomids and daphnids have a reasonable size to work with and do not require specialized optical equipment, unlike copepods, whilst mysid testing requires a flow-through testing system.

The above given information as well as the comparative data presented in the following chapters are aimed to demonstrate the relevance, usefulness and feasibility of the chironomid life cycle test as an additional higher-tier tool for the environmental risk assessment.

2. MATERIALS AND METHODS

The experimental work presented in the next chapter was conducted according to a draft guideline, which is based on the existing test guidelines 218 Sediment-Water Chironomid Toxicity Test Using Spiked Sediment (OECD, 2004a) and 219 Sediment-Water Chironomid Toxicity Test Using Spiked Water (OECD, 2004b). The validation data and background of the 218 and 219 guidelines are described in a BBA booklet edited by Streloke and Köpp (1995).

The life-cycle test is an extension of the OECD TGs 218/219, but unlike these does not end with the emergence of the adult midges. Instead the adult midges (P generation) are carefully collected and put into a breeding cage, so that they can swarm, mate and oviposit in crystallising dishes containing the same spiked water-sediment system they emerged from. Apart from quantitatively following reproduction, fertile egg ropes are selected from the peak of oviposition and allowed to hatch. After hatching, 1st instar larvae (F1 generation) are collected and added to spiked water-sediment vessels again and followed until emergence.

So, in essence the proposed life cycle test covers two generations (P and F1) and exists of a sequence of two OECD TG 218 or 219 tests. The additional information provided by conducting a life-cycle test (in comparison with a normal chronic *Chironomus* study) is therefore:

- Reproduction assessments, both fecundity and fertility.
- Addition of second (filial) generation endpoints (to account for possible higher sensitivity, due to accumulation, carry-over effects etc.)

Since the first part of the test (emergence, development) was already validated in 1995, the ringtest should focus on the additional endpoints as outlined above.

Full experimental details for study conduct can be obtained from the draft guideline (current revised version, including spiked sediment scenario, of February 2, 2010).

3. EXPERIMENTAL WORK

3.1. Overview

After the writing of the draft guideline, various experiments have been conducted, all using the species *Chironomus riparius* MEIGEN. The experiments all followed a NOEC design, since there is no agreement on the effect level to be assessed with the EC_x approach.

The first small scale ringtest was a test in spiked-water design using the general toxicant and reference compound 3,5-dichlorophenol (3,5-DCP). Thereafter, a spiked water test was conducted with the juvenile hormone analogue pyriproxifen (PPF) in anticipation of specific effects on chironomids and also because this compound was selected for validation of other arthropod reproduction tests (with daphnids, copepods, mysids) under development at the OECD (see Gourmelon and Ahtiainen, 2007). The 3,5-DCP and PPF experiments were conducted as small scale ringtests by Syngenta (UK), Bayer CropScience (Germany) and BASF (Germany) and were published by Taenzler et al. (2007). Later, PPF was tested following the same design at the University of Landau (Germany) and published by Tassou and Schulz (2009).

Since it was commented by the UBA that the guideline should also cover other designs, such as a spiked sediment scenario, a small scale ringtest with the insecticide lindane (γ -HCH) was conducted by Bayer CropScience, BASF and the University of Landau (on behalf of Syngenta). Lindane was selected since it had been used previously in the original validation studies for development of the OECD TG 219 (see Strelake and Köpp, 1995). The used concentrations were based on a range finding test according to the OECD TG 218 conducted at BASF. In addition this test was accompanied by chemical analysis of the spiked sediment.

Additionally, the effects of swarm size and also of the sex ratio of adult chironomids in the swarm on egg rope fertility was studied at the BASF laboratory using a slightly different design than described in the draft guideline. Results were presented at a scientific conference by Pupp and Weltje (2006). Similarly, the effect of feeding adult chironomids on egg rope production, egg rope fertility and life span was studied. Results will be presented at a scientific meeting by Weltje and Seliger (2010). The results of these two spin-off projects will be briefly discussed.

3.2. Spiked water test with 3,5-dichlorophenol

The first test carried out according to the draft protocol was a spiked water test using 3,5-dichlorophenol (3,5-DCP), which is a general toxicant that is recommended for reference testing in OECD guidelines such as OECD TG 201 – algae and OECD TG 221 - *Lemna*. The small scale ringtest ($n = 3$) with 3,5-DCP was conducted in the laboratories of Syngenta, Bayer CropScience and BASF. The results were published in Taenzler et al. (2007).

In short, the selected concentrations: 0.0 (dimethylformamide solvent control), 0.0313, 0.125, 0.5 and 2.0 mg/L, based on an acute test with first instar larvae conducted at Bayer CropScience, were too low to induce a significant inhibition of emergence, development or reproduction. Hence, all three laboratories concluded that the NOEC for all endpoints was ≥ 2.0 mg/L. Although no real effect threshold was achieved with the used concentrations, the results were consistent among the participating laboratories. In addition, all experiments were compliant with the validity criteria (possibly one endpoint invalid - see footnote of Table 1). This was judged by the performance of the solvent control containing DMF, since not all laboratories employed a water control. A comparison of the results of the solvent controls are presented below (adapted from Taenzler et al., 2007).

Table 1. Mean endpoint values of the solvent control in the 3,5-DCP experiment

Endpoint	Lab 1	Lab 2	Lab 3
P emergence ratio (both sexes summed) $n = 8$	0.91	0.94	0.81
P development rate (both sexes summed) $n = 8$	0.0667	0.0652	0.0631
P male fraction	0.50	0.58	0.51
Fecundity (egg ropes/female) $n = 2$	0.82	0.79	0.82
Fraction fertile egg ropes $n = 2$	0.97	0.73	0.89
F1 emergence ratio (both sexes summed) $n = 8$	0.88	0.88	0.83
F1 development rate (both sexes summed) $n = 8$	0.0694	0.0592	0.0652
F1 male fraction	0.50	0.52	0.39*

* not valid, when male fraction is interpreted as 0.40 (the guideline says 0.4)

3.3. Spiked water test with pyriproxifen

The second test that was carried out according to the draft protocol was again a spiked water test using the insecticide pyriproxifen (PPF). PPF is a juvenile hormone analogue, which is a specific arthropod toxicant that interferes with the endocrine regulation of the larval phase and the transition into metamorphosis (the pupal phase). PPF has been selected as reference compound for validating reproduction tests with aquatic arthropods under the OECD flag (see Gourmelon and Ahtiainen, 2007). This compound can be regarded as a positive control. The small scale ringtest ($n = 3$) with PPF was conducted in the laboratories of Syngenta, Bayer CropScience and BASF. The first run of the test at Syngenta resulted in a P emergence of 68.1%, which is slightly below 70%, thus invalidating the test. The second run yielded a valid P emergence, but now the F1 emergence was slightly below 70%, thus invalidating the test. However, considering the consistent trend in the data and the valid assessments of reproduction the second test was used for comparison. The selected concentrations were: 0.0 (dimethylformamid solvent control), 0.16, 0.8, 4.0, 20, 100 $\mu\text{g/L}$ (spacing factor 5), which induced the full range of effects. Lab 1 used the same range except for the 100 $\mu\text{g/L}$ treatment, which was replaced with 50 $\mu\text{g/L}$ in anticipation of not a full, but a partial inhibition. The results were published in Taenzler et al. (2007).

Afterwards an identical test was conducted at the University of Landau, using slightly different concentrations: 0.0 (DMF solvent control), 1.0, 3.0, 10, 30, 100 $\mu\text{g/L}$ (spacing factor 3) so that in total four experiments are available for comparison ($n = 4$). The data from the University of Landau were published in Tassou and Schulz (2009). The results are presented in Table 2.

Table 2. Endpoint values (NOECs in $\mu\text{g/L}$) of the PPF experiment

Endpoint	Tassou and Schulz (2009)	Lab 1	Lab 2	Lab 3
P emergence ratio (both sexes summed) $n = 8$	10	4	4	4
P development rate (both sexes summed) $n = 8$	10	4	4	4
P male fraction	≥ 30	≥ 50	4 ¹	≥ 20
Fecundity (egg ropes/female) $n = 2$	10	20	4	≥ 20
Fraction fertile egg ropes $n = 2$	10	20	4	≥ 20
F1 emergence ratio (both sexes summed) $n = 8$	3	0.8	≥ 4	0.8
F1 development rate (both sexes summed) $n = 8$	3	0.8	≥ 4	≥ 20 ²
F1 male fraction	≥ 10	4	≥ 4	≥ 20

¹ NOEC seems rather low in comparison to the other tests

² NOEC seems rather high in comparison to the other tests

The test concentrations induced the full range of effects, with for the highest treatment, 100 $\mu\text{g/L}$, a massive inhibition of the P generation emergence, so that reproduction assessments for this treatment were not possible anymore.

In comparing the endpoints, a consistent pattern is seen. With the exception of two single cases (highlighted in the footnotes of Table 2), the calculated NOEC values are not more than one treatment level apart. For the evaluation of the appropriateness of the endpoints it was extremely helpful to have an additional data set that was generated outside of the framework of the small scale ringtest and which employed different concentrations.

From the work it can be concluded that the second (F1) generation is more sensitive than the first (P) generation, considering emergence and development. Further, there was a trend (only a few significances) in the sex ratio of both generations towards a lower fraction of males. Finally, reproduction was a less sensitive endpoint than emergence or development.

At the same time, chironomid life-cycle experiments were conducted at the University of Aachen (Germany) with the insecticide fenoxycarb (Shinn et al., 2007; 2010), which also belongs to the class of juvenile hormone analogues. Therefore, a comparison with the PPF data could be of interest to study if certain endpoints react sensitively to compounds with this specific mode of action.

In summary, Shinn et al. (2007; 2010) concluded for fenoxycarb that addition of a second generation to the 28-day chronic test increases its sensitivity, as F1 generation NOECs were one level below the P generation NOECs for emergence and development. The same trend was also observed for PPF. A lower male fraction due to fenoxycarb exposure is consistent with the findings on PPF as described by Taenzler et al. (2007). Finally, emergence was more sensitive than reproduction, which is also confirmed for PPF. Taken all the information together it appears that both juvenile hormone analogues display similar patterns in the effects they induce in the two exposed chironomid generations.

3.4. Spiked-sediment test with lindane

Since the guideline should also enable the conduct of other designs, such as a spiked sediment scenario, a small scale ringtest ($n = 3$) with the insecticide lindane (γ -HCH) was conducted by Bayer CropScience, BASF and the University of Landau (on behalf of Syngenta). Lindane, a GABA-gated chloride channel antagonist (i.e. neurotoxic to insects) was selected, because it had been used in the original validation studies for development of the chronic chironomid test guideline (see Strelake and Köpp, 1995) and is since then used as a reference compound for chironomid studies. The used concentrations: 0.0 (acetone solvent control), 0.0375, 0.075, 0.15, 0.3 and 0.6 mg/kg (spacing factor of 2) were based on a range finding test according to the OECD TG 218 conducted at BASF. Lab 2 employed slightly different concentrations of 0.0, 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg/kg. Chemical analysis of the spiked sediment confirmed the correct application of the test substance and showed no contamination of the controls. Therefore, results are based on nominal concentrations. Full details of the chemical analysis are provided in Appendix I. The selected solvent for this test was acetone, which -due to its high volatility- is completely removed from the spiked sediment under a fume hood. The results of the experiment are presented in Table 3.

Table 3. Endpoint values (NOECs in mg/kg dry sediment) of the lindane experiment

Endpoint	Lab 1	Lab 2	Lab 3
P emergence ratio (both sexes summed) $n = 8$	0.075	0.125	0.15
P development rate (both sexes summed) $n = 8$	0.0375	0.03125	0.075
P male fraction	0.075	0.125	0.15
Fecundity (egg ropes/female) $n = 2$	≥ 0.15	≥ 0.25	≥ 0.3
Fraction fertile egg ropes $n = 2$	≥ 0.15	≥ 0.25	0.0375 ¹
F1 emergence ratio (both sexes summed) $n = 8$	≥ 0.15	0.0625 ¹	0.15
F1 development rate (both sexes summed) $n = 8$	0.075	≥ 0.25 ²	0.075
F1 male fraction	≥ 0.15	≥ 0.25	≥ 0.15

¹ NOEC seems rather low in comparison to the other tests

² NOEC seems rather high in comparison to the other tests

The test concentrations induced the full range of effects, with for the highest treatment a massive inhibition of P generation emergence, so that reproduction assessments for this treatment were not possible anymore.

In comparing the endpoints, again a consistent pattern is seen. With the exception of three single cases (highlighted in the footnotes of Table 3), the calculated NOEC values are not more than one treatment level apart.

From the experiments it can be concluded that the second (F1) generation is not more sensitive than the first (P) generation, considering emergence ratio and development rate. Further, there was a trend (only a few significances) in the sex ratio of the P generation towards a lower fraction of males. Finally, reproduction was a less sensitive endpoint than development. This pattern is different from that obtained with the juvenile analogues PPF and fenoxycarb.

3.5. Swarm sex ratio

The work on the effect of sex ratio shifts in the P generation on egg rope fertility was conducted to better understand and interpret the results of the PPF experiments, where effects had been observed on the sex ratio (fewer males) and on egg rope fertility in the higher concentrations. Hence, a lower fertility may be caused by direct toxicant effects or by indirect effects (shift in sex ratio).

Normally the sex ratio in *C. riparius* is evenly distributed. i.e. 1:1 (or 50%:50%) with rather low deviations, provided the number of animals for such an assessment is sufficient.

For the swarm/sex ratio experiments breeding cages were filled with swarms in which the sex ratios were manipulated to 3:1, 2:1, 1:1, 1:2 and 1:3. Each sex ratio was tested with two replicates (i.e. two cages). In addition, swarms were limited to maximally 100 animals (both sexes summed) and the smallest swarm consisted of 36 animals (both sexes summed). In any swarm, the number of females was at minimum 25, to that still valid conclusions on reproduction could be drawn. With the conducted experiments it was also possible to look at the effect of swarm size on fertility, starting from the hypothesis that in a larger swarm there is a better chance of meeting a partner and thus achieve a higher fertility. There were no substances used for these experiments and the sediment consisted of pure quartz sand only.

The results showed clearly and significantly that when the fraction of females in the swarm increases, that the fertility of the egg ropes decreases (see Figure 1 below). Also, the larger swarm size correlates significantly and positively with a higher fertility of the egg ropes (see Figure 2).

In consideration of the results, swarm size should be at least 50 animals (both sexes summed) with a female percentage smaller than 60% to gain a proportion of more than 80% fertile egg ropes.

