Emergence timing and voltinism of phantom midges, *Chaoborus* spp., in the UK

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Abstract. Phantom midges, *Chaoborus* spp. (Diptera: Chaoboridae), are an important taxon in environmental risk assessment of plant protection products due to the sensitivity of their larvae to insecticides. To aid modelling of population responses to xenobiotic exposure, information on emergence timing and voltinism is needed, but definitive evidence to support these parameters is lacking in the literature. We investigated emergence timing of overwintering *Chaoborus* larvae and the number of life cycles occurring per year in two separate, mesh-enclosed outdoor microcosm experiments in the spring and summer of 2017.

Emergence from overwintering larvae of a population of predominantly *C. obscuripes* (99.68 %) commenced on 13 April and peaked on 2 May. The majority of emergence was completed by 3 June. Emergence success ranged from 51.4 % to 66.2 %, indicating that for overwintered *C. obscuripes* larvae, adults emerged contemporaneously in spring, rather than sporadically over the course of spring and summer.

A population of *C. crystallinus* larvae produced up to four discrete generations over the spring and summer months (i.e. were confirmed to be multivoltine), with life cycle durations (egg-to-egg) ranging from 14 to 56 d. The differences in life cycle strategy observed in this study have implications for assessment of the capacity of populations of *Chaoborus* spp. to recover if there are localised impacts due to insecticide exposure or other stressors.

1 Introduction

Phantom midges (*Chaoborus* spp.) are a focal taxon in the environmental risk assessment of some classes of plant protection products due to their high sensitivity to acute exposure and the complexity of their life histories (Dohmen et al., 2016), in addition to their importance as regulators of zooplankton populations (Janz et al., 2016) and ecosystem services such as provision of nutrient subsidies to riparian ecosystems (Parmar et al., 2022). The larval stages are sensitive to the effects of insecticides (Maund et al., 1998; Schroer et al., 2004; van Wijngaarden et al., 2009), and post-exposure population recovery can affect concomitant zooplankton recovery (Hanson et al., 2007). Therefore, *C. crystallinus* has been used in modelling to predict effects and recovery in aquatic invertebrates (Strauss et al., 2016). Life cycle duration, timing and numbers of generations per year are critical variables in modelling populations of aquatic insects (Mebane et al., 2020) and are therefore important for understanding potential recovery following localised extinctions due to chemical exposures (Dohmen et al., 2016; Gergs et al., 2016), in addition to verification of “no-observed-effect concentrations” from microcosm toxicity studies using estimation of minimum detectable difference (Brock et al., 2015; Duquesne et al., 2020).
Chaoboridae are holometabolous Diptera with pelagic larve progressing through four instars, hereinafter L1–L4, preying on zooplankton, including other chaoborids. Habitat preferences for Europe’s more common species are well documented (Parma, 1969a, b, 1971; Borkent, 1981; Arranz et al., 2015). Hatching duration ranges from 190–200 h at 10 °C and 37–50 h at 20 °C (Parma, 1969b). Conspecific chaoborid larvae have variable life spans (Parma, 1971) depending on environmental conditions and zooplankton community structure. The pupal stage for *C. crystallinus* can take 2–4 d at 20, 10–13 d at 10 °C and 30 d at 5 °C. In central Europe, there is a pronounced emergence period between April and May with a second diffuse emergence from late July to October (Parma, 1971).

Life cycle strategies of *Chaoborus* spp. are not so well evidenced in the literature. The presence of larvae throughout the year has prompted assumptions that *Chaoborus* are univoltine in temperate conditions. *C. crystallinus* had been considered univoltine or bivoltine (Parma, 1969b, 1971). Berendonk and Spitzer (2006) state that *C. crystallinus* is typically univoltine in central Europe, although it may be bivoltine in exceptionally hot summers, while Roessink et al. (2005) refer to *C. obscursipes* as multivoltine. However, no supporting empirical evidence is cited in either case. More recently, some empirical evidence for multivoltinism has been reported. Data collected from 19 microcosm studies spanning 14 years at the Technical University of Munich, Germany, showed that L1s and L2s were present from mid-April to early October and L3s from early May to October or November (Janz et al., 2016). Larvae overwintered as L4s, which were present throughout the year. Pupae were found from early April to late August. Ovipositing peaks were assumed to indicate that there were three generations of *C. crystallinus*: one ovipositing in late April/early May, the second in June and the third in late July/early August (Janz et al., 2016). This study refines that approach, tracking development of successive generations by surrounding microcosms with mesh enclosures to exclude the confounding factor of immigrant oviposition and to reduce the reliance on assumptions to delineate completed life cycles.

