

1 Note: this is a pre-publication draft submitted to a refereed journal for consideration on
2 13 November 2018 and has not yet been subject to independent peer-review.

3
4 The raw data supporting this draft manuscript is available [here](#) as an Excel file titled
5 “Chaoborus raw data”

6
7 Title: **Emergence timing and voltinism of phantom midges, *Chaoborus* spp., in the UK.**

8
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18 This work was funded by ADAMA Makhteshim Ltd., P.O. Box 60, Beer-Sheva, 8410001, Israel.

19
20 To make this information widely available the authors wish to apply for “gold” open access for this
21 publication and the fee for this will be paid by the sponsor.

22
23 Declaration of interest: The authors conduct contract research for regulatory purposes.

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25 Key words: aquatic invertebrates, *Chaoborus*, emergence timing, voltinism

26 Abstract

27

28 The emergence timing of adult *Chaoborus* spp. (Diptera: Chaoboridae) was investigated in outdoor
29 freshwater microcosms in the UK in 2017.

30 Adult *Chaoborus* spp. started emerging on 13 April, 14 days after larvae had been introduced, and
31 reached a peak on 2 May, 33 days after introduction of the larvae. The majority of emergence was
32 completed by 3 June (65 days after establishment). Of the 2027 larvae introduced into four
33 microcosms, emergence ranged from 51.4% to 66.2% with a mean of 60.9%. The majority of
34 emerged insects were *C. obscuripes* (99.68%) and the remainder of those identified were *C.*
35 *crystallinus* (0.24%). Males appeared to emerge slightly earlier than females.

36 The number of *Chaoborus* spp. life-cycles per year occurring in the UK was studied in six replicate
37 microcosm groups, each containing four individual units. Populations of zooplankton and benthic
38 invertebrates were established in each mesh-enclosed microcosm (containing 200 L of water) with
39 alder (*Alnus glutinosa*) leaves added to provide a substrate for benthic invertebrates. As soon as the
40 first egg rafts were laid in each microcosm these were removed and introduced onto a fresh unit. The
41 first generations in both replicate groups contained both *C. obscuripes* and *C. crystallinus*, although
42 the larvae sampled from the second to fourth generations in these replicates were all *C. crystallinus*.
43 Since only *C. crystallinus* was found in these later generations, the egg-to-egg development time for
44 *C. obscuripes* could not be determined. For *C. crystallinus*, life cycle times from egg-to-egg ranged
45 from 14 days (replicate group 5, first generation) to 56 days (replicate 3, second generation).

46

47 The results of this study showed that *C. crystallinus* produced up to four discrete generations within
48 the experimental period. The conditions existing in the experimental microcosms may have been
49 unfavourable for oviposition by *C. obscuripes*. This study confirmed that in temperate conditions *C.*
50 *crystallinus* exhibits a multi-voltine life history.

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53 Introduction

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55 The Chaoboridae are Diptera with aquatic larvae that live as pelagic predators, feeding on a wide
56 range of prey including Copepods, Cladocera, Rotifers, Chironomids, mosquito larvae and other
57 chaoborids. The presence of larvae throughout the year has led to the conclusion in published
58 literature that *Chaoborus* spp. are univoltine in temperate conditions, with the implication that a broad
59 spread in life cycle times gives rise to the presence of a range of larval stages throughout the year. In
60 studies conducted in the UK (Young, 1974) and Germany (Strauss et al, 2016) almost every larval
61 stage was found in Spring, Summer and Autumn, with adults emerging over the entire period in field
62 microcosms. In central Europe, *C. crystallinus* was considered to be univoltine and possibly bi-
63 voltine in hot summers (Berendonk and Spitze, 2006; Mumm, 1997; Parma, 1970 and 1971; Ratte,
64 1979).

65

66 There are three genera of Chaoboridae in northern and central Europe; *Chaoborus*, *Mochlonyx* and
67 *Cryophilia*, all of which have four aquatic larval instars (Sæther, 1997; Borkent, 1979; Andersen and
68 Kvifte, 2012). *Chaoborus* species are widely distributed throughout Europe and of the six species
69 that are known *C. flavicans*, *C. obscuripes* and *C. crystallinus* are the most common, the most
70 abundant, and the most studied. Pelagic, third and fourth instar larvae of some species, such as
71 *Chaoborus flavicans*, have adapted to co-exist with fish in larger water bodies and lakes. *C. flavicans*
72 exhibits vertical migration to sediment where they burrow during the daytime to avoid predators and

73 migrate upwards at night to feed. In shallower water or where fish are absent they may be entirely
 74 pelagic (Parma, 1969). Populations of *C. crystallinus* predominantly occur in shallow water bodies
 75 without fish and are mostly pelagic, although larvae have been found in sediment, (Parma 1969).
 76 Females of *C. crystallinus* are able to detect the presence of fish kairomones in water (Berendonk
 77 1999) and so can avoid depositing their eggs in water bodies containing fish.

78
 79 Larger natural or anthropogenic water bodies that do not contain fish have been found to be
 80 dominated by *C. obscuripes* (Lamontagne et al., 1994; Berendonk and Bonsall, 2002). *C. obscuripes*,
 81 *C. crystallinus* and *C. flavicans*, which are common throughout Europe, are the most researched of the
 82 Chaoborid species. Habitat preferences for the more common European species are summarised in
 83 Table 1.