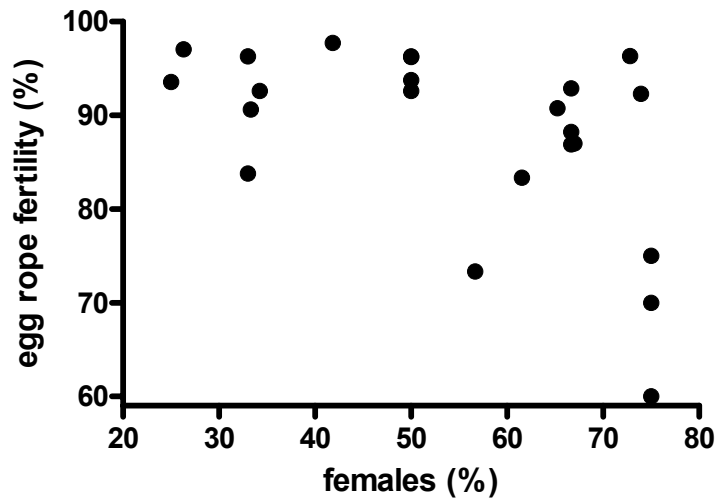


Figure 1. Egg rope fertility against percentage of females in the swarm (Pearson correlation, $r = -0.533$, $P = 0.0088$)

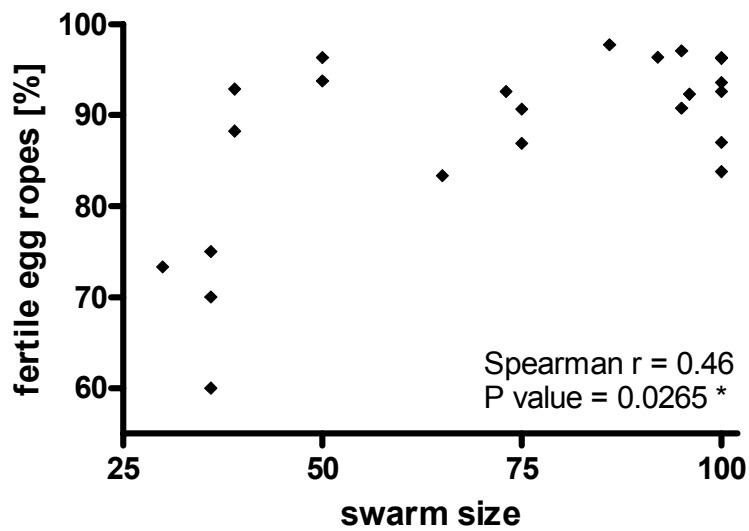


Figure 2. Egg rope fertility against swarm size (Pearson correlation, $r = 0.46$, $P = 0.0265$)

3.6. Effects of adult feeding on reproduction and life span

In the previous experiment it was demonstrated that the sex ratio in the swarm of the adult midges has an influence on the fertility of the egg ropes (more egg ropes were fertile when more males than females were present). Secondly, in a swarm with initially an equal number of each sex, infertile egg ropes were mostly produced at the end of the oviposition period when males start to die, whereas females are still vital (as a result of protandry; emergence of males occurs in advance of female emergence). These facts, combined with scientific literature mentioning an extended life span of adult midges when offered food in the form of a sugar solution, initiated the experiment on the influence of adult feeding on reproduction and life span.

Four replicate cages, two with adult feeding and two without, were employed. Adult feeding was offered in the form of a cotton pad soaked with a sugar solution. To each cage about 75 midges (sexes equally distributed) were added that had emerged from control vessels.

The results showed that adult feeding significantly extends the life span of both sexes (see Figure 3 for females). This leads to an extended swarming period in which both sexes are present in a 1:1 sex ratio. As a result, the reproductive performance of the midges was increased, both in terms of fecundity (number of egg ropes per female) and also fertility (number of fertile egg ropes per female). The unfed females produced on average 1.00 egg masses (and 0.78 fertile egg masses per female). The fed females produced on average 2.34 egg masses (and 1.43 fertile egg masses per female).

It should be noted that towards the end of the experiment the produced egg ropes were quite small.

In the light of these findings it should be considered to feed the adults in the breeding cages of the life-cycle test to improve reproductive output and compliance with the proposed validity criteria.

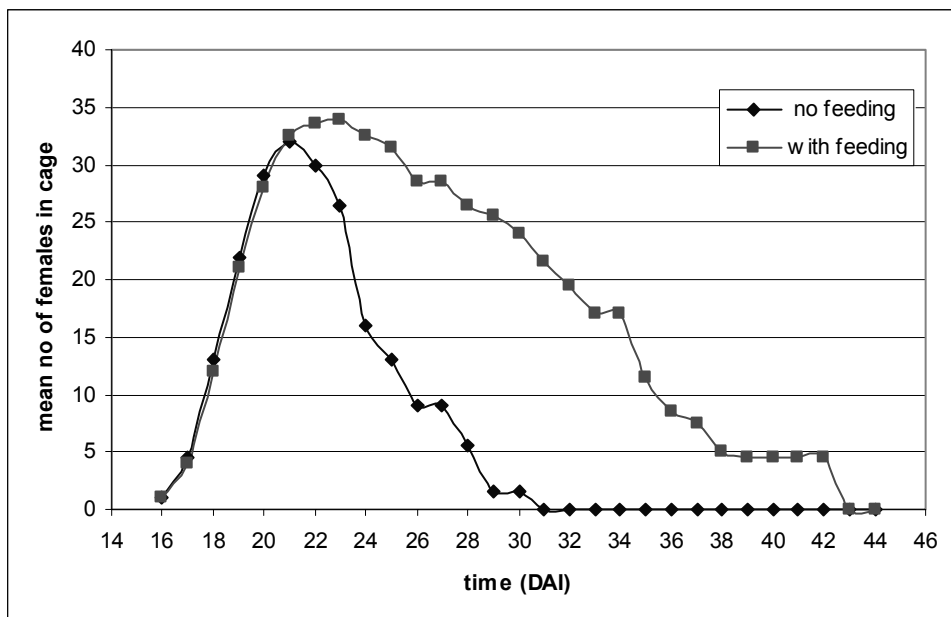


Figure 3. Swarm size development (i.e. survival) in time of fed and unfed females

4. RING TEST ON CONTROLS

4.1. General

Eleven laboratories from the UK, Switzerland, France, the United States and Germany were involved as participants in the ringtest. By email they were provided with the draft guideline (version of July 16, 2009) and an Excel file for data entry. One participant (Lab 7) employed only 2 instead of 4 cages, due to capacity problems. The raw biological data (emergence of P and F1, reproduction) are provided for each lab in Appendix II).

Since the main focus of this ringtest was to study compliance with the proposed validity criteria of the life-cycle test protocol, the following sections will go into each of the criteria separately.

In total, there are eleven validity criteria: three related to physico-chemical parameters (temperature, oxygen and pH); four regarding emergence and development (for each generation separately) and four criteria related to sex ratio (for each generation) and reproduction (fecundity and fertility). The physico-chemical criteria are copied from the OECD TGs 218/219 and therefore not new, but harder to maintain since the life cycle test is of longer duration. The biological criteria concerning emergence and development are also not new, but again harder to achieve when two instead of one generation is studied. The wording of the development criterion was slightly changed to better reflect its practical use (this was also a comment from a national expert). The criteria on sex ratio and reproduction are new in this guideline and as such more in focus than the previous criteria.

4.2. Temperature, oxygen and pH

The physico-chemical data on water temperature and pH are presented in Figures 4 and 5 for each laboratory and generation.

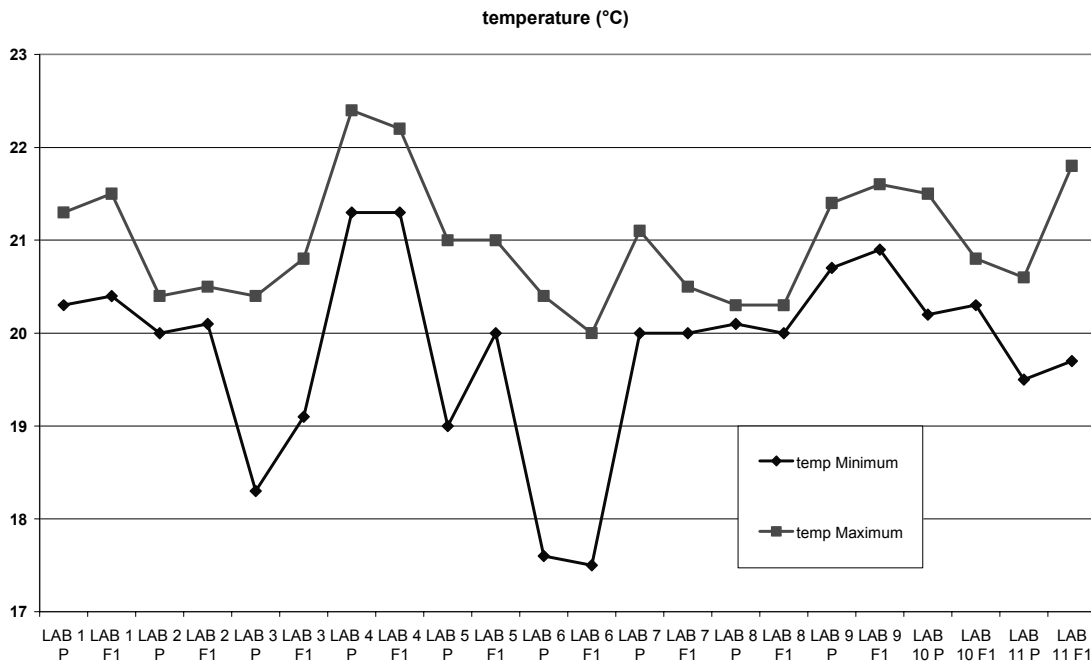


Figure 4. Minimum and maximum measured water temperatures for each laboratory and generation

Temperature was mostly well under control, but Lab 3, 6 and 11 did have some difficulties, most pronounced in Lab 6 for both generations. This participant reported values even lower than those depicted in Figure 4 of around 14 - 15°C in the water bath on multiple occasions. Therefore, this test is considered to fail the temperature validity criterion, which requires a water temperature not differing more than $\pm 1.0^{\circ}\text{C}$. There was only one issue with the water temperature in Lab 3 at the start of the test (P generation). The value was quite low (18.3°C) but it apparently did not affect the development of the animals during the test. In fact, the development rate for the P generation in Lab 3 was one of the highest. Therefore, this measurement at the start of the test is not considered to invalidate the test. In Lab 11 in the F1 generation the spread in temperature values was too high, but like the previous case this was a single event and not likely to have impacted the outcome of the test.

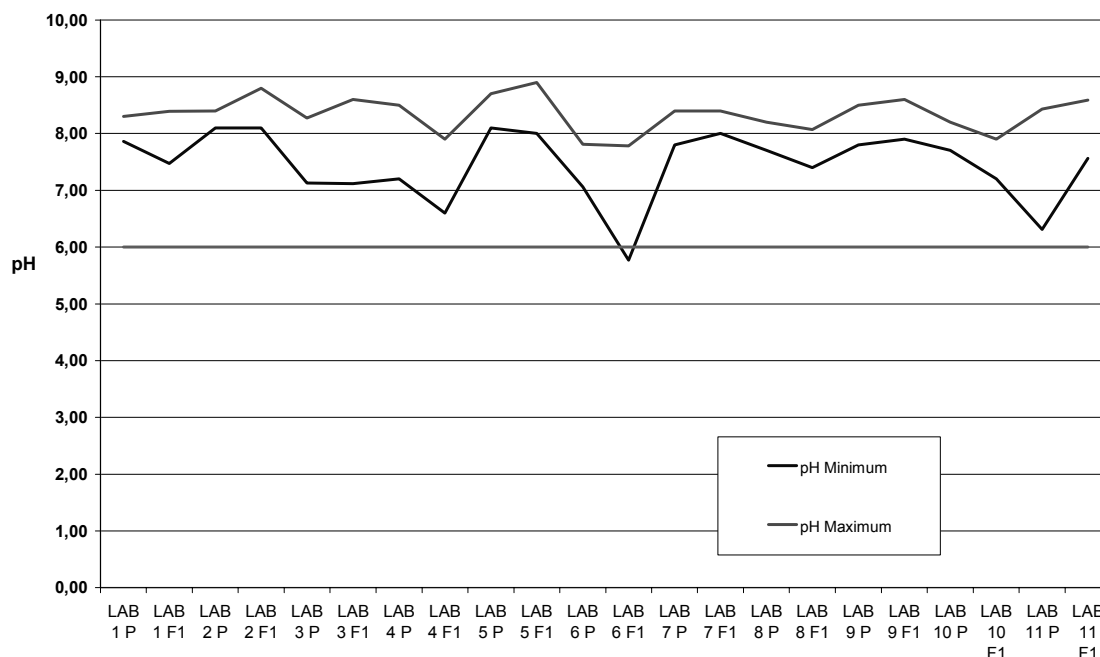


Figure 5. Minimum and maximum measured pH values for each laboratory and generation

The pH is the least critical water parameter and was well under control, except for Lab 6 in the F1 generation where, towards the end of the experiment, lower values were measured.

The dissolved O₂ values (not shown) were above 60% ASV (i.e. 5.46 mg/L at 20°C under standard atmospheric pressure in freshwater), except for Lab 3 (2.00 and 2.13 mg/L at the end of the P generation) and Lab 9 (3.03 mg/L in the F1 generation). These lower oxygen values were of short duration and not likely to have been of influence on the test outcome. In the case of Lab 3 all midges had already emerged from these vessels.

4.3. Emergence and development

The emergence ratio for the P and F1 generations are presented in Figures 6 and 7, respectively. They show that the validity criteria for emergence (a mean value of 70%) in both generations were satisfied. Since the criterion applies to the overall mean emergence and not to each separate vessel or each separate cage it is allowed that some vessels demonstrate lower than 70% emergence (i.e. less than 14 midges) as long as other values are higher, so that the average is > 70%. Further, from Figure 7 it can be seen that in the F1 generation in Lab 6 and to a lesser extent also in Lab 4, emergence rates were lower than the overall average. In Lab 6 the mean was 77%.