The study was intended to evaluate (1) whether emergence of adults from the overwintering cohort of L4 larvae would be protracted over the entire summer season and (2) whether more than two discrete generations could be produced per year (i.e. that pond-dwelling *Chaoborus* spp. are multivoltine).

2 Methods

Two experiments were conducted at AgroChemex Environmental Ltd., Aldhams Farm Research Station, Essex, UK (grid reference TM09943048; grid references are according to the Ordnance Survey National Grid), in 2017, the first to investigate the emergence timing of *Chaoborus* adults from an overwintered cohort of L4s and the second to determine how many discrete generations per year could occur.

Established February–March 2017, the microcosms used in both experiments were of identical design, comprising circular polypropylene tanks 0.8 m in diameter and 0.6 m deep, sunk into turf ground to a depth of 0.5 m. Washed sharp sand (20 L) was added, and each microcosm was filled to a depth of 0.5 m with freshly drawn borehole water. Approximately 10 L of water-saturated lake sediment was added, after sieving to remove invertebrates. Each unit was covered with a 1 mm² aperture mesh to exclude immigrating aquatic flies. Oxygenating macrophytes (*Elodea canadensis*, sourced from Labcorp, Eye, Suffolk, UK) were rinsed thoroughly to remove invertebrates and loosely planted in each microcosm, occupying approximately 25 % of the sediment surface. Zooplankton and benthic detritivores, sourced from a natural pond on site, were added to each microcosm.

Active microcosms were monitored weekly for physicochemical water quality parameters (temperature, pH, dissolved oxygen and conductivity) using a Hach HQ40D multimeter. Water temperatures in one unit per experiment were monitored using calibrated data loggers, with readings every 30 min, and in an unused, unenclosed microcosm from June onwards to compare temperatures in enclosed and unenclosed systems. Climatic conditions were monitored continuously through the experimental season using a Vantage Pro2™ weather station (Davis Instruments Corporation, Hayward, CA, USA) positioned approximately 115 m from the microcosm array (grid reference TM10053044).

2.1 Emergence timing

*Chaoborus* populations were seeded on 22 March 2017 by adding nominally 500 L4s, obtained from an untreated reservoir at Labcorp, Huntingdon, UK, to each of four replicate microcosms. Prior to seeding, the initial mesh coverings were replaced with pop-up frames and insect-proof mesh (Popadome, Harrod Horticultural, Lowestoft, UK) (“enclosure(s”). Emergence of adult *Chaoborus* spp. was monitored daily by collecting insects seen within the enclosure using a vacuum sampler. Specimens were preserved for identification in 70% alcohol. The water surface was inspected daily for oviposition. Eggs were removed immediately to prevent recruitment of F1 larvae.

2.2 Voltinism in *Chaoborus*

This experiment comprised six replicates each of four individual microcosms, established between February and March 2017. Four additional microcosms were prepared to provide the initial eggs. These were covered with enclosures, and each was seeded with approximately 500 *Chaoborus* L4s from the same source as the emergence experiment.

During adult emergence, these microcosms were monitored regularly for the presence of eggs, which are laid as
rafts on the water surface. Eggs were transferred to the first microcosm in each replicate set, and in each seeded microcosm, the initial mesh covering was replaced with an enclosure. Seeded microcosms were monitored for the presence of larvae and pupae, using a 19 cm diameter white disc, attached to a rod, for contrast. Adults were assessed by inspection of the enclosure mesh.

Adults were left within the enclosure to reproduce. Resultant eggs were then transferred to the second microcosm of each replicate set to initiate the subsequent generation. Each established microcosm was inspected three times per week. At the end of September, monitoring frequency was reduced to once weekly. The presence of each life stage of Chaoborus was recorded in each active microcosm. Where larvae were present, the approximate instar (estimated by eye) and the presence of pupae were recorded to ensure that critical development stages were not missed. Once emergence had been observed, at each subsequent assessment, the water surface was inspected for eggs. The observation of the first oviposition was recorded, and those eggs were used to start the next sequential unit within the replicate. Subsequently, additional eggs laid within the active units were also transferred to supplement the next generation, until viable L1s were established.

