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## CORRECTED PROOF

## Rapid Communication

## Recent expansion of the non-indigenous amphipod *Chelicorophium curvispinum* (G.O. Sars, 1895) in the Seine estuary

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## OPEN ACCESS

### Abstract

Recent sampling of the upper part of the Seine estuary (oligohaline, freshwater reaches) has led to the observation of the non-indigenous amphipod *Chelicorophium curvispinum* for the first time in the downstream part of the Seine Basin (between the Tancarville Bridge and Paris). Specimens were collected using a suprabenthic sledge along a salinity gradient ranging from freshwater to mesohaline, with observations of *C. curvispinum* all along this gradient. Samples collected were characterized by a relatively small number of individuals, reaching a maximum abundance of 15 individuals / 100 m<sup>3</sup> when temperatures were the highest, during the summer. The sex ratio was dominated by females, with approximately twice as many females than males. Interestingly, adults reach a size which allowed the reproduction of the species, but no ovigerous females were observed. Despite the rapid expansion of *C. curvispinum* in the Seine estuary, this study cannot assess whether this species should be considered as invasive in the Seine Basin. Nevertheless, as the sampling was carried out within the navigation channel, further observations will be necessary to determine the abundance of this species on soft and hard bottoms of the riverbanks.

**Key words:** freshwater-estuarine continuum, suprabenthos, connectivity, established population

### Introduction

The amphipod *Chelicorophium curvispinum* (G.O. Sars, 1895) originates from brackish environments of the Ponto-Caspian basin, and subsequently showed a rapid extension of its range to Western Europe at the beginning of the 20<sup>th</sup> century. This species firstly colonized rivers in Poland during the 1920s (Jazdzewski and Konopacka 2000), and then spread to the rivers of Central Europe including the Oder, Vistula and Elbe (Jazdzewski 1980). It migrated towards West Germany during the 1950s and was found in the Dortmund-Ems canal in 1977 (Herhaus 1978). Then, it invaded the River Rhine between 1982 and 1989 (Van den Brink et al. 1989), continuing its expansion towards the northwest by colonizing the Meuse (Wouters 1985; Khalanski 1997) and the Moselle (Schöll 1990; Bachmann et al. 1997; Kallenbach et al. 2000). Further to the south, it was first observed in the Saône in 1991 (Genin 1992) and in the Rhône during the early 2000s

(Dessaix and Fruget 2008). Its first observation in the Seine basin was in 1998 in the Picardy canals. Later, in 2009, *C. curvispinum* was observed in the downstream part of Oise and Aisne sub-basin and around Paris (Souben et al. 2014). It remains scarce, present in very rare and isolated cases in the rest of the Seine Basin (Souben et al. 2014) at the time of this study.