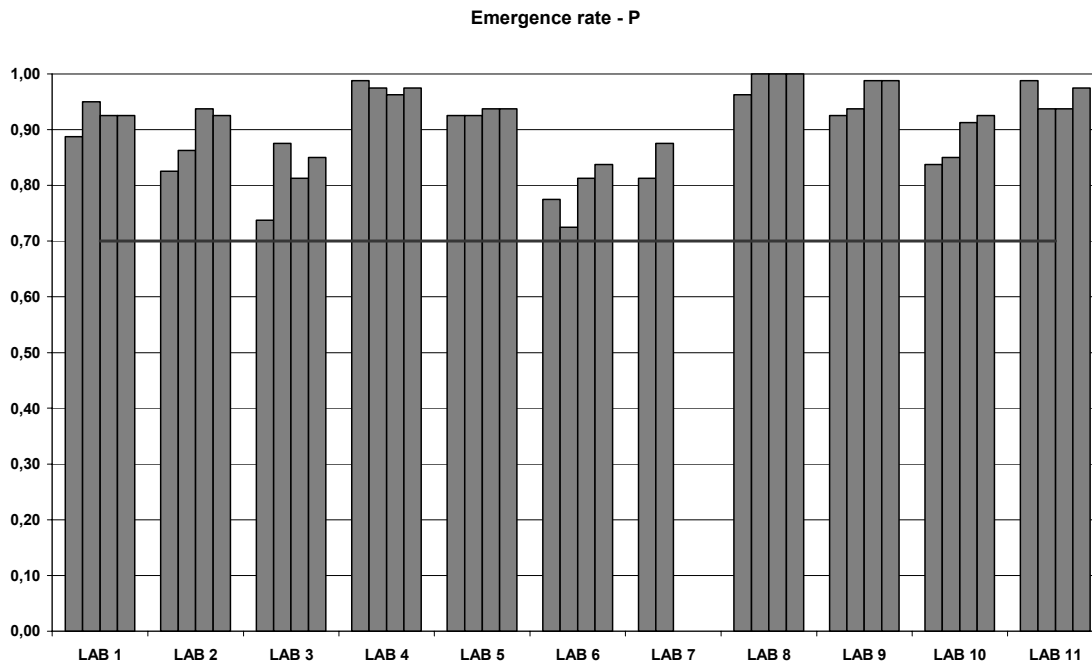


Figure 6. Emergence of the P generation for each laboratory and each cage (i.e. the mean value of four vessels)

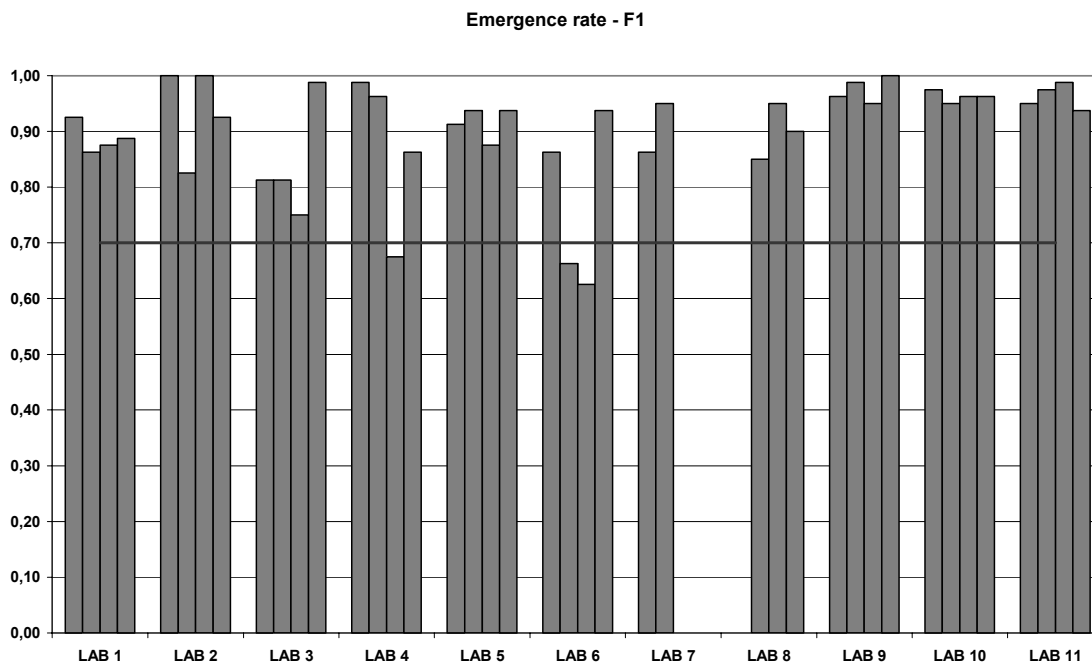


Figure 7. Emergence of the F1 generation for each laboratory and each cage (i.e. the mean value of four vessels)

There is no fixed number for judging the validity the development rate itself, but instead the guideline says that emergence in the control(s) should occur between day 12 and 23 after inserting the larvae into the test vessels. This criterion was slightly adapted to reflect current practice, that single emergence observations on or after day 23 do not invalidate the test (emergence on or before day 12 only rarely occurs). In the

ringtest no emergence before day 12 was observed. Overall, over 95% of the midges of both generations emerged in the time window defined by the validity criterion for development.

4.4. Sex ratio and reproduction

The mean sex ratio (expressed as the male fraction) of the fully emerged and alive adults in the P generation and in the F1 generation were between 0.41 and 0.58 (results not shown), thus fulfilling the validity criterion of the guideline. As expected, no large deviations from the natural sex ratio of 1:1 were observed. However, on an individual cage basis the validity criterion was sometimes breached.

According to the draft guideline, the test is valid when at least 0.6 egg ropes per female are produced (in each breeding cage) and in addition when 60% of the produced egg ropes are fertile. So, taking these two criteria together would mean that at least $(0.6 \times 0.6 =)$ 0.36 fertile egg ropes per female should be produced.

The number of egg ropes per female and the number of fertile egg ropes per female for each of the laboratories are presented in Figure 8 below.

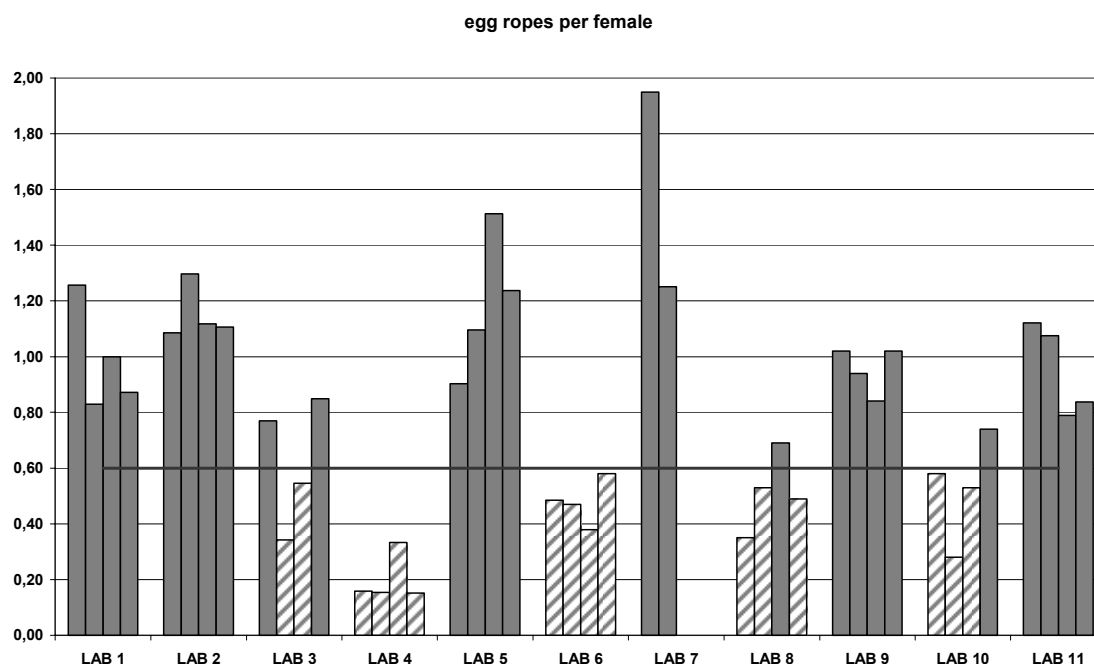


Figure 8. Egg ropes per female in the P generation for each breeding cage

Six of the 11 labs had no problems in achieving the validity criterion of 0.6 egg ropes per female. The highest number of egg ropes occurred in Lab 7, in which the adult midges were fed (information by the participant as a deviation from the draft GL). This observation fits to the results of the adult feeding test, in which it was shown that fed midges have longer life spans and an increase in reproductive output.

On the other hand, five labs had problems in achieving a sufficient number of egg ropes. Lab 3 failed for 2 of the 4 breeding cages, which may be explained by their use of small breeding cages of 15 x 22 x 22 cm, which deviates from the size recommended in the draft guideline: 30 x 30 x 30 cm. Likely the cage was too small for swarming. In addition, this participant had some (minor) issues with phys-chem parameters (see section 4.2). Lab 3 regularly performs chironomid reproduction studies using larger cages and provided

data on their historic controls, which demonstrate that they normally achieve the validity criterion easily (see Figure 9).

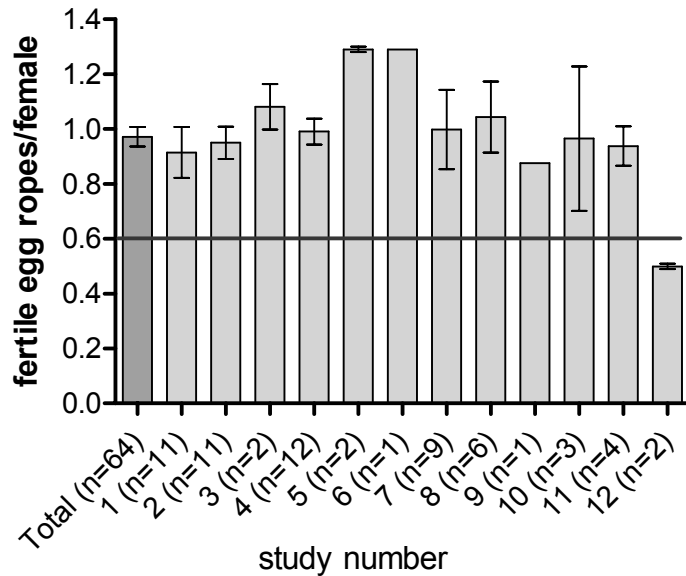


Figure 9. Fertile egg ropes per female (historic controls from Lab 3)

Lab 4 generated the lowest numbers of egg ropes and informed us that soon after the start of the ringtest their culture was replenished with fresh egg ropes from an external culture, since they experienced problems with their chironomid culture quality. Also, life span of the midges in the breeding cages appeared to be short (not quantified). The reproduction in Lab 6 obviously suffered from the low temperatures, for which this test was already invalidated. In labs 8 and 10 the partial failure to meet the validity criteria was not obvious at first, but a detailed inspection of the P generation emergence data showed that the mean emergence time of males and females are ca. 4 and 5 days apart, respectively. Both participants confirmed that this difference was unusually high in their experience and likely to have impacted the egg production. Indeed a difference of 4 to 5 days is likely to be of influence on reproductive parameters, since it impacts the sex ratio in the swarm and fitness of the males.

The fertile fraction of the total egg ropes is shown in Figure 10.

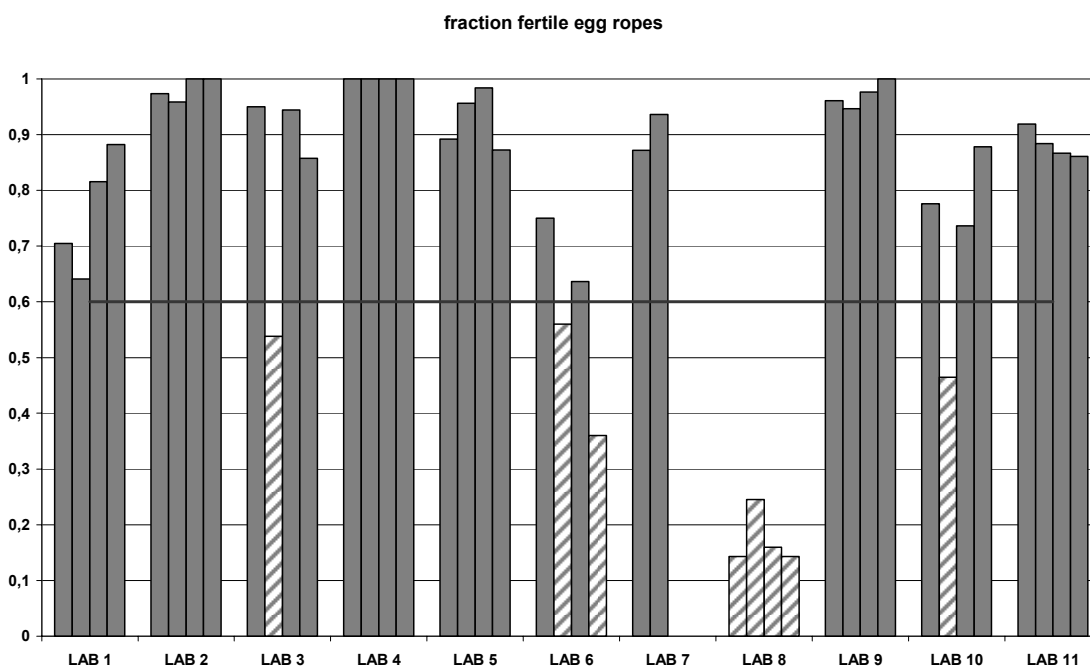


Figure 10. Fraction of fertile egg ropes for each breeding cage

Seven of the 11 labs had no problem in achieving the required fertile fraction of 0.6. Interestingly, in lab 4, where less than 0.6 egg ropes per female were produced (see Figure 8) all of these egg ropes were fertile. Labs 3 and 10 had one breeding cage each with a lower fraction, while lab 6 had 2 cages with a fertile fraction below the validity criterion. In lab 8, none of the breeding cages fulfilled this validity criterion. Thus, apart from Lab 4, all labs that experienced problems with the fecundity criterion, had also problems with the fertility criterion. It should be noted though that the problems with fertility are less than those with fecundity.

5. CONCLUSIONS

5.1. General

The motivation for the development of a chironomid full life-cycle toxicity test has been detailed in the *Introduction* section and will not be repeated here. More important are the conclusions that may be drawn from the supporting experimental work that has been conducted over the last years. Overall, it was demonstrated that both spiked water and spiked sediment designs can be conducted successfully. The larger ringtest with controls and the spin off projects on swarm size, sex ratio and adult feeding provided further insights in and improvements for the life-cycle test.

5.2. Sex ratio and swarm size

The sex ratio in the swarm may have an influence on the egg rope fertility (when fewer males than females are present) and thus to set a validity criterion on the male fraction is justified. Accordingly, when in a test effects on fertility are observed an inspection of the sex ratio may assist in determining if these effects are caused by the lower fraction of males (indirect effect) or by direct compound toxicity. This aspect is highlighted, since males tend to be more sensitive than females (as observed in the lindane, PPF and fenoxycarb experiments) and thus fertility may be impacted by this. The higher sensitivity of males may be related to their accelerated development rate as compared to females (i.e. the characteristic protandry, causing males to emerge earlier than females). The natural phenomenon of protandry also causes a gradual shift in the sex ratio in the breeding cage from 100% males at the beginning of emergence to 100% females at the end (when all males have died and the last emerged females are still alive). This natural shift may be the cause for the observation of infertile egg ropes, which mostly occur at the end of the reproduction phase. The adult feeding experiment demonstrated that life span of emerged adults may be extended by offering them sugar solution as a food source. This would also contribute to maintaining a sex ratio of 1:1 in the swarm for a longer time period and thereby stimulating reproduction, both as fecundity and fertility.

When laboratories had problems with the validity criterion that was proposed for the sex ratio (i.e. male fraction) it was always the case that fewer males were present, never too many males (i.e. more than 60% males never occurred). Therefore, it may be considered to adjust this validity criterion.

Additionally it was shown that the swarm size has an influence on the egg rope fertility (in a larger swarm more fertile egg ropes are produced). This aspect is covered by setting a validity criterion on the control emergence rate of the P generation, ensuring a swarm of at least $(4 \times 20 \times 0.7 \Rightarrow) 56$ individuals in one cage.

5.3. Ringtest on the controls

The ringtest on the controls yielded valuable information for evaluation and improvement of the life-cycle test guideline. First it was demonstrated that the biological validity criteria on emergence and development that were adapted from OECD TGs 218/219 were all met by the ring test participants. The same holds true for the sex ratio criteria.

Some problems occurred with the physico-chemical water parameters oxygen, pH and temperature, that were also adapted from OECD TGs 218/219, but apart from the temperature problems in Lab 6, these were all minor issues. The test of Lab 6 had to be invalidated on these grounds.

The main focus of the ringtest was on reproduction, for which 6 out of 11 labs fulfilled the minimum requirement. However, of the five tests that failed to meet the validity criteria, the tests of two labs were excluded from the analysis, due to the large temperature range (lab 6) and the use of too small breeding cages (lab 3). Hence, 3 tests out of 9 did not comply with the validity criterion for reproduction. For each of these three tests (labs 4, 8 and 10) plausible explanations were given and/or found that can explain the low observed fecundity. The explanation for lab 4 was the poor quality of the culture (which was replenished with fresh egg ropes soon thereafter). Since it is known that laboratory cultures of *C. riparius* are sensitive to genetic impoverishment due to inbreeding (Nowak et al., 2007) it is important to introduce new blood regularly. The quality of the animals could potentially be checked by performing regularly an acute toxicity test with a reference compound, such as lindane or potassium-dichromate.