Monitoring the life cycle was repeated for the subsequent generations, as applicable. The date from the first oviposition in each generation was used to estimate the egg-to-egg life cycle duration. On three occasions, once in July and twice in October, samples of late-instar larvae were preserved in 70% alcohol for taxonomic identification. In the July sampling, only three microcosms contained larvae sufficiently developed for identification. Ten larvae were sampled from each. Larvae sampled from Unit 1 in July were all Chaoborus obscuripes, and male and female Chaoborus obscuripes in each replicate are shown in Fig. 1.

Three specimens of Chaoborus crystallinus were found, the first on 24 April and one each on 1 and 5 September, respectively. A single adult sampled on 22 May was only identifiable to genus level. Therefore, of the emerged adults, 99.68% were Chaoborus obscuripes and 0.32% were Chaoborus crystallinus.

Water temperature, dissolved oxygen, pH and conductivity did not vary significantly between microcosms. Summary statistics and nonparametric comparison results are given in Table S1 in the Supplement, and the raw data are available in the Supplement.

3.2 Voltinism

The development of Chaoborus from the initial eggs introduced is presented in Fig. 2. Eggs seeded into the first microcosms of Replicates 1, 2, 4 and 5 failed to establish at the first attempt, and Replicates 1, 2 and 5 were reinitiated as fresh eggs became available. Reinitiated Replicates 1 and 5 both progressed through to fourth generations. These two replicates contained Chaoborus obscuripes and Chaoborus crystallinus in their respective first units although the larvae sampled from Units 2–4 were all Chaoborus crystallinus. Reinitiation of Replicate 2 produced only two generations, which, when sampled in October, were found to comprise only Chaoborus crystallinus.

Populations of Chaoborus in Replicates 3 and 5 progressed through to third and fourth generations, respectively. Larvae sampled from Unit 1 in July were all Chaoborus obscuripes, although when re-sampled in October, both Chaoborus obscuripes and Chaoborus crystallinus were found. Generations 2 and 3, both sampled in October, comprised only Chaoborus crystallinus. Replicate 6 also did not require reinitiation, but Unit 2 did not establish successfully and its population died out. Six late-instar larvae remained in Unit 1 by the October sampling, all Chaoborus obscuripes.

Egg-to-egg times for Chaoborus crystallinus ranged from 14 to 56 d. The shortest generation time occurred when the water temperature was at its highest in late June and early July. However, the longest observed development time also spanned this period, so other environmental parameters or variables such as prey density are clearly involved in determining the Chaoborus development rate.

Water temperature and dissolved oxygen, recorded weekly during the study, did not differ significantly between replicates. However, there were statistically significant between-replicate differences in pH and conductivity (Table S2).

There was a marked shift in dominance from Chaoborus obscuripes in the first generations to Chaoborus crystallinus in the subsequent generations, although populations of Chaoborus obscuripes persisted in the first units of four out of the five productive replicates (Table 1).

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Figure 1. Emergence timing of male and female *Chaoborus obscuripes*. Males appeared to emerge slightly earlier than females.

Table 1. Taxonomic identification of sampled larvae (sampling date and numbers in parentheses).

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Figure 2. Development of *C. crystallinus* from introduced egg rafts in each microcosm that sustained populations in the life cycle study. Replicate 4 could not be reinitiated with egg rafts as none were available at a suitable time. Monitoring of this unit showed that no larvae were present at any time, confirming the effectiveness of the systems in preventing colonisation by *Chaoborus* spp. from other sources.
4 Discussion

The emergence of adult *C. obscuripes* originating from the pupation of post-overwintering L4s took place over a clearly defined period between mid-April and early June. Males appeared to emerge slightly earlier than females (Fig. 1). In the first week of emergence, 42 males were recorded compared with 8 females. All except four of the emergent adults were *C. obscuripes*, which was consistent with the populations of *Chaoborus* known to inhabit the source pond in previous years.