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Table 1: Habitat preferences for the larvae of European Chaoboridae

Species	Location and Habitat
<i>Mochlonyx velutinus</i>	Larvae found in puddles, springs, pools, wells, tree holes and in temporary or permanent pools
<i>Chaoborus flavicans</i> *	Lakes most often but also ponds
<i>Chaoborus crystallinus</i> *	Shallow ponds without fish, shallow ephemeral water bodies
<i>Chaoborus obscuripes</i> *	Man-made waters bodies without fish, small and shallow waters, also found in deeper lakes
<i>Chaoborus pallidus</i> *	Shaded portion of pools

86 * overwinter as instar IV larvae in permanent water bodies

87

88 Chaoboridae are holometabolous and larvae develop through four growth stages (instars) and then
 89 pupate. *Mochlonyx* and *Cryophilina* and *Chaoborus nyblaei* overwinter in the egg stage which is
 90 resistant to desiccation whilst other temperate species overwinter as fourth instar larvae and pupate
 91 between April to June depending on temperature.

92

93 Oviposition takes place a few hours after emergence and in some species (e.g. *C. flavicans*) eggs can
 94 be held at the surface by a surrounding jelly. *C. crystallinus* lays on average 361 eggs arranged in the
 95 form of floating discs to form a raft. The duration of the egg stage is temperature-dependent and for
 96 *C. crystallinus* can range from 190-200 hours at 10°C and between 37-50 hours at 20°C. First and
 97 second instar larvae develop rapidly over a few weeks whereas the developmental periods of the third
 98 and fourth instar larvae are considerably longer. First and second instar larvae are positively
 99 phototactic (Parma, 1971; Sæther, 1997) at first and stay in the upper layer of water (epilimnion)
 100 which is warmer and has more oxygen. The later instars are generally found deeper in the water
 101 column where they feed on zooplankton.

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103 *Chaoborus* larvae are very tolerant of a wide range of unfavourable environmental conditions. *C.*
 104 *flavicans* can withstand long periods without oxygen and both *C. flavicans* and *C. crystallinus* can
 105 withstand long periods without food. Apart from *C. nyblaei*, which has resistant eggs, *Chaoborus*
 106 species have no resting stages that allow them to survive adverse conditions (von Ende, 1982).
 107 Larvae of the same species can have variable life spans (von Ende, 1982) depending on environmental
 108 conditions and the structure of the community. Life cycles can therefore be univoltine (predominantly
 109 in Europe) bi-voltine (high temperatures in Europe) or multi-voltine (in Japan, Xie et al., 1998).

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The duration of the pupal stage is also temperature dependent and for *Chaoborus crystallinus* this can range from 2-4 days at 20°C, between 10-13 days at 10°C and 30 days at 5°C. In Central Europe, there appears to be a very pronounced emergence period for *Chaoborus* and *Mochlonyx* between April and May with a second, less pronounced emergence from the end of July to October (von Ende, 1982). Females emerge from pupae and mate almost immediately, before their genitalia harden. Male swarming behaviour is commonplace and some species have been shown to be attracted to light. Imagines have no resting periods and live for at most ten days during which they do not feed (Berendonk, 1999; Parma, 1971).

The precise duration of the life-cycle of *Chaoborus* spp. does not appear to have been clearly defined in published studies. Since the larval stages are known to be extremely sensitive to the effects of insecticides and size-dependent sensitivity can play an important role in the survival and recovery of natural populations, the duration and timing of the life cycle has implications for the interpretation of how natural populations recover from exposure to stressors.

Berendonk and Spitze (2006) state in their introduction: '*Chaoborus crystallinus* is univoltine in Central Europe although it may go through two generations in exceptionally hot summers.' However, no citation to support this statement is given in that paper. It appears that this is an assumption sourced from earlier publications and is not based on empirical evidence. The *Chaoborus* found in microcosm studies conducted in central Europe appear to be mostly *C. crystallinus*, as deduced by Janz et al. (2016), who analysed data collected from 19 microcosm studies conducted over 14 years at the University of Munich, Germany. In this study, 1st and 2nd instar larvae were present from mid-April to early-October, 3rd instar larvae from early-May to October/November. Larvae overwintered as 4th instar and these were present during the entire study. Pupae were found from early-April to the end of August. The number of egg-laying peaks was used to indicate the number of generations of *C. crystallinus* in this study (the first at the end of April/early May, the second in June and the third at the end of July beginning of August). Janz et al concluded that there were three generations of *C. crystallinus* each year.

The presence of larvae throughout the year has led to the conclusion in published literature that *Chaoborus* spp are univoltine in temperate conditions. In central Europe, *C. crystallinus* was considered to be univoltine, although possibly bi-voltine in hot summers (Berendonk and Spitze, 2006; Mumm, 1997; Parma 1970 & 1971; Ratte 1979). Verberk et al (2008) considered the dispersal strategy of *Chaoborus crystallinus* to be uni/bi-voltine and typical of those species that have a long period of juvenile development. In Central Europe, there appears to be a very pronounced emergence period for *Chaoborus* and *Mochlonyx* between April and May with a second, less pronounced emergence from the end of July to October (von Ende, 1982). Life cycle strategies of *Chaoborus* spp. reported in the literature are summarised in Table 2.