In case of labs 8 and 10 the large difference between mean emergence times of males and females (4 and 5 days, respectively) was likely responsible for the low fecundity. Normally this difference is ca. 2 days, but when it becomes larger, the males are of lower fitness when the females are introduced to the breeding cages. Soon thereafter they begin to die, while female emergence is not complete yet. This issue can be handled by feeding the adults, since it significantly extends their life span and stimulates reproduction.

All in all, the life-cycle test was successfully conducted in the majority of the laboratories.

5.4. Effect patterns and sensitivity

The pattern of effects induced by juvenile hormone mimics (PPF and fenoxycarb) was different from that induced by the insecticide lindane. As further data become available in the future it would be interesting to study if specific effect patterns correlate with specific (insecticidal) modes of action.

In terms of sensitivity, the addition of a 2nd generation made the test more sensitive than the OECD test 218 or 219 as demonstrated with the test on juvenile hormone mimics. Interestingly, reproduction was mostly not the most sensitive endpoint, but equally sensitive or less sensitive than emergence and/or development (see PPF and lindane experiments). Due to statistical issues with regard to the reduced number of replicates, an EC_X approach may be more useful for the reproduction assessments, but there needs to be an agreement on the level of X (the size of the effect) first to facilitate a comparison with obtained NOEC values.

For emergence and development, it should also be considered that this test is potentially more sensitive than the classic chronic OECD chironomid test, because it employs eight replicates per treatment instead of the usual six. Thus, its discriminating power to pick up smaller effects is increased.

Further, since the life-cycle test is designed to assess in total eight endpoints there is the increased possibility of statistical significant effects, which may be merely due to chance (i.e. type I error). Therefore, the final outcome of the test should be based on an integrated interpretation of the collected endpoints, instead of focussing on a single value.

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APPENDIX I

Chemical analysis of lindane in spiked sediment

Chemical Analysis

With lindane a spiked sediment chironomid life-cycle test has been performed. Due to the physico-chemical properties of the test item and the used spiking procedure the focus of the accompanying chemical analysis has been on the sediment.

Before the biological phase of the chironomid life cycle tests with lindane were initiated, the three participating laboratories agreed on performing an accompanying chemical analysis of lindane in the sediment. The accompanying chemical analysis was performed non-GLP by the contract laboratory Currenta in Leverkusen, Germany, according to the method 2030-0608001-05D (Bestimmung von polychlorierten Biphenylen (PCB) aus Extrakten von Böden und Abfall") which is equivalent to DIN 38414-20. The equivalence of the method 2030-0608001-05D has been verified by a ring test.

The method and laboratory performing the chemical analysis was not yet selected when the tests were started. Therefore the minimum sample size for the sediment was not exactly known to the participating laboratories. Unfortunately the sediment sample size delivered by the University of Landau did not fulfill the requirements of the used analytical method.

Therefore the samples of the parental and filial generation for the different measurement points in time had to be combined (e.g. Day 0 FI and Day 0 P generation). Thereby the available sample amounts were increased, but still did not reach the standard amount, resulting in higher limits of detection. Nevertheless, by using this procedure a determination of the lindane content was possible.

The nominal test concentrations used in the three participating laboratories differ slightly as the results of the laboratories who started with the test were used to adapt the concentrations in the laboratories which started later.

Table 1. Nominal lindane concentrations investigated in the three participating laboratories

Laboratory	[mg/kg dry sediment]
BASF	0.0375
BASF	0.075
BASF	0.15
BASF	0.3
Bayer CropScience	0.03125
Bayer CropScience	0.0625
Bayer CropScience	0.125
Bayer CropScience	0.25
Bayer CropScience	0.5
University of Landau	0.0375
University of Landau	0.075
University of Landau	0.15
University of Landau	0.3
University of Landau	0.6

Method

Extraction: A defined amount of the sediment sample (5 – 50 g depending on the available sample amount) was extracted for 2 hours by using acetone/cyclohexane (1:1). The samples which have been reanalyzed were treated with acetone/cyclohexane (1:1) for a 24 hour period to increase the yield of the extraction as far as possible.

The samples gained by the extraction step were analyzed via a Gas-Chromatograph-Mass-Spectrometer (GC-MS) according to the method 2030-0608001-05D ("Bestimmung von polychlorierten Biphenylen (PCB) aus Extrakten von Böden und Abfall") which is equivalent to DIN 38414-20. The equivalence of the method 2030-0608001-05D has been verified by a ring test. For that purpose an external lindane standard has been used and the method has been validated for precision, correctness, linearity, limit of detection and limit of quantification.

The limit of detection (LoD) was 1 µg/kg in case of 50 g sediment samples. This was the case for samples originating from BASF and BCS. For the smaller sediment samples the LoD was 7 - 22 µg/kg.

Results

The results of the chemical analysis are summarized in the Tables 2 to 5 presented below.

Table 2. Results of the lindane analysis for sediment samples from Landau

Sample	Generation	Day	nominal concentration [mg/kg DS]	Lindane [mg/kg DS]	% of nominal
S1 plus S13	P & FI	0	solvent control	<0.003	/
S2 plus S14	P & FI	0	0.0375	0.0259	69.1
S3 plus S15	P & FI	0	0.075	0.0588	78.4
S4 plus S16	P & FI	0	0.15	0.104	69.0
S5	P	0	0.3	0.259	86.3
S6	P	0	0.6	0.503	83.8
S7	P	13	solvent control	<0.007	/
S8	P	13	0.0375	0.0233	62.1
S9	P	13	0.075	0.0443	59.1
S10	P	13	0.15	0.132	88.0
S11	P	13	0.3	0.227	75.7
S12	P	13	0.6	0.455	75.8
S17/S23/S24/S33	P & FI	28	solvent control	<0.,002	/
S18/S25/S26/S34	P & FI	28	0.0375	0.0229	61.1
S19/S27/S28/S35	P & FI	28	0.075	0.0306	40.8
S20/S29/S30/S36	P & FI	28	0.15	0.0691	46.1
S21/S31/S32/	P & FI	28	0.3	0.119	39.7
S22	P	28	0.6	0.318	53.0

DS = Dry Sediment; Day = experimental day, minus values are used for days before insertion of the larvae, P = parental generation; FI = second generation

Table 3. Results of the lindane analysis for sediment samples from BASF

Sample	Generation	Day	nominal concentration [mg/kg DS]	Lindane [mg/kg DS]	% of nominal
DAI 0/ DAT 2/ 5.11.08	P	0	solvent control	<0.001	/
DAI 0/ DAT 2 / 5.11.08	P	0	0.0375	0.046	122.4
DAI 0/ DAT 2 / 5.11.08	P	0	0.075	0.079	105.5
DAI 0/ DAT 2 . / 5.11.08	P	0	0.15	0.174	116.0
DAI 0/ DAT 2 / 5.11.08	P	0	0.3	0.347	115.7
DAI 28/ DAT 30 / 3.12.08	P	28	solvent control	<0.001	/
DAI 28/ DAT 30 / 3.12.08	P	28	0.0375	0.039	104.5
DAI 28/ DAT 30 / 3.12.08	P	28	0.075	0.074	98.9
DAI 28/ DAT 30 / 3.12.08	P	28	0.15	0.146	97.3
DAI 28/ DAT 30 / 3.12.08	P	28	0.3	0.284	94.7
DAI 14/ DAT 16 Cryst. 3.12.08	P	14	solvent control	<0.001	/
DAI 14/ DAT 16 Cryst. 3.12.08	P	14	0.0375	0.139	370.7
DAI 14/ DAT 16 Cryst. 3.12.08	P	14	0.075	0.256	341.3
DAI 14/ DAT 16 Cryst. 3.12.08	P	14	0.15	0.260	173.3
DAI 0/ DAT 2 28.11.08	F1	0	solvent control	<0.001	/
DAI 0/ DAT 2 28.11.08	FI	0	0.0375	0.018	48.3
DAI 0/ DAT 2 28.11.08	FI	0	0.075	0.041	55.1
DAI 0/ DAT 2 28.11.08	FI	0	0.15	0.158	105.3
DAI 21 DAT 23 19.12.08	FI	21	solvent control	<0.001	
DAI 21 DAT 23 19.12.08	FI	21	0.0375	0.015	41.1
DAI 21 DAT 23 19.12.08	FI	21	0.075	0.026	35.1
DAI 21 DAT 23 19.12.08	FI	21	0.15	0.096	64.3

DS = Dry Sediment; Day = experimental day, minus values are used for days before insertion of the larvae, P = parental generation; FI = second generation

Table 4. Results of the lindane analysis for sediment samples from BCS

Sample	Generation	Day	nominal concentration [mg/kg DS]	Lindane [mg/kg DS]	% of nominal
F0/0d/3	P	0	solvent control	<0.001	/
F0/0d/6	P	0	0.03125	0.007	22.4
F0/0d/9	P	0	0.0625	0.025	39.5
F0/0d/12	P	0	0.125	0.062	49.8
F0/0d/15	P	0	0.25	0.100	39.8
F0/0d/18	P	0	0.5	0.215	43.0
F0/7d/3	P	7	solvent control	<0.001	/
F0/7d/6	P	7	0.03125	0.007	21.4
F0/7d/9	P	7	0.0625	0.019	30.6
F0/7d/12	P	7	0.125	0.053	42.7
F0/7d/15	P	7	0.25	0.111	44.4
F0/7d/18	P	7	0.5	0.232	46.4
F0/28d/3	P	28	solvent control	<0.001	/
F0/28d/6	P	28	0.03125	0.005	15.7
F0/28d/9	P	28	0.0625	0.017	27.7
F0/28d/12	P	28	0.125	0.044	35.4
F0/28d/15	P	28	0.25	0.097	38.7
F0/28d/18	P	28	0.5	0.176	35.2
F1/0d/3	FI	0	solvent control	<0.001	/
F1/0d/6	FI	0	0.03125	0.009	29.8
F1/0d/9	FI	0	0.0625	0.026	41.3
F1/0d/12	FI	0	0.125	0.059	47.2
F1/0d/15	FI	0	0.25	0.114	45.6
F1/7d/3	FI	7	solvent control	<0.001	/
F1/7d/6	FI	7	0.03125	0.008	25.3
F1/7d/9	FI	7	0.0625	0.021	33.8
F1/7d/12	FI	7	0.125	0.054	43.2
F1/7d/15	FI	7	0.25	0.094	37.4
F1/28d/3	FI	28	solvent control	<0.001	/
F1/28d/6	FI	28	0.03125	0.007	23.4
F1/28d/9	FI	28	0.0625	0.020	31.4
F1/28d/12	FI	28	0.125	0.043	34.4
F1/28d/15	FI	28	0.25	0.092	36.7

DS = Dry Sediment; Day = experimental day, minus values are used for days before insertion of the larvae, P = parental generation; FI = second generation

The results obtained for sediment samples from BASF and the University of Landau revealed recoveries between 69 and 122.4% of nominal at the start of the exposure period. However, the sediment samples from BCS resulted in lower values. Due to the limited available sediment samples amounts it was not possible to reanalyze all samples with recoveries below 80% of nominal. Lindane is known to be a stable compound which adsorbs to the sediment when applied to water sediment systems. Based on its physico-chemical properties high recovery values were expected. In case of BCS, samples on the day of spiking were taken as well. For the parental generation this was day -2 and for the filial generation this was day -3. For these two sampling days sufficient material was available for a reanalysis. As the original values were lower as expected it was decided to enlarge the extraction period from 2 hours to 24 hours. Extraction was carried out as “Soxhlet-extraction” using the acetone/cyclohexane (1:1) for 24 h. The new values increased as presented in Table 5.

Based on these additional results it can be assumed that the relatively low recoveries observed especially for the BCS samples was related to a strong binding of the test item lindane to the sediment, which resulted in a relatively low extraction yield. One reason for the observed strong binding is presumably the relatively long storage time (ca. one year) of the deep frozen samples before measurement.

The results obtained for the samples from BCS on day -2 and day -3 are presented in Table 5.

Table 5. Results of the lindane analysis for sediment samples taken at day of spiking / BCS

Laboratory	Sample	Generation	Day	nominal concentration [mg/kg DS]	Lindane [mg/kg DS]	% of nominal
BCS	F0/2d/3	P	-2	solvent control	<0,001	/
BCS	F0/2d/6	P	-2	0.03125	0.002	6.4
BCS	F0/2d/9	P	-2	0.0625	0.007	11.2
BCS	F0/2d/12	P	-2	0.125	0.017	13.3
BCS	F0/2d/15	P	-2	0.25	0.059	23.8
BCS	F0/2d/18	P	-2	0.5	0.093	18.5
Results of the second analysis after 24 hours of extraction						
BCS	F0/2d/3	P	-2	solvent control	<0,001	/
BCS	F0/2d/6	P	-2	0.03125	0.009	28.8
BCS	F0/2d/9	P	-2	0.0625	0.0257	41.1
BCS	F0/2d/12	P	-2	0.125	0.0692	55.4
BCS	F0/2d/15	P	-2	0.25	0.191	76.4
BCS	F0/2d/18	P	-2	0.5	0.399	79.8
Results of the second analysis after 24 hours of extraction						
BCS	F1/3d/3	FI	-3	solvent control	<0,001	/
BCS	F1/3d/6	FI	-3	0.03125	0.006	17.6
BCS	F1/3d/9	FI	-3	0.0625	0.012	19.2
BCS	F1/3d/12	FI	-3	0.125	0.027	21.5
BCS	F1/3d/15	FI	-3	0.25	0.042	16.6
Results of the second analysis after 24 hours of extraction						
BCS	F1/3d/3	FI	-3	solvent control	<0,001	/
BCS	F1/3d/6	FI	-3	0.03125	0.0134	42.9
BCS	F1/3d/9	FI	-3	0.0625	0.0347	55.5
BCS	F1/3d/12	FI	-3	0.125	0.0842	67.4
BCS	F1/3d/15	FI	-3	0.25	0.175	70

With only a few exceptions, the biological results were similar in all three participating laboratories. Lindane is known to be stable under the used test and storage conditions. The samples from BASF and the University of Landau resulted in recoveries between 69 and 122.4% of nominal. The reanalysis of the BCS samples by using a longer extraction period resulted in clearly higher extraction yields. This demonstrates that the strong binding of lindane to sediment particles resulted in a non-complete and thereby non-consistent extraction of the test item. Therefore it can be assumed that the test item has been correctly applied in all three participating laboratories.

Taking these results and arguments into consideration it was decided to base the biological results on the nominal concentrations.