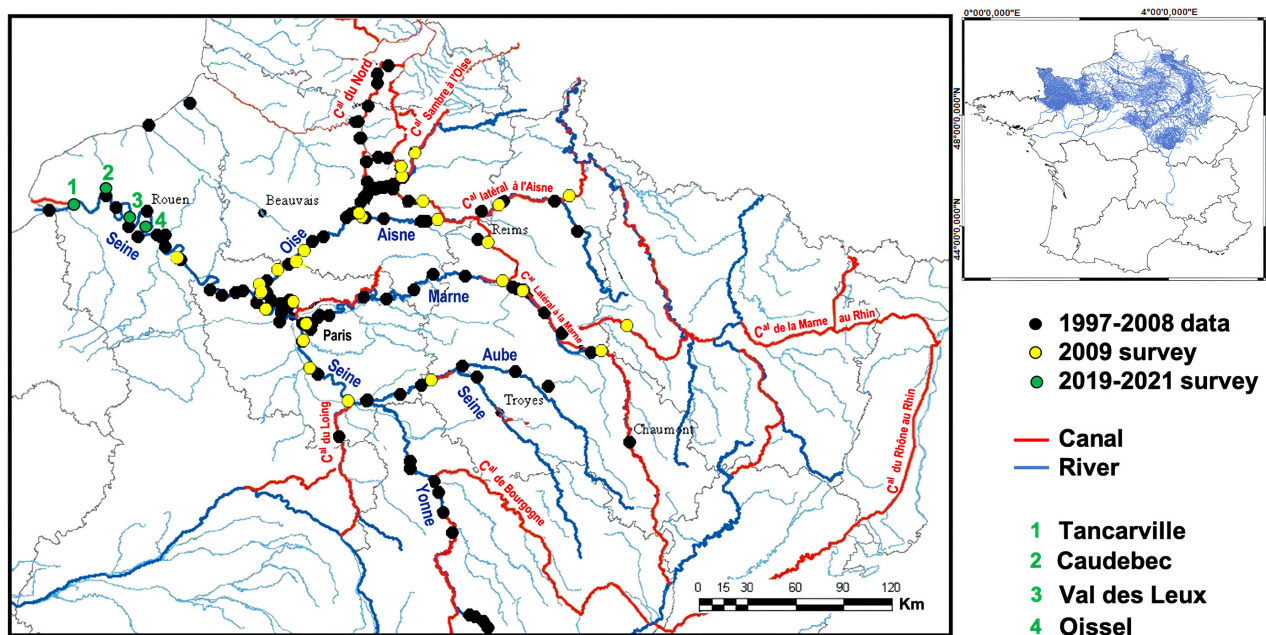
A 2018 survey conducted by Pezy et al. (2021) reported a total of 152 Non-Indigenous Species. In this checklist, *C. curvispinum* was not recorded in the lower part of the Seine estuary (based on previous observations). Moreover, a more recent benthic and suprabenthic survey conducted by Pezy et al. (2017) found no evidence of *C. curvispinum* in the lower part of the Seine estuary. The suprabenthos contains the organisms that migrate at night in the lower water layers and remain in the sediment during the day (Brunel et al. 1978). As a result, this faunal compartment has often been neglected in coastal research projects due to the difficulty of collecting samples just above the sea bottom without contamination by surficial sediments (Dauvin et al. 2000; Dauvin and Vallet 2006). The suprabenthic community is primarily characterized by Peracarida (amphipods, mysids, cumaceans, isopods and tanaids) and decapods. The suprabenthos in the Seine estuary has been studied in its downstream part (*i.e.*, downstream the Tancarville Bridge) since 1993, within the framework of phases 1 to 3 of the Seine-Aval programs (Wang and Dauvin 1994; Mouny et al. 2000; Dauvin and Pezy 2013; Pezy et al. 2017). In the Seine estuary, the suprabenthos is always present in the lower water layers due to the high turbidity of this system. During successive studies over 25 years, no *C. curvispinum* has ever been observed.

With the aim of understanding the functioning of the suprabenthos in the previously neglected upstream part (*i.e.*, upstream the Tancarville Bridge) of the Seine estuary, the SARTRE project was set up to describe the structure (distribution ranges, abundance, biomass and interaction with environmental variables) of the suprabenthos and associated food web by studying different compartments (organic matter, phytoplankton, zooplankton, suprabenthos and ichthyofauna). This project has confirmed the upstream expansion of the invasive mysid *Neomysis americana* (N. Chauvel *personal observations*), which has been reported further downstream in the estuary (Pezy et al. 2019). It has also led to the observation of *C. curvispinum* individuals along an upstream-downstream transect from freshwater to oligohaline zones, which is the focus of this present paper. The aim of this study is therefore to describe the main features of the *C. curvispinum* population in this part of the Seine estuary, and to establish the first hypotheses regarding its possible invasive character based on demographic observations (abundance, reproduction) of its population.