152 **Table 2: Number of generations per year for *Chaoborus* spp. from published literature**
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Species	Number of generations
<i>C. flavicans</i>	Univoltine or bivoltine (Regmi et al., 2013) Univoltine (Jäger et al., 2011) Univoltine (Liljendahl-Nurminen et al., 2002) Univoltine (Schröder, 2013) Univoltine (Berendonk and Bonsall, 2002)
<i>C. crystallinus</i>	Univoltine (Sardella and Carter, 1983; Sæther, 1997) Univoltine (Verberk et al., 2008) Univoltine (Berendonk and Spitze, 2006) and bivoltine in hot summers (possibly) Univoltine (Mumm, 1997; Parma, 1971; Ratte, 1979) Univoltine (Berendonk and Bonsall, 2002) Univoltine (Sæther, 1997) Multi-voltine (Strauss et al., 2016) Multi-voltine (Janz et al., 2016)
<i>C. obscuripes</i>	Multivoltine (Zafar et al., 2011) Univoltine (van Wijngaarden et al., 2006)

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 156 As shown in Table 2, publications up to 2008 have considered *C. crystallinus* to be univoltine. On the
 157 other hand, the two most recent papers (both published in 2016) are unequivocal in the conclusion
 158 that *C. crystallinus* is multivoltine (in temperate conditions). We would surmise that the disparity
 159 between the earlier and most recent articles is that the conclusions of the latter are based on empirical
 160 evidence, whereas the statements on voltinism in the other papers appear to originate from
 161 an assumption in earlier publications. The empirical evidence in Strauss et al. (2016) and Janz et al.
 162 (2016) is essentially the observed prevalence of the various *C. crystallinus* life-stages over time in
 163 outdoor microcosms. The experiment described in this publication is a refinement of this approach.
 164 The difference is that this new work excludes the confounding factor of egg deposition by adults
 165 which have emerged from other water bodies. This was achieved by the enclosing microcosms in
 166 ‘tents’ made of ‘insect-proof’ netting. The only *C. crystallinus* inoculants were egg rafts placed by
 167 the experimenters. These rafts come from the previous (also enclosed) generation. This could be
 168 described as a ‘temporal chain’, each link in the chain being an artificial transfer of egg rafts from one
 169 enclosed microcosm to the next.

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 171 *C. crystallinus* is considered to be highly sensitive to the effects of certain insecticides and has been
 172 used in individual based models to predict their potential effects and recovery of aquatic invertebrates
 173 (Dohmen et al., 2015). An evaluation of the number of generations of *C. crystallinus* per year would
 174 be relevant for understanding the recovery potential of *Chaoborus* spp. in freshwater systems
 175 following possible reduction by pesticides.

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 177
 178 **Materials and Methods**

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 180 Two separate trials were conducted at AgroChemex Environmental Ltd., Aldhams Farm Research
 181 Station in Essex, U.K. (Grid Reference TM 099 305) in 2017, one to investigate the emergence timing
 182 of *Chaoborus* spp. adults and one to determine how many generations per year could occur in the UK.
 183

184 Microcosms of the same design were used in both trials. Each microcosm consisted of a circular
185 plastic tank approximately 0.8 m in diameter and 0.6 m deep (with a volume of 227 litres), sunk into
186 turfed ground to a depth of approximately 50 cm. 20 litres of washed sharp sand was added and each
187 microcosm was filled to a depth of approximately 50 cm (the same level as surrounding soil) with
188 approximately 200 L of freshly-drawn borehole water. Approximately 10 litres of water-saturated
189 lake sediment was poured in and allowed to settle in an even layer over the sand base and each unit
190 was covered with insect-proof mesh (1 mm² aperture) to prevent the entry of aquatic flies, particularly
191 *Chaoborus* spp. An oxygenating submerged aquatic macrophyte (*Elodea canadensis*, sourced from
192 Envigo, Eye Research Laboratory, Suffolk, UK) was loosely planted in each microcosm to occupy an
193 area of approximately ¼ of the sediment surface. The plant material was rinsed thoroughly prior to
194 introduction into the systems to remove any invertebrates.

195

196 Populations of zooplankton typically comprising rotifers, copepods, daphnids and detritus-feeding
197 benthic invertebrates (e.g. *Asellus* and *Gammarus*) were established in each microcosm using material
198 collected from a natural pond on the site at Aldhams Farm Research Station. Following the addition
199 of invertebrates, a handful of alder (*Alnus glutinosa*) leaves was added to each microcosm to provide
200 a substrate for benthic invertebrates. Alder leaves had originally been collected from Fen Alder Carr
201 (a local nature reserve established in 1982), Suffolk, UK, and then dried and stored. The alder leaves
202 were soaked for >7 days in clean borehole water and roughly shredded with scissors prior addition to
203 the microcosms.