The voltinism study showed that *C. crystallinus* produced up to four discrete generations within the experimental period. Since both replicates producing four generations were reinitiated several weeks after the season’s oviposition commenced, one more generation may have been possible. There were some differences in development times, voltinism or reproductive success between species, since no *C. obscuripes* were sampled from second or later generations, despite their presence in the first generations and persistence until the end of the experiment. Despite the substantial overlap in the natural distributions of the two species (Borkent, 1981), it is possible that elements of the study conditions favoured *C. crystallinus*. Interspecific variation in larval tolerance to UV radiation has been reported (Nagiller and Sommaruga, 2009), while UV radiation has also been demonstrated to reduce the kairomone-induced defences against predation by *C. obscuripes* in the cladoceran *Daphnia pulex* (Sterr and Sommaruga, 2008), mediated by the effects of UV on the chemical integrity of kairomones released by the predator. Additionally, feeding in *C. obscuripes* larvae has been shown to be adversely affected by increasing pCO₂ (Kowalewska et al., 2020), so it seems possible that night-time releases of CO₂ from macrophytes, which were not measured but were likely to have been substantial given the daytime extremes of dissolved oxygen (see the Supplement), may have impaired *C. obscuripes* reproduction in this study. However, since all reinitiated replicates contained only *C. crystallinus*, this could indicate that all mating and ovipositing in *C. obscuripes* were completed by the time the eggs used for reinitiating were harvested. If so, this would indicate that *C. obscuripes* is truly univoltine, a view supported by the evidence from the emergence study described here, in which no *C. obscuripes* larvae from the overwintering initial cohort remained after mid-June, and from the life cycle study, in which larvae of *C. obscuripes* were found in the first units of some replicates, even though the mesh enclosures remained in place, preventing immigrating adults from ovipositing.

The time for development from egg to egg ranged from 14 to 56 d with a mean of 32.8 d. As significant causal links between environmental conditions and development time were limited, it seems most likely that varying prey quality or availability contributed to the wide range of development times.

5 Conclusions

This study confirmed that under temperate conditions, emergence of overwintering *C. obscuripes* larvae is not protracted over an entire summer and that *C. crystallinus* is multivoltine. This may indicate a higher capability for populations to recover from localised extinction. Recovery in *C. obscuripes* has been documented previously (e.g. Van Wijngaarden et al., 2006; Brock et al., 2010). The former authors, however, showed that recovery in *C. obscuripes* was much more pronounced after a spring application than in late summer, which appears to have been supported by the finding that emergence in overwintering L4s of this species is largely complete by mid-summer. Recovery, therefore, appears to be enhanced during periods of emergence and immigration from unaffected neighbouring sources, which suggests that *C. crystallinus* metapopulations may be better adapted to withstand localised extinctions than *C. obscuripes*.

The number of life cycles in *C. crystallinus* was positively correlated with daily minimum (but not mean or maximum) air temperature and negatively correlated with the UV index and solar radiation. These three parameters in combination explained a significant portion of the variation in voltinism, but none, taken in isolation, made a significant contribution. Therefore, it is possible that variation in these parameters acted only indirectly on the performance of the *C. crystallinus* populations, possibly by affecting food web interactions. For example, Tseng et al. (2021) showed that temperature has a significant effect on synthesis of important fatty acids.
at the primary producer level, which could have a concomitant influence on nutritional quality for secondary and tertiary consumers. Sentis et al. (2017) found a significant reduction in prey handling time (i.e. more effective feeding) in *C. obscuripes* with an increase in water temperature from 16 to 20 °C. Lastly, Lindholm et al. (2016) found the congener *C. nyblaei* to need attenuation of UV by substantial discoloration of the water in order to act as a significant top-down predator.

The life cycle duration (in this study ranging from 14–56 d for *C. crystallinus*) has implications for intraspecific variation in the sensitivity of the larvae to xenobiotics. For example, it could be hypothesised that the longer the life cycle duration, the longer each larva spends in each development stage, and, in particular, long durations of early instars with smaller body sizes may render them more sensitive to toxicants (Gergs et al., 2015).

**Data availability.** Supporting water quality data and values required to plot Fig. 1 are given in the Supplement and at https://doi.org/10.5281/zenodo.7293656 (Cockroft et al., 2022).

**Supplement.** The supplement related to this article is available online at: https://doi.org/10.5194/we-22-101-2022-supplement.

**Author contributions.** RJGC, WRJ and SN designed the experiments, and RJGC carried them out. AGI advised on test organism identification. KCB prepared Fig. 1 and an earlier version of the manuscript with contributions from all co-authors. RJGC prepared this version of the paper with contributions from all co-authors.

**Competing interests.** The contact author has declared that none of the authors has any competing interests.

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