APPENDIX II

Raw data (biology) of each participating laboratory:

1. Emergence P generation
2. Emergence F1 generation
3. Reproduction

LAB 1 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)					
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27
gender	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f						
1	cage A					1	3	1	1	2	1	1	1	1	3			1																17	9	8	0.85	1.17	0.0608	
2						2	1		4	1	1	1		2	1	1			5														20	10	10	1.00	1.57	0.0584		
3							4	3				1		1	2			5																17	10	7	0.85	1.17	0.0589	
4						1	3		3		2	3	1	1	3																			17	7	10	0.85	1.17	0.0620	
5	cage B					1	2		2	2	1	1	1	4	3		2		1															20	13	7	1.00	1.57	0.0595	
6						1	3		2		1	1		2	5		2		1		1													19	12	7	0.95	1.35	0.0586	
7							3		8		1	1		1	2		1		2				1											20	8	12	1.00	1.57	0.0601	
8						1	1		1	2		6		1	2		3																		17	14	3	0.85	1.17	0.0600
9	cage C						4		4		4	1		2	1				2															18	6	12	0.90	1.25	0.0613	
10						1	2		5	1	1	3		2	1		2																	18	9	9	0.90	1.25	0.0618	
11						1	4		1	2		3		2	2		1		2																18	12	6	0.90	1.25	0.0609
12					1		1	2	1	4	3	1	3		1		1		2																20	11	9	1.00	1.57	0.0634
13	cage D					4	3		2	2		2	3	1	1																			18	8	10	0.90	1.25	0.0646	
14							3			2		5	2	2		1		1																16	13	3	0.80	1.11	0.0601	
15					1		2	6		3		1	1		4	2																		20	7	13	1.00	1.57	0.0647	
16						5	3		1	1	1	4		2		1			2																21	11	10	1.00	1.57	0.0629

* DAI = Day After Insertion of the larvae

LAB 1 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)								
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28	
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f								
17	cage A							2	1	2			1	1	4	1			2	2															19	12	7	0.95	1.35	0.0563			
18								3			1	1	1	2	2	2		1	1		1														19	10	9	0.95	1.35	0.0571			
19								6		4	1		1	2	1	3																				19	10	9	0.95	1.35	0.0654		
20								5	2			1		2	1	2		3	1																	17	9	8	0.85	1.17	0.0635		
21	cage B							1	2	2		1			3	1	2	2	2	1																17	10	7	0.85	1.17	0.0578		
22								1	3	2	1		3	1	1		3	1																		17	10	7	0.85	1.17	0.0603		
23												1	6	1		1		2	1	4	1	1															18	9	9	0.90	1.25	0.0650	
24											1	2	3	1		1	1		2	2	3	1															17	10	7	0.85	1.17	0.0618	
25	cage C							1	5					1		3	1	3	1																		15	8	7	0.75	1.05	0.0612	
26								3	3			4		2	1	2		2	1		1																20	13	7	1.00	1.57	0.0617	
27									1	3			3	1			3	1	2	1		2																17	11	6	0.85	1.17	0.0599
28										3	2			2	1	3	1	1		4	1																	18	10	8	0.90	1.25	0.0582
29	cage D							2	4			3	1	2		2	2																				16	9	7	0.80	1.11	0.0639	
30								2	4	3	1	1	2		1		3	1																			19	9	10	0.95	1.35	0.0620	
31									4	1	1	1		3			5	2		1																		19	13	6	0.95	1.35	0.0599
32									2	7	1	1	1	2		1				2																		17	6	11	0.85	1.17	0.0648

* DAI = Day After Insertion of the larvae

LAB 1 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
16.08.09	18	4			1	5	4			1	5	5			5	8					8
17.08.09	19	5			1	6	6		1	1	8	10			10	5					5
18.08.09	20		1			1	6			1	7	5			5	11					11
19.08.09	21	3	2		1	6	5	1	1	7	4			1	5	4	3				7
20.08.09	22	9		2	1	12	4	1	1	6	1	3	1	1	5	1					1
21.08.09	23	1	1			2		1		1		1	1	2							0
22.08.09	24	2				2		2		2		1		1			1				1
23.08.09	25	1	1		1	3			2	2		1		1							0
24.08.09	26	4	1		1	6				0	1		1	2							0
25.08.09	27		1			1		1		1			1	1							0
26.08.09	28					0				0	1			1	1	1					1
	total	29	7	2	6	44	25	6	2	6	39	27	6	4	1	38	30	4	0	0	34
			females in cage	35	egg ropes per female	1.26		females in cage	47	egg ropes per female	0.83		females in cage	38	egg ropes per female	1.00		females in cage	39	egg ropes per female	0.87
Fertile egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
16.08.09	18	4				4	4			4	5	5			5	8					8
17.08.09	19	5				5	5		1	1	7	9			9	5					5
18.08.09	20		1			1	3			3	5			5	11						11
19.08.09	21	2	2		1	5	4	1	1	6	4			4	4	1					5
20.08.09	22	6		1		7	1		1	2	1	3			4						0
21.08.09	23	1	1			2		1		1		1	1	2							0
22.08.09	24	2				2		2		2		1		1			1				1
23.08.09	25	1	1		1	3				0		1		1							0
24.08.09	26		1		1	2				0				0							0
	total	21	6	1	3	31	17	4	2	2	25	24	6	1	0	31	28	2	0	0	30
			females in cage	35	fertile egg ropes per female	0.89		females in cage	47	fertile egg ropes per female	0.53		females in cage	38	fertile egg ropes per female	0.82		females in cage	39	fertile egg ropes per female	0.77
			males in cage	35	male fraction in cage	0.50		males in cage	29	male fraction in cage	0.38		males in cage	34	male fraction in cage	0.47		males in cage	36	male fraction in cage	0.48

ENV/JM/MONO(2010)35
LAB 2 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)								
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28	
		gender	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m							f	m	f	m	f			
1	cage A								2	2	3	2	4	1																					14	7	7	0.70	0.99	0.0609			
2									3	3	1	5	3	2	1																				18	11	7	0.90	1.25	0.0608			
3										3	5	3	3	3																						17	6	11	0.85	1.17	0.0626		
4										3	2	2	1	3	2	2	1	1																			17	11	6	0.85	1.17	0.0606	
5	cage B								3	6	1	4	3			1	2																			20	10	10	1.00	1.57	0.0609		
6									3	2			1	4	4	1	1																			16	10	6	0.80	1.11	0.0586		
7										1	7	1	2	3	1	3	1																				19	9	10	0.95	1.35	0.0601	
8											5	1	4	4																							14	8	6	0.70	0.99	0.0610	
9	cage C								6	3	1	3	3	1	3																					20	8	12	1.00	1.57	0.0627		
10										2	2	3	3	1	2	1	2	1																		17	7	10	0.85	1.17	0.0570		
11										3	7		4	1	2		2	1																			20	10	10	1.00	1.57	0.0605	
12										1	2	2	5	2	5		1																				18	9	9	0.90	1.25	0.0603	
13	cage D								2	3	1	1	4	6		1																				18	12	6	0.90	1.25	0.0607		
14									4	4	2	3	5		1																					19	11	8	0.95	1.35	0.0622		
15										5	2	4	2	2			1	1																		17	5	12	0.85	1.17	0.0616		
16										2	8		3	5				2																			20	10	10	1.00	1.57	0.0610	

* DAI = Day After Insertion of the larvae

LAB 2 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)					
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f					
17	cage A						2		6		2	6		3																					20	10	10	1.00	1.57	0.0657
18									6		4	3		1	4		2																		20	9	11	1.00	1.57	0.0641
19					1		1		7		4	1		1	4																				20	6	14	1.00	1.57	0.0658
20									2		6			1	2		3	1		5															20	8	12	1.00	1.57	0.0603
21*)	cage B															1		2				1		2										6	1	5	0.30	0.58	0.0523	
22							5		5	1		1	4		3			1																	20	6	14	1.00	1.57	0.0673
23					1		2		4	1		2	1		2			2		5															20	11	9	1.00	1.57	0.0636
24							2		4	1		4	4		4			1																		20	10	10	1.00	1.57
25	cage C						3		3		2	2				5		3		1	1														20	11	9	1.00	1.57	0.0619
26					2			3		8	1		3		2			1																	20	7	13	1.00	1.57	0.0649
27							5		5	3		4		2						1															20	10	10	1.00	1.57	0.0676
28							1		9		2	2		2		1	1		2																	20	7	13	1.00	1.57
29	cage D						5		4	2		1	4		1	1																			18	7	11	0.90	1.25	0.0682
30					3		2		6	1		2	1		1																				16	4	12	0.80	1.11	0.0701
31							5		6	1		1	4		3																				20	8	12	1.00	1.57	0.0679
32								2		4	1		4		1				4		2	2													20	7	13	1.00	1.57	0.0591

* DAI = Day After Insertion of the larvae

*) Due to unknown reasons the emergence in this test vessel was reduced. For statistical evaluation it may be excluded.

ENV/JM/MONO(2010)35

LAB 2 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
24.09.09	17	1				1	1				1				0						0
25.09.09	18	1				1	5			5	1				1	1					1
26.09.09	19	9	2			11	8	1		9	15				15	14					14
27.09.09	20	9				9	9			9	5				5	12					12
28.09.09	21	6				6	6			6	4	1			5	2					2
29.09.09	22		4			4	4	7		11	1	6			7	1	8				9
30.09.09	23		4			4		6		6		4			4	1	3				4
01.10.09	24		2			2		1		1	1				1						0
	total	26	12	0	0	38	33	15	0	0	48	27	11	0	0	38	31	11	0	0	42
			females in cage	35	egg ropes per female	1.09		females in cage	37	egg ropes per female	1.30		females in cage	34	egg ropes per female	1.12		females in cage	38	egg ropes per female	1.11
Fertile egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
24.09.09	17	1				1	1				1				0						0
25.09.09	18	1				1	5			5	1				1	1					1
26.09.09	19	9	2			11	8	1		9	15				15	14					14
27.09.09	20	9				9	9			9	5				5	12					12
28.09.09	21	5				5	4			4	4	1			5	2					2
29.09.09	22		4			4	4	7		11	1	6			7	1	8				9
30.09.09	23		4			4		6		6		4			4	1	3				4
01.10.09	24		2			2		1		1	1				1						0
	total	25	12	0	0	37	31	15	0	0	46	27	11	0	0	38	31	11	0	0	42
			females in cage	35	fertile egg ropes per female	1.06		females in cage	37	fertile egg ropes per female	1.24		females in cage	34	fertile egg ropes per female	1.12		females in cage	38	fertile egg ropes per female	1.11
			males in cage	31	male fraction in cage	0.47		males in cage	32	male fraction in cage	0.46		males in cage	41	male fraction in cage	0.55		males in cage	36	male fraction in cage	0.486

LAB 3 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)								
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28	
		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f							m	f	m	f	m	f		
1	cage A									3	4	2	1																							10	5	5	0.50	0.79	0.0633		
2								3		3	6	3	2																							17	9	8	0.85	1.17	0.0637		
3								5		6	1															1										13	1	12	0.65	0.94	0.0645		
4								1	2	5	4	1	1	1									2	1			1									19	11	8	0.95	1.35	0.0606		
5	cage B							4	1	2	7	1	2									1	1													19	11	8	0.95	1.35	0.0634		
6										5	6	4													1											16	11	5	0.80	1.11	0.0624		
7								2	1	7	6	1																								17	7	10	0.85	1.17	0.0651		
8								1		6	3	1	5	1										1												18	10	8	0.90	1.25	0.0622		
9	cage C									7	2	1	5																							15	7	8	0.75	1.05	0.0630		
10								4	3	3	2	1																							13	5	8	0.65	0.94	0.0666			
11								3		7	6	1	3																							20	9	11	1.00	1.57	0.0644		
12								3	2	2	6	2	1										1													17	12	5	0.85	1.17	0.0640		
13	cage D							6	6		1	3																								16	10	6	0.80	1.11	0.0671		
14								1		5	3	3	5	1								1													19	9	10	0.95	1.35	0.0619			
15									6		5	4	1																							16	5	11	0.80	1.11	0.0659		
16									2	1	5	8		1																						17	9	8	0.85	1.17	0.0649		

* DAI = Day After Insertion of the larvae

ENV/JM/MONO(2010)35
LAB 3 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)								
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28	
		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f							m	f	m	f	m	f		
17	cage A										6	1	1	5	2	1																			16	9	7	0.80	1.11	0.0596			
18									3	4	1	6	2																						17	10	7	0.85	1.17	0.0620			
19									1	5		4	3	2																						15	9	6	0.75	1.05	0.0609		
20										5	2	2	1	4	2																						17	8	9	0.85	1.17	0.0591	
21	cage B								1	1	4	1	1	3	1	3																				15	8	7	0.75	1.05	0.0621		
22									2		4	3	1	3	1	3																					17	7	10	0.85	1.17	0.0604	
23									2		5	1	2	2	2	3			2		2		1														18	10	8	0.90	1.25	0.0595	
24														4	3	2	4	1	1																			15	7	8	0.75	1.05	0.0534
25	cage C								5	2	2	3		2																							15	6	9	0.75	1.05	0.0644	
26									1	2	2	1	1	1	1		1																				11	7	4	0.55	0.84	0.0591	
27									2		7	2		1	1	3																						19	14	5	0.95	1.35	0.0590
28								3	3	2		3	1	1																								15	7	8	0.75	1.05	0.0652
29	cage D									5		4	1	3	6	1																					20	14	6	1.00	1.57	0.0585	
30									2	1	8	2	3	1	1		1																				19	6	13	0.95	1.35	0.0627	
31							1	2	1	4	1	1	3	3	3																						20	12	8	1.00	1.57	0.0613	
32								1	1		4		4	1	2	3	1	1	2																			20	9	11	1.00	1.57	0.0552

* DAI = Day After Insertion of the larvae

LAB 3 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
01.10.09	17	4				4	1				1	8				8	7			1	8
02.10.09	18	6	1			7	8				8	5				5	13				13
03.10.09	19					0					0					0					0
04.10.09	20					0					0					0					0
05.10.09	21	7			1	8		2		2	4	1			5	5			1	6	
06.10.09	22	1				1	1	1		2					0				1	1	
07.10.09	23					0				0					0						0
08.10.09	24					0				0					0						0
09.10.09	25					0				0					0						0
10.10.09	26					0				0					0						0
	total	18	1	0	1	20	10	3	0	0	13	17	1	0	0	18	25	0	0	3	28
			females in cage	26	egg ropes per female	0.77		females in cage	38	egg ropes per female	0.34		females in cage	33	egg ropes per female	0.55		females in cage	33	egg ropes per female	0.85
Fertile egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
01.10.09	17	4				4	1				1	8				8	7			1	8
02.10.09	18	6	1			7	6				6	5				5	12				12
03.10.09	19					0					0					0					0
04.10.09	20					0					0					0					0
05.10.09	21	7				7				0	3	1			4	4					4
06.10.09	22	1				1				0					0						0
07.10.09	23					0				0					0						0
08.10.09	24					0				0					0						0
09.10.09	25					0				0					0						0
10.10.09	26					0				0					0						0
	total	18	1	0	0	19	7	0	0	0	7	16	1	0	0	17	23	0	0	1	24
			females in cage	26	fertile egg ropes per female	0.73		females in cage	38	fertile egg ropes per female	0.18		females in cage	33	fertile egg ropes per female	0.52		females in cage	33	fertile egg ropes per female	0.73
			males in cage	33	male fraction in cage	0.56		males in cage	31	male fraction in cage	0.45		males in cage	32	male fraction in cage	0.49		males in cage	35	male fraction in cage	0.51