204

205 Microcosms in both trials were monitored weekly for temperature, pH, dissolved oxygen and
206 conductivity using probes and a calibrated data logger. Water temperature in one unit was monitored
207 with readings every 30 minutes, using a calibrated data logger. Additionally, the water temperature in
208 an unused, unenclosed microcosm was also monitored continually from June onwards, to allow a
209 comparison of temperatures in enclosed and unenclosed systems. Climatic conditions on the
210 microcosm site were recorded throughout the study using a Davis field weather station, situated
211 approximately 100 metres from the study site.

212

213 **Emergence timing.**

214

215 This study was conducted to determine whether the first spring emergence of adult *Chaoborus* from
216 overwintered 4th instar larvae took place over a defined period, or was protracted, with adults
217 emerging and reproducing at intervals throughout the year.

218

219 Four microcosms were used for this study. Populations of *Chaoborus* spp. were established on 22
220 March 2017 in each microcosm by the addition of approximately 500 4th instar larvae, obtained from
221 an untreated field reservoir at Envigo Ltd Wooley Road, Alconbury, Huntingdon, Cambridgeshire,
222 UK. *Chaoborus* were collected using a sweep net and transferred to a covered holding vessel
223 containing water from the source reservoir for transportation to the field site. Each container held
224 larvae collected from several sweeps of the water column, just below the water surface. On arrival,
225 larvae were held outdoors in their original containers with loosely fitting covers. On the day of
226 initiation, groups of approximately 50 larvae were transferred into a tray, counted and then added to
227 one of the replicate microcosms. This process was repeated until the four microcosms contained 502,
228 503, 510 and 512 larvae respectively. Before the start of the study, the microcosms were covered with
229 insect-proof netting to prevent colonisation by the local populations of *Chaoborus*.

230 The emergence of adult *Chaoborus* sp. was monitored daily in the four microcosms by collecting
231 emerged insects seen on the walls of the enclosure or on the surface of the ground around the
232 microcosm using a vacuum sampler. Collected insects were preserved in 70% ethanol and stored for

233 subsequent identification to species level. The water surface was inspected daily for the presence of
234 egg rafts deposited by emerged females that had mated. Any egg rafts observed were removed as
235 soon as practically possible to prevent the introduction of fresh larvae to the microcosms. From day
236 88 (after introduction) onwards, the presence of larvae and pupae in the water column was also
237 recorded, to give an indication of whether further emergence could be expected to occur.

238

239 **Voltinism in *Chaoborus***

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241 The experimental system consisted of six replicates each of four individual microcosms established
242 between February and March 2017 at the field site. An additional set of four microcosms was
243 established to provide the initial egg rafts and these were covered with a pop-up frame and insect
244 proof mesh.

245

246 Each of the four microcosms established for the production of egg rafts were initiated with
247 approximately 500 4th instar *Chaoborus* spp. larvae from an untreated man-made field reservoir
248 (approx. 63 m³ and 2m deep) at Envigo Ltd Wooley Road, Alconbury, Huntingdon, Cambridgeshire,
249 UK and covered with insect-proof netting. Following the emergence of adult *Chaoborus* spp., these
250 egg generation microcosms were regularly monitored for the presence of egg rafts on the water
251 surface. When egg rafts were found they were transferred to the first of the four microcosms in each
252 replicate set. The production of egg rafts in the egg generation microcosms was monitored until no
253 more egg rafts were required. Each of the first of the four microcosms in each replicate set containing
254 egg rafts was inspected at least three times each week for the appearance and development of larvae,
255 pupation, emergence of adults and deposition of egg rafts. The presence of larvae and their
256 approximate instar together with the estimated numbers of pupae and emerged adults was recorded
257 together with the date and numbers of any egg rafts produced.

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259 Emerged adult insects were allowed to remain within the enclosure, reproduce and deposit egg rafts
260 on the water surface. These eggs were then collected and added to the second microcosm of each
261 replicate set to initiate populations. Established microcosms were inspected three times per week until
262 the end of September when the monitoring frequency was reduced to once per week. Assessments
263 were carried out visually by inserting a 19 cm diameter white disc attached to a rod to provide
264 contrast for assessing both at the surface (early-instar larvae) and at depth (late instar larvae and
265 pupae). The presence or absence of each life stage of *Chaoborus* was recorded in each active
266 microcosm. Where present, an approximation of the size range of larvae visible was recorded, mainly
267 to facilitate monitoring of egg hatching success and the rate of development, to ensure that critical
268 development stages were not missed. Once adult emergence had been observed, at each subsequent
269 assessment, the water surface was inspected for the presence of egg rafts deposited by emerged
270 females that had mated. The observation of the first deposition of egg rafts was recorded and those
271 egg rafts used to initiate the next sequential unit within the replicate. Subsequently, additional egg
272 rafts produced within the active units were also transferred to supplement the next unit's population,
273 until it was considered that no more were required.

274 On three occasions, once in July and twice in October, samples of late-instar larvae were taken and
275 preserved in 70% alcohol for identification to species level. In the July sampling, only three
276 microcosms contained larvae considered sufficiently developed for identification and ten larvae were
277 sampled from each. In October, where larvae were abundant, approximately 30 were sampled and if
278 fewer than this were seen, all larvae which could be captured were preserved.