## Materials and methods

### *Study area*

The Seine estuary is located on the northern coast of France, opening onto the eastern part of the English Channel. The Seine estuary is a system under



**Figure 1.** Location of sites included in the historical dataset (1997–2009) and the four sites (Tancarville, Caudebec, Val des Leux and Oissel) sampled during the SARTRE project between 2019 and 2021 (modified from Souben et al. 2014). Additional information concerning samplings are available in Supplementary material Table S1.

tidal influence (8 m at spring tide near the mouth), with a tidally-influenced section extending 160 km upstream from the mouth to the Poses dam.

### Sampling strategy

A total of six surveys were conducted between 2019 and 2021 on four sites (Tancarville, Caudebec, Val des Leux and Oissel) representative of the range of salinities in the upstream Seine estuary (Figure 1). Samplings were all conducted during the winter (March 2021 survey), the spring (May 2021 surveys) and the summer (June 2019 and 2021, July 2019 and September 2020 surveys). Suprabenthos organisms were all carried out by day under similar tidal conditions, during the middle of the tidal cycle at slack water of flood tide. The suprabenthos was collected using a suprabenthic sledge filtering the water at 0.10–0.40 m above the sea bottom. The sledge was equipped with a WP2 plankton net (0.5 mm mesh size) with a flow meter at its centre to measure the volume of water filtered, allowing the standardization of abundance per 100 m<sup>3</sup>. At each station and for all surveys, three replicates were collected for a sampling duration of 5 minutes at a speed of approximately 2.8 km/h (1.5 knots). Environmental parameters (water temperature, salinity, oxygen concentration, turbidity) were measured at each station and for all surveys using a CTD profiler. After collection, the specimens were sieved on a 500- $\mu$ m square mesh sieve and fixed using 70% ethanol. They were then identified under a dissecting microscope at the laboratory.

The morphological characters of the *C. curvispinum* specimens sampled in the Seine estuary are in agreement with those given by Sars (1895) in the original description primarily for the head and the antennae. Thus, the cephalon shows a frontal edge slightly angular in the middle. In the female,



**Figure 2.** *Chelicorophium curvispinum* (G.O. Sars, 1895): male, profile (a), antennae 2 male (b), antennae 2 female (c). Scale bar: 2 mm. Photo by N. Chauvel.

the first antennae are short, attaining only 1/3 of the length of the total body. The first article of the peduncle is about the length of the other articles combined, and is armed below with 4–5 small spines. The second article has a similar spinule in the middle of the posterior edge. The flagellum is shorter than the peduncle and has less than 10 articles.

In the male, these antennae are more developed, and have the peduncle densely clothed with fascicles of slender bristles. The second article is elongated, fully equalling in length the first article and narrower. The flagellum is composed of about 12 articles. The antennae 2 are most developed in the male than in the female, attaining about the length of the whole body. The distal ventrolateral angle of peduncle article 4 of these antennae also shows one large process associated with two small accessory processes, which is characteristic of this species (Figure 2, right pictures). The last article of the peduncle is produced at the end posteriorly into a strongly incurved spiniform projection with a slightly bilobed expansion. The flagellum is shorter than the last article of the antennae, and possess numerous long setae (Figure 2).

A total of 5 specimens of *C. curvispinum* are lodged in the crustacean collection of the National Museum of Natural History in Paris under three collection numbers: MNHN-IU-2022-401, MNHN-IU-2022-3308 and MNHN-IU-2022-3309.

### *Statistical analyses*

A two-way ANOVA was used to test whether there was a difference in abundance between the different locations, dates and for the interaction of these two factors. Shapiro and Bartlett tests were used to check the normality and homoscedasticity of data. Tukey's HSD tests were then used to determine whether there were differences between the different factor levels.

### **Results**

A total of 117 individuals of *C. curvispinum* were collected at the four stations on the six sampling dates (Table 1). 71 females and 46 males were observed, with a mean sample male/female ratio of  $0.42 \pm 0.44$ , but no ovigerous

**Table 1.** Environmental parameters (S: salinity; T: temperature °C; Tu: turbidity NTU; O: Oxygen concentration in mg.L<sup>-1</sup>) and biotic parameters (N : number of *C. curvispinum* collected; A: abundance, mean number of *C. curvispinum* per 100 m<sup>3</sup> with standard deviation) measured on the six sampling dates.