ENV/JM/MONO(2010)35
LAB 4 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)								
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28	
		gender	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m							f	m	f	m	f			
1	cage A				0	0	4	0	7	2	1	0	1	2	0	2	0	0	0	0		0	0	0	1	0	0	0	0	0	0	0	0	0	20	7	13	1.00	1.57	0.0661			
2					0	0	0	0	2	5	0	2	3	2	0	2	0	0	2	1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	12	7	0.95	1.35	0.0623		
3					1	0	1	0	3	2	1	4	0	1	0	1	0	5	0	1		0	2	0	0	0	0	0	0	0	0	0	0	0	0	22	16	6	1.00	1.57	0.0615		
4					0	0	2	3	1	3	1	1	4	2	2	0	0	0	1	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0652		
5	cage B				1	0	2	4	3	1	0	0	3	3	1	1	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	9	10	0.95	1.35	0.0673		
6					0	0	0	1	5	2	2	0	1	4	1	2	0	0	0	0		0	0	1	1	0	0	0	0	0	0	0	0	0	0	20	10	10	1.00	1.57	0.0625		
7					0	0	0	0	0	4	0	1	4	1	2	2	1	0	2	2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	10	9	0.95	1.35	0.0595	
8					1	0	1	0	3	2	1	1	3	3	1	1	0	0	0	3		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	10	10	1.00	1.57	0.0630	
9	cage C				0	0	0	0	3	2	0	3	3	1	2	0	0	0	2	2		0	0	0	1	0	0	0	0	0	0	0	0	0	0	19	9	10	0.95	1.35	0.0602		
10					0	0	4	0	2	3	1	1	2	2	2	0	0	0	0	2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	8	11	0.95	1.35	0.0647		
11					0	0	3	2	2	2	1	0	3	2	0	3	0	0	0	0		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	19	10	9	0.95	1.35	0.0647	
12					2	0	2	2	7	3	1	1	0	2	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	9	12	1.00	1.57	0.0689	
13	cage D				0	0	0	1	2	2	1	3	3	1	0	2	0	2	0	2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	13	6	0.95	1.35	0.0619		
14					0	0	1	3	3	1	0	2	2	2	0	2	2	2	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	12	8	1.00	1.57	0.0637		
15					0	0	3	1	6	2	1	6	0	2	1	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	11	11	1.00	1.57	0.0672		
16					0	0	0	1	3	4	1	1	0	2	4	0	1	0	0	2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	10	9	0.95	1.35	0.0628		

* DAI = Day After Insertion of the larvae

LAB 4 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																																				total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)						
		11		12		13		14		15		16		17		18		19		20		21		22		23		24		25		26		27		28													
		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f												
17	cage A					2	0	1	2	2	0	0	2	2	2	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	11	9	1.00	1.57	0.0605					
18						1	1	0	1	0	1	0	0	3	4	3	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	12	7	0.95	1.35	0.0583				
19						2	0	0	2	1	2	0	3	3	1	4	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	10	10	1.00	1.57	0.0606				
20						2	0	0	2	2	0	1	1	4	3	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	10	11	1.00	1.57	0.0600			
21	cage B					4	4	4	5	0	0	0	0	2	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	10	11	1.00	1.57	0.0682			
22						3	2	3	5	0	0	0	1	3	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	10	9	0.95	1.35	0.0664			
23						3	2	0	2	0	1	0	2	4	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	12	8	1.00	1.57	0.0628		
24						4	3	1	2	0	0	0	0	3	1	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	9	9	0.90	1.25	0.0647		
25	cage C					0	0	0	1	0	0	1	1	4	2	4	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	8	10	0.90	1.25	0.0563		
26						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	#DIV/0!				
27						2	0	0	2	4	1	2	0	2	3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0616	
28						0	0	0	0	0	1	0	1	4	2	2	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	10	6	0.80	1.11	0.0559
29	cage D					0	0	0	0	1	0	0	0	3	2	3	4	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	9	8	0.85	1.17	0.0549	
30						0	0	0	0	1	2	2	1	5	2	2	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	9	10	0.95	1.35	0.0577	
31						0	0	0	0	0	0	1	0	3	1	3	5	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	10	8	0.90	1.25	0.0543
32						0	0	0	0	0	0	0	0	5	3	1	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	9	6	0.75	1.05	0.0553

* DAI = Day After Insertion of the larvae

ENV/JM/MONO(2010)35

LAB 4 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
01.11.09	18	1				1	1				1	5			5	1					1
02.11.09	19					0				0					0						0
03.11.09	20					0				0					0						0
04.11.09	21					0				0					0						0
05.11.09	22	2				2	2			2	2	2			2	2	2				2
06.11.09	23	3				3	2			2	5				5	4					4
07.11.09	24	1				1	1			1					0						0
	total	7	0	0	0	7	6	0	0	6	12	0	0	0	12	7	0	0	0	7	
			females in cage	44	egg ropes per female	0.16		females in cage	39	egg ropes per female	0.15		females in cage	36	egg ropes per female	0.33		females in cage	46	egg ropes per female	0.15
Fertile egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
01.11.09	18	1				1	1				1	5			5	1					1
02.11.09	19					0				0					0						0
03.11.09	20					0				0					0						0
04.11.09	21					0				0					0						0
05.11.09	22	2				2	2			2	2	2			2	2	2				2
06.11.09	23	3				3	2			2	5				5	4					4
07.11.09	24	1				1	1			1					0						0
	total	7	0	0	0	7	6	0	0	6	12	0	0	0	12	7	0	0	0	7	
			females in cage	44	fertile egg ropes per female	0.16		females in cage	39	fertile egg ropes per female	0.15		females in cage	36	fertile egg ropes per female	0.33		females in cage	46	fertile egg ropes per female	0.15
			males in cage	37	male fraction in cage	0.46		males in cage	39	male fraction in cage	0.50		males in cage	42	male fraction in cage	0.54		males in cage	34	male fraction in cage	0.425

LAB 5 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)					
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f					
1	cage 1					1	4	4		2	3	2	3																					19	8	11	0.95	1.35	0.0625	
2							3	3			1	5	1	3	2																			18	11	7	0.90	1.25	0.0592	
3							4	1			2	4	4			1	1	2																19	14	5	0.95	1.35	0.0574	
4						1	3	5		1	1	3	2	2																				18	8	10	0.90	1.25	0.0615	
5	cage 2					0	3	4	1	2	1	1	4	2	1																			20	9	11	1.00	1.57	0.0599	
6							1	1		1	1	6	1	3																				17	13	4	0.85	1.17	0.0548	
7						1	3	1		3	2	2	1	4	1	1																		19	10	9	0.95	1.35	0.0596	
8						1	2	1		2	2	4	2	1		1																		18	10	8	0.90	1.25	0.0575	
9	cage 3						5	5		1	2	4	1	1	1																			20	8	12	1.00	1.57	0.0619	
10						1	2	1	3		1	2	4	3	1																			18	11	7	0.90	1.25	0.0610	
11						3	2	1			2	1	9	1																					19	12	7	0.95	1.35	0.0617
12						1	5	2			2	5	2	1																					18	10	8	0.90	1.25	0.0619
13	cage 4					2	4	1			2	6	1		2																				18	10	8	0.90	1.25	0.0616
14						1	5	1			3	8																							18	11	7	0.90	1.25	0.0624
15						4	6	2		2	3	1	1																						19	5	14	0.95	1.35	0.0660
16						2	4	1	2	1	3	4	3																						20	12	8	1.00	1.57	0.0625

* DAI = Day After Insertion of the larvae

ENV/JM/MONO(2010)35
LAB 5 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)								
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28	
		gender	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m							f	m	f	m	f			
1	cage 1						1		2		3		2		3		2		1		1														17	11	6	0.85	1.17	0.0609			
2							1		6		1		2		4		4		2															20	12	8	1.00	1.57	0.0630				
3							2		4		2		0		3		3				2		2		1									19	11	8	0.95	1.35	0.0607				
4							2		4		1		2		2		1		3		1		1											17	9	8	0.85	1.17	0.0639				
5	cage 2				1		6		4			1		5		1		2																20	9	11	1.00	1.57	0.0667				
6							5		3		1		2		4				2		2												19	11	8	0.95	1.35	0.0647					
7					1		6		4		3			3				1															18	6	12	0.90	1.25	0.0699					
8							3		2				2		3		1		2		1		3				1						18	11	7	0.90	1.25	0.0618					
9	cage 3						3		2		4		1		2		4																16	9	7	0.80	1.11	0.0679					
10							3		4		2		3		2		1		2							1						18	11	7	0.90	1.25	0.0645						
11							6		3		3		3		3				1		1				1							18	8	10	0.90	1.25	0.0671						
12					1		5		2		2		3		3				1		1											18	10	8	0.90	1.25	0.0671						
13	cage 4						2		3		2		4		3		2		2				2										20	13	7	1.00	1.57	0.0622					
14								3		2			2		4		3		2		1		3									20	13	7	1.00	1.57	0.0588						
15							2				1		1		3		4		1		1		2		1							16	13	3	0.80	1.11	0.0586						
16							3		2		2		3		5		3				1											19	12	7	0.95	1.35	0.0636						

* DAI = Day After Insertion of the larvae

LAB 5 - reproduction

Total egg ropes		cage 1					cage 2					cage 3					cage 4				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
04.10.09	17					0					0					1					1
05.10.09	18	3	1			4	2				2	1	1			2	4				4
06.10.09	19	6	2			8	10	1			11	19	2		1	22	10	1			11
07.10.09	20	11	1			12	13		1		14	7	2			9	8	4			12
08.10.09	21	5	3			8	4	3			7	5	7		1	13	7	2			9
09.10.09	22		3			3	2	6		1	9	7	4	2		13	3	4			7
10.10.09	23		2			2		1			1		1			1		2		1	3
11.10.09	24					0	1	1			2				1	1					0
	total	25	12	0	0	37	32	12	1	1	46	40	17	2	3	62	33	13	0	1	47
			females in cage	41	egg ropes per female	0.90		females in cage	42	egg ropes per female	1.10		females in cage	41	egg ropes per female	1.51		females in cage	38	egg ropes per female	1.24
Fertile egg ropes		cage 1					cage 2					cage 3					cage 4				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
07.10.09	20					0					0	1				1	1				1
08.10.09	21	4	1			5	2				2	1	1			2	4				4
09.10.09	22	12	2			14	16	1			17	24	3		1	28	16	1			17
10.10.09	23	4	1			5	7		1		8		1			1	2	4			6
11.10.09	24	3	3			6	4	2			6	8	8		1	17	6	1			7
12.10.09	25		2			2	2	5		1	8	6	2	1		9	1	4			5
13.10.09	26					0					0					0					0
14.10.09	27					0					0					0					0
15.10.09	28		1			1	1	2			3		1		1	2		1			1
	total	23	10	0	0	33	32	10	1	1	44	40	16	1	3	60	30	11	0	0	41
			females in cage	41	fertile egg ropes per female	0.80		females in cage	42	fertile egg ropes per female	1.05		females in cage	41	fertile egg ropes per female	1.46		females in cage	38	fertile egg ropes per female	1.08
			males in cage	33	male fraction in cage	0.45		males in cage	32	male fraction in cage	0.43		males in cage	34	male fraction in cage	0.45		males in cage	37	male fraction in cage	0.49

ENV/JM/MONO(2010)35
LAB 6 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)							
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f							
1	cage A	0	0	0	0	0	0	5	0	1	1	1	2	0	2	1	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	17	9	8	0.85	1.17	0.0647	
2		0	0	0	0	0	0	2	0	2	4	1	4	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	10	6	0.80	1.11	0.0662
3		0	0	0	0	0	0	1	0	4	1	0	1	1	3	0	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	9	7	0.80	1.11	0.0625
4		0	0	0	0	0	0	2	0	5	0	1	1	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	5	8	0.65	0.94	0.0657
5	cage B	0	0	0	0	0	0	0	0	3	0	2	1	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	9	2	0.55	0.84	0.0615	
6		0	0	0	0	0	0	2	0	4	1	1	1	0	1	1	3	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	16	7	9	0.80	1.11	0.0634	
7		0	0	0	0	0	0	1	0	1	4	1	1	1	0	0	2	0	2	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	16	12	4	0.80	1.11	0.0610	
8		0	0	0	0	0	0	2	0	3	0	3	0	0	4	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	7	8	0.75	1.05	0.0638	
9	cage C	0	0	0	0	0	0	3	0	5	1	2	0	2	1	0	1	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	7	12	0.95	1.35	0.0641	
10		0	0	0	0	1	0	4	1	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	3	9	0.60	0.89	0.0706		
11		0	0	0	0	0	0	3	0	1	4	2	3	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	11	7	0.90	1.25	0.0657	
12		0	0	0	0	0	0	3	0	2	2	1	1	0	5	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	10	6	0.80	1.11	0.0649	
13	cage D	0	0	0	0	0	0	2	0	2	2	4	4	1	0	0	0	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	19	8	11	0.95	1.35	0.0638	
14		0	0	0	0	0	0	1	0	3	0	1	5	0	1	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	14	9	5	0.70	0.99	0.0639		
15		0	0	0	0	0	0	1	0	1	1	4	0	1	4	0	2	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	17	10	7	0.85	1.17	0.0614	
16		0	0	0	0	0	0	0	0	3	2	1	2	2	2	0	4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	17	11	6	0.85	1.17	0.0622	

* DAI = Day After Insertion of the larvae

LAB 6 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																				total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)															
		11		12		13		14		15		16		17		18		19		20								21		22		23		24		25		26		27		28
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f							
17	cage A	0	0	0	0	0	0	3	0	3	1	2	3	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	15	6	9	0.75	1.05	0.0659
18		0	0	0	0	0	0	0	0	3	4	1	5	0	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	14	5	0.95	1.35	0.0646
19		0	0	0	0	0	0	1	0	1	2	2	2	0	3	2	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	17	11	6	0.85	1.17	0.0619
20		0	0	0	0	0	0	2	0	5	0	3	5	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	18	7	11	0.90	1.25	0.0650
21	cage B	0	0	0	0	0	0	2	2	2	1	0	2	0	3	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	9	5	0.70	0.99	0.0620	
22		0	0	0	0	0	0	2	0	5	0	1	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	5	8	0.65	0.94	0.0668	
23		0	0	0	0	0	0	2	0	4	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	3	7	0.50	0.79	0.0687	
24		0	0	0	0	0	0	0	0	5	0	1	3	0	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	10	6	0.80	1.11	0.0635	
25	cage C	0	0	0	0	0	0	1	0	0	1	0	3	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	10	1	0.55	0.84	0.0633		
26		0	0	0	0	0	0	5	0	0	2	0	2	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	8	5	0.65	0.94	0.0674		
27		0	0	0	0	0	0	6	0	2	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	3	9	0.60	0.89	0.0705		
28		0	0	0	0	0	0	3	0	5	0	0	0	0	3	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	6	8	0.70	0.99	0.0655		
29	cage D	0	0	0	0	0	0	6	0	2	1	1	1	0	2	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	10	9	0.95	1.35	0.0645		
30		0	0	0	0	0	0	0	0	4	0	4	0	0	1	0	1	0	7	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	18	10	8	0.90	1.25	0.0601		
31		0	0	0	0	0	0	0	0	7	0	0	0	0	1	0	6	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	11	7	0.90	1.25	0.0612		
32		0	0	0	0	0	0	3	0	5	0	1	0	1	5	0	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	10	10	1.00	1.57	0.0637		