279

280 The process of monitoring the appearance and development of larvae, presence of pupae, the
281 emergence of adults and deposition of egg rafts was repeated for the second, third and fourth
282 generations when applicable. In each case, the date from the first appearance of egg rafts in any
283 generation was used to estimate the duration of the life cycle time from egg-to-egg of each generation.
284 Larvae were sampled and identified to species level to determine the population composition in each
285 microcosm.

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288 **Results**

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290 **Emergence timing**

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292 Of the 1228 emergent adults all but four were identified as *C. obscuripes*. Counts of the numbers of
293 emerged adult male and female *C. obscuripes* in each of the four microcosms are shown in Figure 1.
294 Three specimens of *C. crystallinus* were found, the first (in Replicate 2) on 24 April 2017 (Day 25
295 post-initiation) and one each on 01 and 05 September 2017 (Days 154 and 158, respectively, both in
296 Replicate 1). A single adult *Chaoborus* sp. sampled from Replicate 1 on 22 May 2017 (Day 53) could
297 not be identified to species level. Therefore, of the successfully emerged adults, 99.68% were *C.*
298 *obscuripes* and only 0.24% were *C. crystallinus*.

299

300 Males appeared to emerge slightly earlier than females (Figure 1). In the first week of emergence 42
301 males were recorded compared with 8 females. Before 22 May there were generally more freshly
302 emerged males than females whereas the reverse was observed after this date.

303

304 Mean values for water temperature, dissolved oxygen, pH and conductivity measured weekly in each
305 microcosm are summarised in Table 3. Water temperature was measured every 30 minutes
306 throughout the study in one microcosm in replicate 1. The raw data for these environmental condition
307 readings are available in a data file “*Chaoborus* raw data” and have been summarised as daily
308 maximum, minimum and mean values in Figure 2.

Figure 1: Emergence timing of male and female *Chaoborus obscuripes*

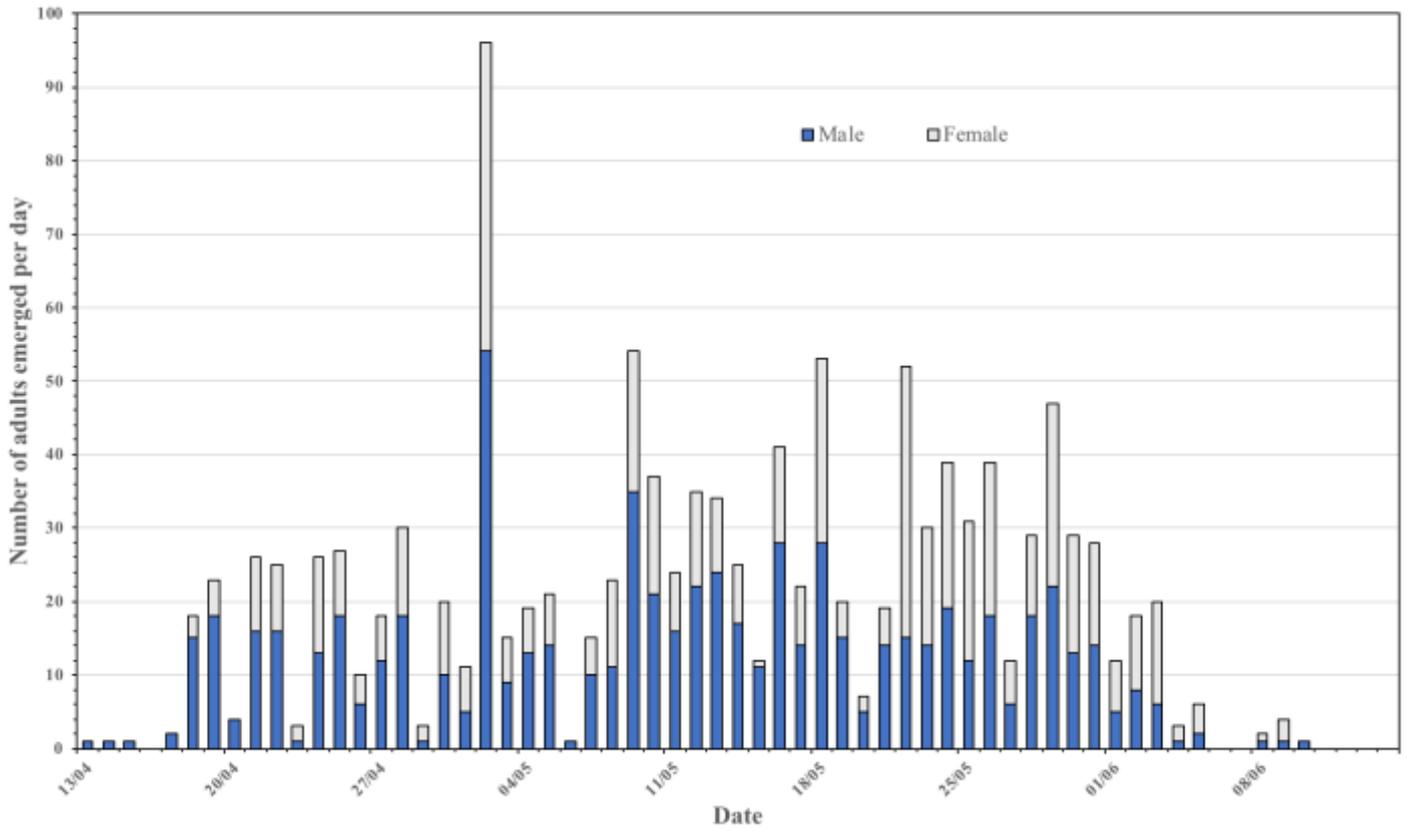


Plate 1. Microcosms with mesh-covered frames



Plate 2. Adult male *Chaoborus* spp.