Station	Date	S	T	Tu	O	N	A
Tancarville	June 2019	2.7	19.7	487.4	4.5	–	–
	July 2019	12.5	21.5	487.5	4.0	2	0.83 ± 0.91
	September 2020	14.2	19.4	478.8	3.4	6	1.33 ± 0.06
	March 2021	0.4	8.0	117.8	5.3	2	0.97 ± 1.00
	May 2021	2.9	14.4	487.7	7.1	–	–
	June 2021	2.9	19.8	332.6	5.9	2	0.90 ± 1.56
Caudebec	June 2019	0.3	19.9	11.4	5.7	1	0.23 ± 0.40
	July 2019	0.3	23.2	280.1	6.3	–	–
	September 2020	0.3	20.3	487.7	4.6	6	2.23 ± 1.12
	March 2021	0.3	9.0	52.3	5.3	–	–
	May 2021	0.2	14.6	77.9	7.8	3	1.03 ± 1.05
	June 2021	0.3	20.9	37.3	5.8	11	2.57 ± 1.08
Val des Leux	June 2019	0.3	20.0	94.5	5.2	1	0.60 ± 1.04
	July 2019	0.3	23.4	300.0	4.7	13	11.43 ± 9.28
	September 2020	0.3	20.4	125.1	7.4	10	2.37 ± 2.08
	March 2021	0.3	9.2	23.0	5.3	–	–
	May 2021	0.3	14.3	12.9	5.4	1	0.73 ± 1.27
	June 2021	0.3	20.2	13.8	3.5	6	4.50 ± 6.70
Oissel	June 2019	0.3	21.0	9.9	4.2	–	–
	July 2019	0.3	23.6	4.5	4.5	36	15.00 ± 9.95
	September 2020	0.3	20.4	8.4	6.7	13	3.97 ± 1.59
	March 2021	0.3	9.2	23.4	5.2	–	–
	May 2021	0.2	15.3	5.8	5.0	–	–
	June 2021	0.3	21.1	9.2	4.0	4	1.57 ± 0.50

females were observed. The date, location and interaction between these factors showed a significant effect ( $F_{(15,48)} = 2.815$ ,  $p < 0.01$ ) on abundance of *C. curvispinum*. Abundances were the highest in July 2019 (Tukey HSD,  $p < 0.05$ ), and the two most upstream stations (Val des Leux and Oissel) showed higher abundances than those measured at Tancarville (Tukey HSD,  $p < 0.05$ ), suggesting a decreasing density gradient from upstream to downstream (Table 1). It is also noteworthy that moults of this species were also recorded during the surveys of September 2020, March and May 2021, as far as Tancarville.

The species was observed at salinities ranging from 0.2 to 14.2, temperatures from 8.0 to 23.6 °C, turbidity from 4.5 to 487.7 NTU and oxygen concentration between 3.4 to 7.8 mg.L<sup>-1</sup> (Table 1). Male body length (rostrum-telson length) measured between 1 and 5 mm, and between 1 and 8 mm for females.

## Discussion

Historically, the Seine Basin has been studied at 170 stations from 1997 to 2008 (Souben et al. 2014). These authors have presented a bibliographic review of hydrobiological data obtained at 141 stations between 1997 and 2008. In 2009, these authors also carried out a field monitoring campaign including 29 stations to establish the status of *C. curvispinum* in the Seine Basin watercourses. At each station, two artificial hard substrates [keepnet

(0.2 × 0.3 m) filled with three stones] were immersed for three weeks. Their results highlight the fact that *C. curvispinum* occurs in the Oise and Aisne basins and, more recently, within the Paris area due to fluvial navigation. However, in 2009, this species was not detected in the Seine River downstream from its confluence with the Oise River. Thus, Souben et al. (2014) propose that *C. curvispinum* should not be considered as an invasive species in the Seine Basin, because this species has a low population density, with a slow rate of settlement, and it appears to have no significant effect on the native macrofauna (Souben et al. 2014), as observed in the Loire (Piscart et al. 2010).