* DAI = Day After Insertion of the larvae

ENV/JM/MONO(2010)35
LAB 6 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
08.10.09	17	2	0	0	1	3	2	0	0	0	2	2	0	0	1	3	1	0	0	0	1
09.10.09	18	0	0	0	0	0	3	0	0	0	3	2	0	0	0	2	5	0	0	0	5
10.10.09	19	6	0	0	0	6	2	0	0	0	2	1	0	0	0	1	4	0	0	0	4
11.10.09	20	3	0	0	0	3	5	0	0	0	5	3	0	0	0	3	2	0	0	0	2
12.10.09	21	1	0	0	0	1	0	0	0	0	0	2	0	0	0	2	2	0	0	0	2
13.10.09	22	2	0	0	0	2	1	0	0	0	1	0	0	0	0	0	1	1	0	1	3
16.10.09	25	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.10.09	27	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	0	1	3
	total	14	0	0	2	16	14	0	0	0	14	10	0	0	1	11	17	1	0	2	20
			females in cage	33	egg ropes per female	0.48		females in cage	34	egg ropes per female	0.41		females in cage	29	egg ropes per female	0.38		females in cage	38	egg ropes per female	0.53
Fertile egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
08.10.09	17	2	0	0	1	3	2	0	0	0	2	1	0	0	0	1	1	0	0	0	1
09.10.09	18	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	2	0	0	0	2
10.10.09	19	5	0	0	0	5	2	0	0	0	2	1	0	0	0	1	3	0	0	0	3
11.10.09	20	1	0	0	0	1	4	0	0	0	4	2	0	0	0	2	1	0	0	0	1
12.10.09	21	1	0	0	0	1	0	0	0	0	0	2	0	0	0	2	1	0	0	0	1
13.10.09	22	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16.10.09	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.10.09	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	total	11	0	0	1	12	9	0	0	0	9	7	0	0	0	7	8	0	0	0	8
			females in cage	33	fertile egg ropes per female	0.36		females in cage	34	fertile egg ropes per female	0.26		females in cage	29	fertile egg ropes per female	0.24		females in cage	38	fertile egg ropes per female	0.21
			males in cage	25	male fraction in cage	0.43		males in cage	20	male fraction in cage	0.37		males in cage	30	male fraction in cage	0.51		males in cage	26	male fraction in cage	0.406

ENV/JM/MONO(2010)35

LAB 7 - emergence F1 generation

		emerged midges [per day]																																																
		DAI*		11		12		13		14		15		16		17		18		19		20		21		22		23		24		25		26		27		28												
Vessel ID	gender	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)							
F1 1	cage A				1		4		1	2		1	4		1		1	1																					16	4	12	0.80	1.11	0.0636						
F1 2							3		4		1		3		1			2	3																					18	8	10	0.90	1.25	0.0612					
F1 3					1		2		1	1	1		1	2		2	3	1	2			2																				19	8	11	0.95	1.35	0.0602			
F1 4							5		2			1		2	3			1	1		1																						16	6	10	0.80	1.11	0.0631		
F1 5	cage B						5		2			4		3		2	1	1				1																						19	11	8	0.95	1.35	0.0642	
F1 6				1			4		3		2	2	2	2		2																												18	6	12	0.90	1.25	0.0669	
F1 7							3		2	3	2	3	1	3			1	1		1																									20	11	9	1.00	1.57	0.0646
F1 8				1			3		2			2	1	2		2	4			1			1																							19	9	10	0.95	1.35

* DAI = Day After Insertion of the larvae

LAB 7 - reproduction

Total egg ropes		cage A					cage B				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
16.11.09	17	3	1			4					0
17.11.09	18	6				6	3	1			4
18.11.09	19	4	3			7	5	1			6
19.11.09	20	6	2			8	4				4
20.11.09	21	5	7			12	6	3			9
21.11.09	22	4	8			12	1	4			5
22.11.09	23	2	3			5	1	2			3
23.11.09	24	2	1			3		5			5
24.11.09	25	4	3			7		2			2
25.11.09	26	1	2			3	2				2
26.11.09	27		3			3	2	1			3
27.11.09	28					0					0
28.11.09	29		2			2	1	1			2
						0					0
	total	37	35	0	0	72	25	20	0	0	45
			females in cage	37	egg ropes per female	1.95		females in cage	36	egg ropes per female	1.25
Fertile egg ropes		cage A					cage B				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
16.11.09	17	3	0			3					0
17.11.09	18	6				6	3	1			4
18.11.09	19	4	3			7	5	1			6
19.11.09	20	6	2			8	4				4
20.11.09	21	4	6			10	6	3			9
21.11.09	22	4	7			11	1	3			4
22.11.09	23	2	3			5	1	2			3
23.11.09	24	1	0			1		4			4
24.11.09	25	4	2			6		2			2
25.11.09	26	1	2			3	2				2
26.11.09	27		2			2	2	0			2
27.11.09	28					0					0
28.11.09	29		1			1	1	1			2
						0					0
	total	35	28	0	0	63	25	17	0	0	42
			females in cage	37	fertile egg ropes per female	1.70		females in cage	36	fertile egg ropes per female	1.17
			males in cage	28	male fraction in cage	0.43		males in cage	34	male fraction in cage	0.49

ENV/JM/MONO(2010)35
LAB 8 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)							
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f							
1	cage A					0	0	0	0	6	0	2	0	0	0			0	4	1	3	0	2	0	1	0	0	0	0			0	0	0	0	19	10	9	0.95	1.35	0.0583	
2						0	0	0	0	4	0	1	0	1	1			0	2	0	4	0	3	1			0	1	0	0			0	0	0	1	19	12	7	0.95	1.35	0.0552
3						0	0	0	0	6	0	3	0	1	0			1	3	0	3	0	2	0	1	0	0	0	0			0	0	0	0	20	9	11	1.00	1.57	0.0591	
4						0	0	0	0	4	0	5	0	1	0			0	3	0	3	0	1	0	1	0	0	0	1			0	0	0	0	19	9	10	0.95	1.35	0.0586	
5	cage B					0	0	0	0	5	0	5	0	1	0			1	2	0	1	0	2	0	2	0	1	0	0			0	0	0	0	20	8	12	1.00	1.57	0.0588	
6						0	0	2	0	3	0	6	0	0	0			1	4	0	1	0	1	0	1	0	1	0	0			0	0	0	0	20	8	12	1.00	1.57	0.0602	
7						0	0	0	0	6	0	4	0	2	0			1	1	1	0	0	1	1	1	1	1	1	0	0			0	0	0	0	20	4	16	1.00	1.57	0.0592
8						0	0	1	0	3	0	3	0	1	0			1	1	0	0	1	3	0	1	0	1	0	2			0	2	0	0	20	10	10	1.00	1.57	0.0545	
9	cage C					0	0	0	0	5	0	3	0	3	1			0	1	0	2	1	1	1	1	0	1	0	0			0	0	0	0	20	7	13	1.00	1.57	0.0586	
10						0	0	0	0	6	0	2	0	1	1			1	0	1	4	0	3	0	1	0	0	0	0			0	0	0	0	20	9	11	1.00	1.57	0.0584	
11						0	0	0	0	3	0	4	0	2	0			1	4	0	3	0	0	0	2	0	1	0	0			0	0	0	0	20	10	10	1.00	1.57	0.0574	
12						0	0	0	0	7	0	3	0	0	0			1	4	0	2	0	0	0	2	0	0	0	1			0	0	0	0	20	9	11	1.00	1.57	0.0592	
13	cage D					0	0	0	0	3	0	1	0	1	0			1	3	1	2	1	4	0	3	0	0	0	0			0	0	0	0	20	12	8	1.00	1.57	0.0543	
14						0	0	2	0	3	0	1	0	1	1			1	6	0	2	0	1	0	1	0	1	0	0			0	0	0	0	20	12	8	1.00	1.57	0.0581	
15						0	0	0	0	4	0	5	0	0	0			1	2	1	3	0	2	0	1	0	1	0	0			0	0	0	0	20	9	11	1.00	1.57	0.0577	
16						0	0	0	0	4	0	5	0	0	0			1	1	1	3	1	1	0	2	0	1	0	0			0	0	0	0	20	8	12	1.00	1.57	0.0573	

* DAI = Day After Insertion of the larvae

LAB 8 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)								
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28	
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f								
17	cage A																																	0	0	0	0.00	0.00	#DIV/0!				
18																																			0	0	0	0.00	0.00	#DIV/0!			
19																																				0	0	0	0.00	0.00	#DIV/0!		
20																																				0	0	0	0.00	0.00	#DIV/0!		
21	cage B					8	0	2	0	1	1	0	3							0	4												0	0	20	9	11	1.00	1.57	0.0645			
22						2	0	1	0	1	1	0	3							0	6												0	0	14	10	4	0.70	0.99	0.0597			
23						2	0	4	0	1	0	1	1							0	5												0	0	18	10	8	0.90	1.25	0.0580			
24						2	0	0	1	2	4	0	3							0	4													0	0	16	12	4	0.80	1.11	0.0619		
25	cage C		0	0	0	0	4	0				4	3	0	2	0	2	0	0														0	0	18	9	9	0.90	1.25	0.0598			
26			0	0	5	0	2	0				1	1	0	3	0	5	0	0														0	1	0	0	18	10	8	0.90	1.25	0.0642	
27			0	0	3	0	3	0				3	3	0	2	0	4	0	0															0	2	0	0	20	11	9	1.00	1.57	0.0623
28			0	0	4	0	2	0				2	1	2	2	1	4	0	0															0	2	0	0	20	9	11	1.00	1.57	0.0619
29	cage D		0	0	3	0	5	0				2	2	2	3	0	1	0	1														0	0	0	0	19	7	12	0.95	1.35	0.0655	
30			0	0	4	0	0	0				1	2	0	3	0	4	0	4														0	0	0	0	18	13	5	0.90	1.25	0.0608	
31			0	0	5	0	1	0				2	3	0	5	0	2	0	1														0	0	0	0	19	11	8	0.95	1.35	0.0643	
32			0	0	6	0	2	0				2	2	0	3	1	0	0	0														0	0	0	0	16	5	11	0.80	1.11	0.0685	

* DAI = Day After Insertion of the larvae

ENV/JM/MONO(2010)35

LAB 8 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
29.11.09	19	1				1					0					0					0
01.12.09	21					0					1					1					1
02.12.09	22			1		1					0	2				2					0
03.12.09	23					0					2	2				2	1	1			2
04.12.09	24	1			1	2	3		2	5	3					3	2				2
07.12.09	27	4	1	3		8	6	1	2	9	11			1	12	8	1	3			12
08.12.09	28	2				2				0	3	1			4	1					1
09.12.09	29					0				0					0	1					1
10.12.09	30					0				0					0	1					1
	total	8	1	4	1	14	11	1	2	2	16	22	1	0	1	24	15	2	3	0	20
			females in cage	40	egg ropes per female	0.35		females in cage	30	egg ropes per female	0.53		females in cage	35	egg ropes per female	0.69		females in cage	41	egg ropes per female	0.49
* the female died during deposit of egg ropes																					
Fertile egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
01.12.09	21	1				1					0					0					0
03.12.09	23					0					0	1				1					0
04.12.09	24					0					0	1				1	2				2
07.12.09	27	1				1	3			3	2					2		1			1
10.12.09	30					0		1		1					0						0
	total	2	0	0	0	2	3	1	0	0	4	4	0	0	0	4	2	1	0	0	3
			females in cage	40	fertile egg ropes per female	0.05		females in cage	30	fertile egg ropes per female	0.13		females in cage	35	fertile egg ropes per female	0.11		females in cage	41	fertile egg ropes per female	0.07
			males in cage	37	male fraction in cage	0.48		males in cage	50	male fraction in cage	0.63		males in cage	45	male fraction in cage	0.56		males in cage	39	male fraction in cage	0.488

LAB 9 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)					
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f					
1	cage A	0	0	0	0	0	0	0	0	3	1	4	0	1	4	0	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	19	11	8	0.95	1.35	0.0615
2		0	0	0	0	0	0	0	0	0	0	6	2	1	6	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	20	12	8	1.00	1.57	0.0608
3		0	0	0	0	0	0	0	0	1	0	0	0	0	7	0	5	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	16	15	1	0.80	1.11	0.0585
4		0	0	0	0	0	0	0	0	4	0	1	0	0	7	0	5	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	19	14	5	0.95	1.35	0.0608
5	cage B	0	0	0	0	0	0	1	0	1	0	5	1	4	2	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	8	12	1.00	1.57	0.0617	
6		0	0	0	0	1	0	2	0	1	0	3	1	2	3	0	4	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	20	11	9	1.00	1.57	0.0623	
7		0	0	0	0	0	0	0	0	1	0	8	1	3	1	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	18	6	12	0.90	1.25	0.0619	
8		0	0	0	0	0	0	0	2	1	3	2	1	2	0	3	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	17	11	6	0.85	1.17	0.0611	
9	cage C	0	0	0	0	0	0	1	0	4	0	1	3	1	3	0	3	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	20	12	8	1.00	1.57	0.0615	
10		0	0	0	0	2	0	2	0	1	0	4	5	0	2	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	10	10	1.00	1.57	0.0652	
11		0	0	0	0	0	0	2	0	4	1	1	2	1	7	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	13	8	1.00	1.57	0.0639	
12		0	0	0	0	0	0	1	0	3	0	3	5	1	4	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	19	9	10	0.95	1.35	0.0635	
13	cage D	0	0	0	0	0	0	2	0	6	1	1	3	1	3	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	19	9	10	0.95	1.35	0.0654		
14		0	0	0	0	0	0	0	4	1	5	5	1	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	10	10	1.00	1.57	0.0641		
15		0	0	0	0	0	0	1	0	1	0	6	1	0	6	0	3	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	21	12	9	1.00	1.57	0.0613		
16		0	0	0	0	0	0	1	0	3	2	4	4	0	1	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	11	9	1.00	1.57	0.0639		

* DAI = Day After Insertion of the larvae

LAB 9 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D																
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes												
26.10.09	16	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0	2	-	-	-	2												
27.10.09	17	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0												
28.10.09	18	9	-	-	-	9	6	-	-	-	6	17	-	-	-	17	16	-	-	-	16												
29.10.09	19	19	-	-	-	19	10	-	-	-	10	12	-	-	-	12	10	-	-	-	10												
30.10.09	20	10	-	-	-	10	10	-	-	-	10	5	-	-	-	5	7	-	-	-	7												
31.10.09	21	8	-	-	-	8	1	-	-	-	1	1	-	-	-	1	4	-	-	-	4												
01.11.09	22	1	-	-	-	1	2	-	-	-	2	0	-	-	-	0	1	-	-	-	1												
02.11.09	23	5	1	-	-	6	4	-	-	-	4	1	-	-	-	1	3	-	-	-	3												
03.11.09	24	0	-	-	-	0	1	-	-	-	1	1	-	-	-	1	0	-	-	-	0												
	total	52	1	0	0	53	34	0	0	0	34	37	0	0	0	37	43	0	0	0	43												
			females in cage		52	egg ropes per female			1.02			females in cage		36	egg ropes per female		0.94			females in cage		44	egg ropes per female		0.84			females in cage		42	egg ropes per female		1.02

Fertile egg ropes		cage A					cage B					cage C					cage D																
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes												
26.10.09	16	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0	2	-	-	-	2												
27.10.09	17	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0												
28.10.09	18	9	-	-	-	9	6	-	-	-	6	17	-	-	-	17	16	-	-	-	16												
29.10.09	19	19	-	-	-	19	10	-	-	-	10	12	-	-	-	12	10	-	-	-	10												
30.10.09	20	9	-	-	-	9	9	-	-	-	9	5	-	-	-	5	7	-	-	-	7												
31.10.09	21	7	-	-	-	7	1	-	-	-	1	1	-	-	-	1	4	-	-	-	4												
01.11.09	22	1	-	-	-	1	1	-	-	-	1	0	-	-	-	0	1	-	-	-	1												
02.11.09	23	5	1	-	-	6	4	-	-	-	4	0	-	-	-	0	3	-	-	-	3												
03.11.09	24	0	-	-	-	0	1	-	-	-	1	1	-	-	-	1	0	-	-	-	0												
	total	50	1	0	0	51	32	0	0	0	32	36	0	0	0	36	43	0	0	0	43												
			females in cage		52	fertile egg ropes per female			0.98			females in cage		36	fertile egg ropes per female		0.89			females in cage		44	fertile egg ropes per female		0.82			females in cage		42	fertile egg ropes per female		1.02
			males in cage		22	male fraction in cage			0.30			males in cage		39	male fraction in cage		0.52			males in cage		36	male fraction in cage		0.45			males in cage		38	male fraction in cage		0.48