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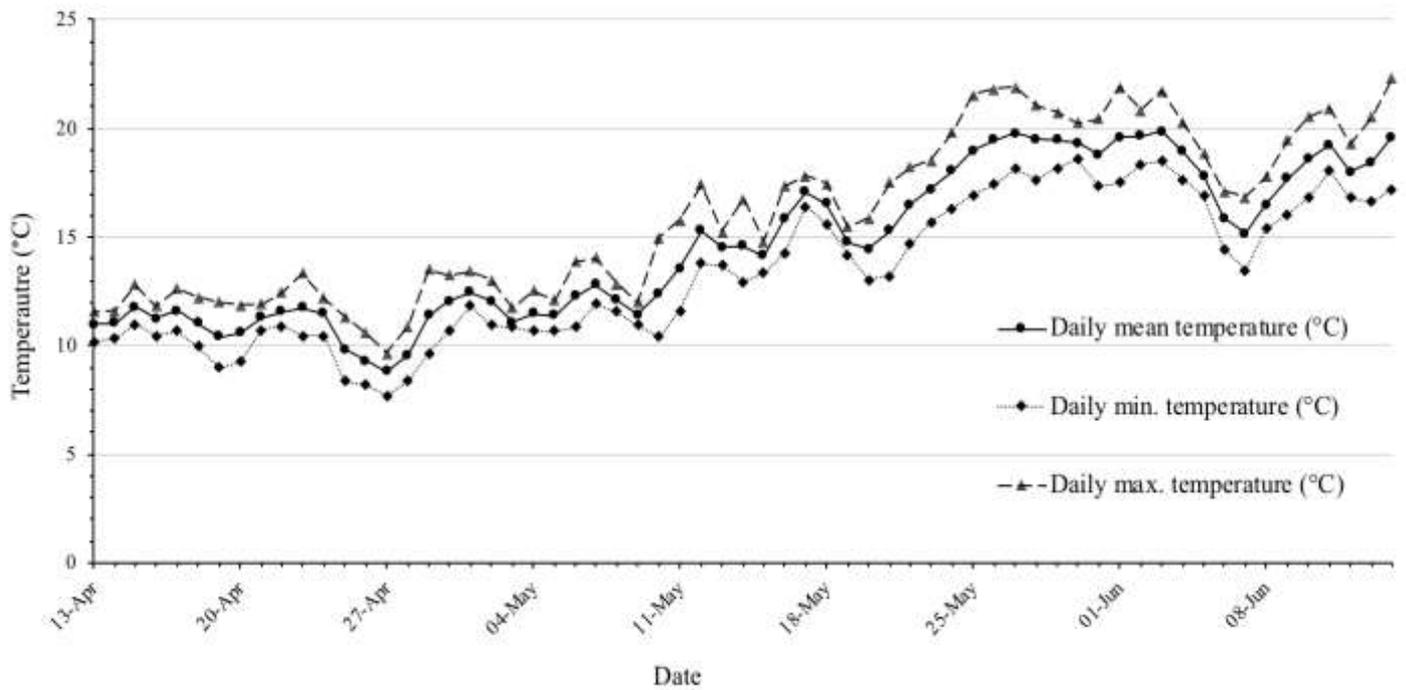
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Table 3: Weekly water conditions during the *Chaoborus* emergence study

Date measured	Mean Water Temp. (°C) (Standard Deviation)	Mean Water pH (Standard Deviation)	Mean DO ₂ (%) (Standard Deviation)	Mean Cond. (µS cm ⁻¹) (Standard Deviation)
05-Apr	13.50 (0.24)	7.81 (0.04)	92.98 (8.54)	961.25 (9.18)
12-Apr	12.25 (0.13)	8.17 (0.09)	127.45 (18.54)	854.00 (16.02)
19-Apr	9.20 (0.23)	8.09 (0.11)	124.23 (7.23)	814.00 (5.72)
27-Apr	8.33 (0.10)	8.71 (0.12)	134.05 (2.82)	766.00 (6.98)
03-May	11.00 (0.14)	9.32 (0.07)	138.875 (2.17)	745.00 (4.55)
11-May	14.60 (0.61)	9.74 (0.01)	147.30 (4.20)	733.25 (6.34)
19-May	15.35 (0.10)	9.74 (0.05)	133.95 (0.83)	678.75 (8.18)
26-May	17.30 (0.32)	9.78 (0.14)	132.05 (7.27)	683.25 (9.57)
02-Jun	19.83 (0.33)	10.20 (0.35)	152.78 (19.52)	682.25 (10.69)
09-Jun	20.48 (0.26)	10.38 (0.15)	171.50 (6.88)	637.25 (11.73)
16-Jun	21.68 (0.15)	10.33 (0.02)	178.58 (14.48)	653.00 (11.92)