Our study reveals that the colonization of the downstream part of the Seine (from Oissel to Tancarville) by *C. curvispinum* has taken place over the past 10 years. Although the sampling technique used by Souben et al. (2014) is very different from the present study, our results also suggest that this species should not be considered as an invasive species in the River Seine. The colonization of this species from the Oise River to Tancarville could be explained by maritime traffic or by an extension of its distribution from downstream to upstream in the Seine. However, it is surprising to observe the rather slow and limited expansion of this introduced species within the Seine Basin, as this species colonized the River Rhine in seven years (Van den Brink et al. 1989), becoming the dominant macroinvertebrate in just two years in its downstream part (Den Hartog et al. 1992; Paffen et al. 1994) with a strong invasive character. Even if the current evidences from the Seine estuary does not seem to indicate *C. curvispinum* is invasive in this range, evidence from the Rhine cannot rule out that possibility. This warrants that further examinations of this part of the Seine is appropriate. This is truer if we consider that the methodology used here was not primarily intended to study this species specifically, but rather to study the suprabenthos as a whole.

Similarly, it is very likely that the abundance of this species is greatly underestimated in our study. Indeed, *C. curvispinum* lives on stable bottoms, composed of hard substrates (Van den Brink et al. 1993) such as rocks, shells or branches. However, our samples were collected in the deepest part of the channel of the Seine estuary on a seabed composed of soft sediments regularly disturbed by maintenance dredging, which is believed to be an unsuitable habitat for this species (Van den Brink et al. 1993). Sampling of the river banks and observation of *in situ* hard substrates or sunken hard blocks could help to test this hypothesis. The presence of this species on hard substrates can be problematic if the species has a high density. Indeed, *C. curvispinum* is an allogenic ecosystem engineer able to build mud tubes that can sometimes form real “corophiid grounds” favourable for other tubedwelling species such as *Corophium volutator* (also observed in the estuary). However, their development can have a negative effect on species that are attached to bare rocks such as *Dreissena* spp. (Haas et al. 2002). The presence of such “corophiid grounds” remains to be ascertained in this part of the estuary.

The absence of ovigerous females in this study is quite unexpected, especially as the sex ratio is in favour of females, in agreement with the observations of Van den Brink et al. (1993). In the Rhine, ovigerous females have been observed over a period extending from April to September, with sometimes more than 50% ovigerous females being present (Van den Brink et al. 1993). To ensure that it is the same species (and not a cryptic one), genetic studies could also be carried out to determine whether the individuals collected in the Seine estuary are genetically close to those collected in the Rhine, but also to those from the species' native range (Caspian Sea). This would allow us to determine whether or not this population is distinct from the Rhine population and whether it was founded by a different introduction. These differences can also possibly be explained by the fact that the samples were collected outside the natural habitat of this species (corophiid grounds), where ovigerous females probably shelter. It is also possible that the reproduction of this species does not occur so far downstream in the Seine Basin, the slightly saline conditions of the environment being possibly detrimental to this activity. Also, the small number of individuals sampled may not be sufficient to favour the observation of such features. For the moment, however, the absence of reproductive females does not allow us to consider the population of *C. curvispinum* as established in this downstream part of the Seine Basin, but further observations are needed.

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### Author's contribution

NC: investigation and data collection, data analysis and interpretation, writing – original draft. AR: review and editing. JCD: investigation and data collection, review and editing. JPP: investigation and data collection, research conceptualization, sample design and methodology, writing – original draft.

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#### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Details of the sampling carried out during the SARTRE project in the upper Seine estuary.