ENV/JM/MONO(2010)35
LAB 10 - emergence P generation

Vessel ID	DAI*	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)							
		11		12		13		14		15		16		17		18		19		20		21		22		23		24								25		26		27		28
gender		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f							
1	cage A	0	0	0	0	2	0	0	0	3	0	1	2	0	0	2	0	1	1	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	16	7	9	0.80	1.11	0.0613		
2		0	0	0	0	1	0	0	0	2	0	1	1	2	0	1	0	0	2	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	0	15	8	7	0.75	1.05	0.0580	
3		0	0	2	0	0	0	0	0	0	1	1	1	1	0	1	1	2	0	1	1	0	2	0	0	0	1	0	2	0	0	0	0	0	0	0	17	9	8	0.85	1.17	0.0579
4		0	0	1	0	0	0	1	0	0	0	1	1	0	1	4	0	0	1	1	0	1	2	0	0	0	1	0	0	0	2	0	0	0	1	0	1	19	10	9	0.95	1.35
5	cage B	0	0	1	0	0	0	0	0	1	3	1	3	1	3	0	0	0	0	1	0	3	0	1	0	0	0	0	0	2	0	0	0	0	0	0	20	10	10	1.00	1.57	0.0577
6		0	0	1	0	2	0	0	0	1	0	1	0	2	0	0	1	0	0	0	2	0	1	0	1	0	0	0	2	0	0	0	0	0	0	14	7	7	0.70	0.99	0.0601	
7		0	0	1	0	1	0	1	0	1	0	1	0	1	0	3	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	15	6	9	0.75	1.05	0.0604	
8		0	0	1	0	0	0	0	0	0	0	4	1	1	1	2	0	0	2	1	2	0	2	0	2	0	0	0	0	0	0	0	0	0	0	19	10	9	0.95	1.35	0.0578	
9	cage C	0	0	0	0	1	0	1	0	2	0	1	0	3	1	0	1	0	3	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	17	9	8	0.85	1.17	0.0601	
10		0	0	0	0	1	0	2	0	0	0	1	0	1	0	0	0	3	0	2	1	0	3	0	2	0	0	0	1	0	0	0	0	0	0	17	7	10	0.85	1.17	0.0560	
11		0	0	1	0	1	0	0	0	0	0	3	3	4	0	0	1	0	1	0	3	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	20	11	9	1.00	1.57	0.0600
12		0	0	1	0	1	0	1	0	1	0	2	0	1	1	0	1	1	0	2	1	0	3	0	1	0	1	0	1	0	0	0	0	0	0	0	19	9	10	0.95	1.35	0.0582
13	cage D	0	0	1	0	2	0	0	0	1	0	2	0	1	0	3	0	0	1	0	2	0	2	0	1	0	1	0	0	0	0	0	0	0	0	17	7	10	0.85	1.17	0.0601	
14		0	0	1	0	2	0	1	0	1	0	3	0	1	1	2	0	1	1	1	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0	19	6	13	0.95	1.35	0.0616	
15		0	0	1	0	1	0	1	0	1	1	1	1	2	0	0	0	1	0	1	3	0	2	0	3	0	1	0	0	0	0	0	0	0	0	20	11	9	1.00	1.57	0.0585	
16		0	0	0	0	3	0	1	0	2	0	1	0	0	0	1	0	2	0	0	2	1	1	0	2	0	2	0	0	0	0	0	0	0	0	18	7	11	0.90	1.25	0.0591	

* DAI = Day After Insertion of the larvae

LAB 10 - emergence F1 generation

		emerged midges [per day]																																													
		DAI*		11		12		13		14		15		16		17		18		19		20		21		22		23		24		25		26		27		28									
Vessel ID	gender	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)				
1	cage A	0	0	0	0	0	0	0	0	1	0	5	0	4	0	1	4	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0590			
2		0	0	0	0	0	0	0	0	2	0	3	0	7	0	2	0	0	2	0	1	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	20	5	15	1.00	1.57	0.0585			
3		0	0	0	0	0	0	0	0	0	0	1	0	5	0	1	0	2	0	0	0	1	4	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	19	9	10	0.95	1.35	0.0529		
4		0	0	0	0	0	0	0	0	0	0	3	0	2	0	3	0	1	0	1	3	1	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	19	8	11	0.95	1.35	0.0536			
5	cage B	0	0	0	0	0	0	0	0	0	0	5	0	4	0	1	0	0	0	1	1	0	5	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0554		
6		0	0	0	0	0	0	0	0	2	0	5	0	2	0	1	0	0	2	0	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	6	10	0.80	1.11	0.0589		
7		0	0	0	0	0	0	0	0	1	0	0	0	6	0	2	0	1	0	0	0	0	2	1	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0534	
8		0	0	0	0	0	0	0	0	0	0	1	0	7	0	1	0	0	0	1	0	0	2	1	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0532	
9	cage C	0	0	0	0	0	0	0	0	1	0	1	0	2	0	4	0	3	0	1	0	0	4	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	19	7	12	0.95	1.35	0.0539		
10		0	0	0	0	0	0	0	0	0	0	2	0	2	0	2	0	2	0	0	2	0	3	0	3	0	2	0	1	0	0	0	0	0	0	0	0	0	0	19	11	8	0.95	1.35	0.0522		
11		0	0	0	0	0	0	0	0	0	0	3	0	4	0	1	0	2	1	1	1	0	1	0	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0541	
12		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	3	1	1	1	1	5	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	19	12	7	0.95	1.35	0.0486	
13	cage D	0	0	0	0	0	0	0	0	0	0	3	0	3	0	2	0	1	0	0	2	3	3	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	20	8	12	1.00	1.57	0.0538		
14		0	0	0	0	0	0	0	0	0	0	6	0	3	0	0	0	1	0	1	2	1	2	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	20	8	12	1.00	1.57	0.0553		
15		0	0	0	0	0	0	0	0	0	0	3	0	5	0	1	0	1	0	1	2	0	3	0	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	20	9	11	1.00	1.57	0.0543		
16		0	0	0	0	0	0	0	0	0	0	1	0	2	0	3	0	0	0	0	1	1	5	0	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	17	10	7	0.85	1.17	0.0517	

* DAI = Day After Insertion of the larvae

ENV/JM/MONO(2010)35

LAB 10 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
08.11.09	18	1		1		2	0				0	0			0	1					1
09.11.09	19	1				1	1				1	3			1	4			1		1
10.11.09	20	1	1			2			1		1	2			2						0
11.11.09	21	1			1	2	2				2	1			1	1					1
12.11.09	22	1		1		2					0	3		1	4	1	2				3
13.11.09	23	3				3	1				1		3		1	4	4			3	7
14.11.09	24		1		1	2					0	1			1	2			1		3
15.11.09	25				1	1	4				4	1	1		2	1	2				3
16.11.09	26			1	1	2					0				0					2	2
17.11.09	27				1	1					0				0						0
18.11.09	28					0					0				0						0
19.11.09	29			1		1					0				0	1					1
20.11.09	30					0					0				0					1	1
	total	8	2	4	5	19	8	0	1	0	9	11	4	2	2	19	11	4	2	6	23
			females in cage	34	egg ropes per female	0.56		females in cage	33	egg ropes per female	0.27		females in cage	36	egg ropes per female	0.53		females in cage	31	egg ropes per female	0.74
Fertile egg ropes		cage A					cage B					cage C					cage D				
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes
08.11.09	18	1		1		2	0				0	0			0	1					1
09.11.09	19	1				1	1				1	3			1	4			1		1
10.11.09	20	1	1			2					0	2			2						0
11.11.09	21	1			1	2	2				2	1			1	1					1
12.11.09	22			1		1					0	3		1	4	1	2				3
13.11.09	23	2				2	1				1		2		1	2	4			3	7
14.11.09	24		1		1	2					0				0	2			1		3
15.11.09	25				1	1					0		1		1	1	2				3
16.11.09	26					0					0				0					1	1
17.11.09	27				1	1					0				0						0
18.11.09	28					0					0				0						0
19.11.09	29			1		1					0				0						0
	total	6	2	3	4	15	4	0	0	0	4	9	3	1	1	14	10	4	2	4	20
			females in cage	34	fertile egg ropes per female	0.44		females in cage	33	fertile egg ropes per female	0.12		females in cage	36	fertile egg ropes per female	0.39		females in cage	31	fertile egg ropes per female	0.65
			males in cage	33	male fraction in cage	0.49		males in cage	35	male fraction in cage	0.51		males in cage	37	male fraction in cage	0.51		males in cage	43	male fraction in cage	0.581

LAB 11 - emergence P generation

Vessel ID	gender	emerged midges [per day]																												total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)						
		DAI*		11		12		13		14		15		16		17		18		19		20		21		22		23								24		25		26	
1	cage A	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	20	10	10	1.00	1.57	0.0642
2		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	20	8	12	1.00	1.57	0.0652
3		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	20	10	10	1.00	1.57	0.0629
4		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	19	7	12	0.95	1.35	0.0643
5	cage B	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	19	10	9	0.95	1.35	0.0660
6		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	18	9	9	0.90	1.25	0.0627
7		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	18	10	8	0.90	1.25	0.0646
8		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	20	11	9	1.00	1.57	0.0616
9	cage C	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	18	10	8	0.90	1.25	0.0640
10		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	20	10	10	1.00	1.57	0.0633
11		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	18	11	7	0.90	1.25	0.0623
12		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	19	9	10	0.95	1.35	0.0645
13	cage D	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	20	14	6	1.00	1.57	0.0621
14		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	19	12	7	0.95	1.35	0.0619
15		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	22	8	14	1.00	1.57	0.0646
16		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	19	9	10	0.95	1.35	0.0639

* DAI = Day After Insertion of the larvae

ENV/JM/MONO(2010)35

LAB 11 - emergence F1 generation

Vessel ID	DAI*	emerged midges [per day]																				total midges	total females	total males	Emergence ratio	arcsin√ER	development rate (d ⁻¹)																
		11		12		13		14		15		16		17		18		19		20								21		22		23		24		25		26		27		28	
		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f							m	f	m	f	m	f	m	f	m	f	m	f	m	f		
17	cage A	0	0	0	0	0	0	0	0	5	0	1	3	0	4	0	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	12	6	0,90	1,25	0,0623	
18		0	0	0	0	0	0	3	0	3	1	1	2	0	4	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	13	7	1,00	1,57	0,0637	
19		0	0	0	0	0	0	4	0	5	2	2	2	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	6	12	0,90	1,25	0,0673	
20		0	0	0	0	0	0	4	1	4	0	3	3	1	4	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	11	12	1,00	1,57	0,0654	
21	cage B	0	0	0	0	1	0	10	1	3	3	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	7	15	1,00	1,57	0,0707	
22		0	0	0	0	0	0	6	2	5	2	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1,00	1,57	0,0688	
23		0	0	0	0	0	0	3	1	3	5	1	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	12	7	0,95	1,35	0,0680	
24		0	0	0	0	0	0	6	0	1	4	1	4	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	10	9	0,95	1,35	0,0679	
25	cage C	0	0	0	0	0	0	7	1	5	2	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	6	14	1,00	1,57	0,0690	
26		0	0	0	0	0	0	3	2	5	6	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1,00	1,57	0,0692	
27		0	0	0	0	0	0	2	0	6	2	4	3	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	6	13	0,95	1,35	0,0668	
28		0	0	0	0	0	0	2	1	3	2	2	7	0	1	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	15	7	1,00	1,57	0,0652
29	cage D	0	0	0	0	0	0	3	0	6	0	2	2	0	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	9	11	1,00	1,57	0,0656
30		0	0	0	0	0	0	7	2	0	6	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	10	7	0,85	1,17	0,0711	
31		0	0	0	0	0	0	7	1	2	1	0	4	0	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	11	9	1,00	1,57	0,0676	
32		0	0	0	0	0	0	6	0	5	0	1	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	6	12	0,90	1,25	0,0687	

* DAI = Day After Insertion of the larvae

LAB 11 - reproduction

Total egg ropes		cage A					cage B					cage C					cage D																								
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes																				
30.09.09	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1																				
01.10.09	16	4	0	0	0	4	3	0	0	0	3	1	0	0	0	1	1	0	0	0	1																				
02.10.09	17	10	3	0	0	13	12	0	0	0	12	8	2	1	0	11	6	1	0	0	7																				
03.10.09	18	4	0	0	0	4	8	2	0	1	11	10	2	0	1	13	10	2	0	0	12																				
04.10.09	19	2	1	0	3	6	4	0	0	1	5	0	0	0	0	0	1	3	0	0	4																				
05.10.09	20	1	0	0	0	1	4	2	0	3	9	0	0	0	0	0	0	2	0	0	2																				
06.10.09	21	2	3	0	0	5	1	1	0	1	3	1	2	0	0	3	1	1	0	0	2																				
07.10.09	22	0	1	1	0	2	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1																				
08.10.09	23	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1																				
09.10.09	24	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	1	4	0	0	5																				
10.10.09	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																				
total		23	10	1	3	37	32	5	0	6	43	22	6	1	1	30	23	13	0	0	36																				
		females in cage				33	egg ropes per female				1,12	females in cage				40	egg ropes per female				1,08	females in cage				38	egg ropes per female				0,79	females in cage				43	egg ropes per female				0,84
Fertile egg ropes		cage A					cage B					cage C					cage D																								
date	DAI for P generation	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes	normal size, normal appearance	small size, normal appearance	normal size, abnormal appearance	small size, abnormal appearance	sum egg ropes																				
30.09.09	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1																				
01.10.09	16	4	0	0	0	4	3	0	0	0	3	1	0	0	0	1	1	0	0	0	1																				
02.10.09	17	9	3	0	0	12	12	0	0	0	12	8	2	1	0	11	6	1	0	0	7																				
03.10.09	18	4	0	0	0	4	8	2	0	1	11	8	2	0	1	11	10	1	0	0	11																				
04.10.09	19	2	1	0	3	6	4	0	0	1	5	0	0	0	0	0	1	3	0	0	4																				
05.10.09	20	1	0	0	0	1	3	1	0	1	5	0	0	0	0	0	0	1	0	0	1																				
06.10.09	21	2	2	0	0	4	1	1	0	0	2	1	2	0	0	3	1	1	0	0	2																				
07.10.09	22	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1																				
08.10.09	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1																				
09.10.09	24	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2																				
10.10.09	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																				
total		22	9	0	3	34	31	4	0	3	38	18	6	1	1	26	22	9	0	0	31																				
		females in cage				33	fertile egg ropes per female				1,03	females in cage				40	fertile egg ropes per female				0,95	females in cage				38	fertile egg ropes per female				0,68	females in cage				43	fertile egg ropes per female				0,72
		males in cage				44	male fraction in cage				0,57	males in cage				35	male fraction in cage				0,47	males in cage				35	male fraction in cage				0,48	males in cage				37	male fraction in cage				0,46