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Figure 2: Daily maximum, minimum and mean water temperature recorded in replicate 1 during the emergence study



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328 **Voltinism**

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330 Daily maximum, mean and minimum water temperatures recorded during the emergence trial are
 331 presented in Figure 2. The development of *Chaoborus* from the initial egg rafts introduced into each
 332 unit are presented for each replicate in Figure 3. Egg rafts added to the first microcosms of replicates
 333 1, 2, 4 and 5 failed to establish at the first attempt and replicates 1, 2 and 5 were re-initiated at
 334 intervals as fresh egg rafts became available. The re-initiated replicates 1 and 5 both progressed
 335 through to a fourth generation. These two replicates were found to contain both *C. obscuripes* and *C.*
 336 *crystallinus* in their respective first units although the larvae sampled from units 2 – 4 in both
 337 replicates were all *C. crystallinus*. In both replicates, *C. obscuripes* were only found in July, while the
 338 specimens of *C. crystallinus* were only found in October. Replicate 4 could not be re-initiated with
 339 egg rafts as none were available at a suitable time. Monitoring of this unit showed that no larvae were
 340 present at any time, confirming the effectiveness of the systems in preventing immigration of
 341 *Chaoborus* spp. from outside. The re-initiation of replicate 2 produced only two generations which,
 342 when sampled in October, were found to consist only of *C. crystallinus*.

343

344 Populations of *Chaoborus* in replicates 3 and 5 progressed through to a third and fourth generations
 345 respectively. Larvae sampled from the first unit in July were found to be *C. obscuripes*, although
 346 when re-sampled in October, both *C. obscuripes* and *C. crystallinus* were found to be present.
 347 Generations 2 and 3, both sampled in October, were found to consist only of *C. crystallinus*. Replicate
 348 6 also did not require re-initiation but as the second unit did not establish successfully, larvae were not
 349 sampled from Unit 1 until October, in order to give the maximum opportunity for more egg
 350 deposition to restart Unit 2. In practice, a second production of egg rafts did not occur in Unit 1 and
 351 therefore, Unit 2 could not be re-started. Only six late-instar larvae remained in Unit 1 by the October
 352 sampling and all were found to be *C. obscuripes*.

353

354 Egg-to-egg times for *C. crystallinus* are summarised in Table and ranged from 14 days (Replicate 5,
 355 Unit 1) to 56 days (Replicate 3, Unit 2). As only *C. crystallinus* was found in the second, third and
 356 fourth generations of any replicate, it is not possible to draw any conclusions regarding the egg-to-egg
 357 timings for *C. obscuripes*. The shortest generation time of 14 days occurred when the water
 358 temperature was at its highest (Fig. 3) in late June and early July. However, the longest observed
 359 development time of 56 days also spanned this period so other variables such as prey density are
 360 clearly involved in determining *Chaoborus* development rate.

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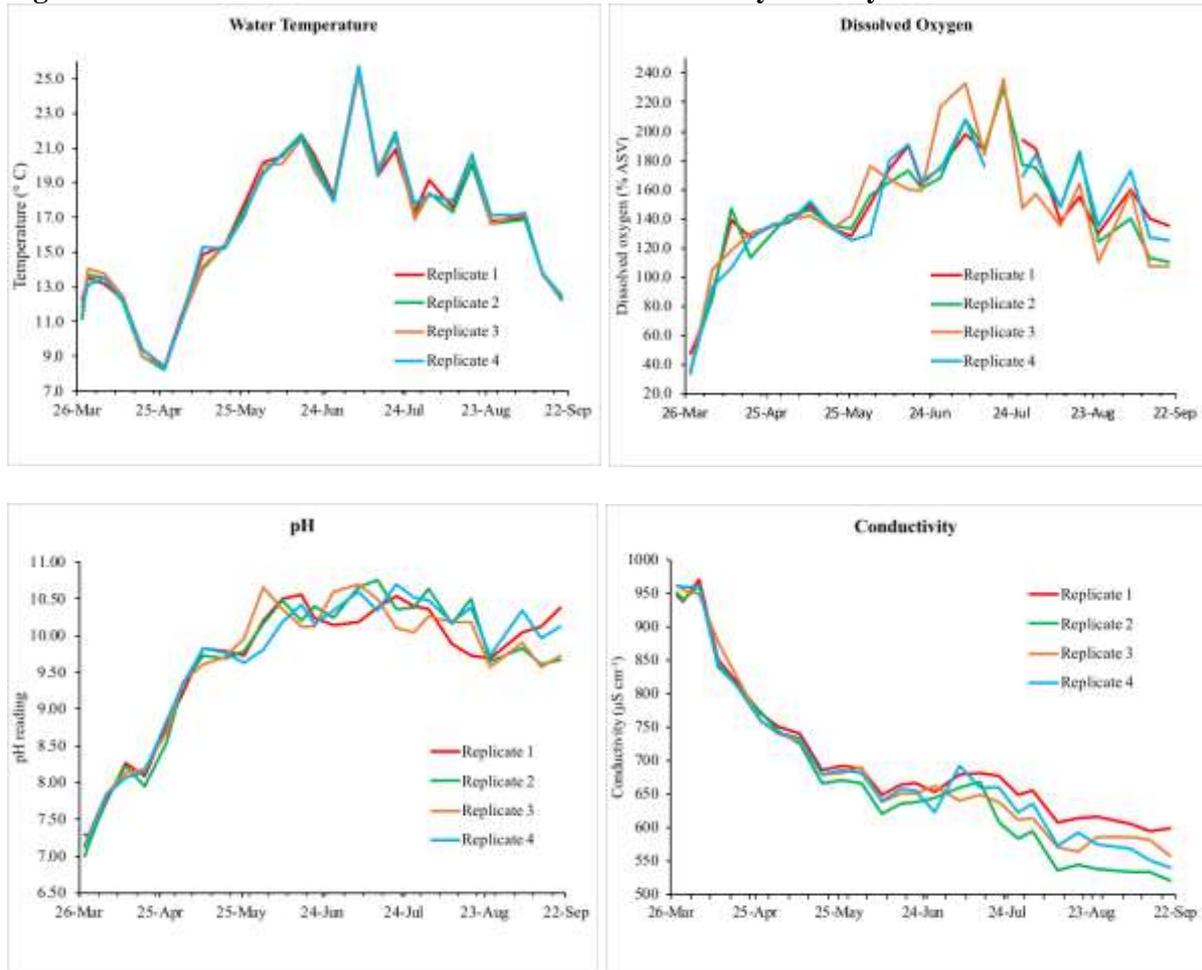
362 **Table 4: Egg to egg development times of *Chaoborus crystallinus***

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	Unit 1		Unit 2		Unit 3	
	Dates / Duration (days)		Dates / Duration (days)		Dates / Duration (days)	
Replicate 1	21 June-17 July	(26)	17 July- 24 Aug.	(38)	24 Aug-15 Sept*	(22)
Replicate 2	28 July - 29 Aug.	(32)	No successful second generation		No successful third generation	
Replicate 3	8 May-23 June	(46)	23 June - 18 Aug.	(56)	No successful third generation	
Replicate 5	21 June- 5 July	(14)	5-28 July	(23)	28 July - 24 Aug.*	(27)
Replicate 6	10 May-23 June	(44)	No successful second generation		No successful third generation	
Mean	32.4		39.0		24.5	
Overall mean	32.8 (S.D.13.0 days)					

364

379 **Fig. 4: Environmental conditions in microcosms in the life cycle study**



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Discussion

387 Emergence of adult *Chaoborus* spp. from overwintered larvae in South Eastern England commenced
 388 on 13 April 2017 (14 days after being introduced to artificial microcosms, Day 0) and peaked on 2
 389 May 2017 (Day 33). The majority of emergence was completed by early June (the last occasion when
 390 emergence of *Chaoborus* spp. occurred in all four replicates was 3 June 2017, Day 65). Of the 2027
 391 fourth instar larvae introduced into the microcosms, the mean emergence success was 60.9%, ranging
 392 from 51.4% in Replicate 4 to 66.2% in Replicate 1. As indicated by Strauss et al (2016) it is likely
 393 that carnivory was responsible for the emergence success being less than 100%. All except four of the
 394 emergent adults were *C. obscuripes* which was consistent with the populations of *Chaoborus* spp.
 395 known to inhabit the source pond in previous years. These results show that the emergence of adult
 396 *C. obscuripes* originating from the pupation of post-overwintering fourth instar larvae took place over
 397 a clearly defined period between mid-April and early June. Males appeared to emerge slightly earlier
 398 than females (Figure 1). In the first week of emergence 42 males were recorded compared with 8
 399 females.

400
 401 The results of the voltinism study showed that *C. crystallinus* produced up to four discrete generations
 402 within the experimental period. Since two replicate microcosm groups exhibiting four generations
 403 were both re-initiated several weeks after the season's egg deposition commenced, one more
 404 generation may have been possible. There may be some differences in developmental times or

405 reproductive success between species since from the second generation onwards populations were
406 dominated by *C. crystallinus*, despite the presence of *C. obscuripes* in the first generations. It is
407 possible that the test units or their conditions may have been favourable for oviposition by *C.*
408 *crystallinus* but not by *C. obscuripes*. In "the wild" *C. obscuripes* is almost always associated with
409 larger bodies of water (large ponds, small lakes and reservoirs).
410

411 The time for development from egg-to-egg ranged from 14 days to 56 days with a mean of 32.8 days
412 (Standard Deviation 13.0 days) indicating a high degree of phenotypic plasticity in *C. crystallinus* life
413 history strategies. Given that environmental conditions within the microcosms were all very similar it
414 seems most likely that differing levels of prey density contributed to the wide range of development
415 times.

416 This study confirmed that under temperate conditions *C. crystallinus* exhibits a multi-voltine life
417 history.

418

419 **References**

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