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New data on the introduction of the invasive signal crayfish *Pacifastacus leniusculus* (Dana, 1852) (Crustacea, Decapoda) and ectosymbiont branchiobdellidans (Annelida, Clitellata) in NW Iberian Peninsula

Nuevos datos sobre la introducción del cangrejo señal *Pacifastacus leniusculus* (Dana, 1852) (Crustacea: Decapoda) y branquiobdelidos ectosimbiontes (Annelida, Clitellata) en el NO de la Península Ibérica

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Abstract

The non-native signal crayfish *Pacifastacus leniusculus* (Dana, 1852) has successfully been established in many catchments of Europe, causing detrimental impacts on both native flora and fauna. The aim of this paper was to report the anthropogenic introduction of *P. leniusculus* and its ectosymbionts (family Branchiobdellidae) in the River Tambre (NW Spain). Seventy one individuals of *P. leniusculus* were captured in the Barrié de la Maza reservoir in November 2016. The presence of several age classes, including young of the year individuals, and ovigerous females suggests that the population is already established and viable. Most individuals (96%) presented branchiobdellidans and 4% exhibited white spots probably caused by a virus. Melanised spots caused by the oomycete *Aphanomyces astaci* (Schikora, 1906) (crayfish plague) were not found in this sample. This population is expected to disperse through the River Tambre basin. The Tambre population may also be a source of individuals for new introductions in the region, where currently no *P. leniusculus* populations are known at a minimum radio of 150 km in straight line. Therefore, immediate management and preventive actions should be pursued to control the dispersion and growth of this population and to avoid new introductions (e.g. ban of crayfish fishing).

Keywords: Astacidae, Branchiobdellidae, distribution, invasive species, River Tambre, Galicia, Spain.

Resumen

El cangrejo señal *Pacifastacus leniusculus* (Dana, 1852), especie introducida en Europa, se ha establecido con éxito en muchas cuencas europeas, causando impactos perjudiciales en la flora y fauna autóctonas. El objetivo de este trabajo fue documentar la introducción antropogénica de *P. leniusculus* y sus ectosimbiontes (familia Branchiobdellidae) en el río Tambre (NO de España). Se capturaron 71 individuos de *P. leniusculus* en el embalse de Barrié de la Maza en noviembre de 2016. La presencia de varias clases de edad (incluida la cohorte 0+) y hembras ovígeras sugiere que la población ya está establecida y es viable. La mayoría de los individuos (96%) presentaron ectosimbiontes y un 4% presentaron manchas blancas probablemente causadas por virus. No se encontraron manchas melánicas, generalmente causadas por el hongo *Aphanomyces astaci* (Schikora, 1906), responsable la plaga del cangrejo de río (afanomicosis). Es de esperar que esta población aumente su distribución dentro de la cuenca del río Tambre y pueda ser una fuente de individuos para nuevas introducciones en la región, donde no se conoce la presencia de *P. leniusculus* en un radio mínimo de 150 km. Por lo tanto, es recomendable que se apliquen medidas de gestión con el fin de controlar la dispersión y el crecimiento de esta población y evitar nuevas introducciones (por ejemplo, a través de la prohibición de la pesca recreativa del cangrejo señal).

Palabras clave: Astacidae, Branchiobdellidae, distribución, especies invasoras, río Tambre, Galicia, España.

INTRODUCTION

The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) is a native species in North America and was introduced in Europe, Russian Federation, and Japan (Schuster *et al*., 2010). In Europe, the species was first introduced in Sweden in 1959 (Dunn, 2012) and thereafter in many other countries, and is now found widespread across the continent (Fig. 1). *Pacifastacus leniusculus* is an invasive species with a high impact on the aquatic biota and the habitat where they live (Griffiths *et al*., 2004; Souty-Grosset *et al*., 2006). In addition, *P. leniusculus* is a common carrier of other organisms like viruses, ectosymbiont branchiobdellidans, fungus, etc. (Ohtaka *et al*., 2005; Souty-Grosset *et al*., 2006). Thus, *P. leniusculus* is a chronic carrier of the oomycete *Aphanomyces astaci* (Schikora, 1906) that causes the crayfish plague, the main cause of decline of native freshwater crayfish species in Europe (Souty-Grosset *et al*., 2006; Dunn, 2012).

Within the Iberian Peninsula, the southern edge of the alien distribution of this species in Europe, *P. leniusculus* were first recorded between the years 1973-1974 (Diéguez-Uribeondo, 2006; Cobo *et al*., 2010). The species is currently widely distributed in the centre and the north of this territory. However, its presence in Galicia (NW Iberian Peninsula) is reduced to one population in the River Sil basin (Mouriño, 2005). There was an additional potential observation of this species in this region, specifically in the international section of the River Miño (Fernández-Martínez & Carrera, 2003). However, this particular observation was found to be erroneous by the authors (Fernández-Martínez pers. comm.).

Pacifastacus leniusculus is currently included in the Spanish Catalogue of Invasive Alien Species (BOE, 2013), which stipulates the prohibition of its possession, transport, trade, and introduction in the natural environment. In addition, specimens extracted from nature by any procedure cannot be returned to the natural environment and captured individuals have to be killed (BOE, 2013). Nonetheless, fishing of this species is still allowed in Spain which, in retrospect, is well known to favour invasive crayfish introductions (Diéguez-Uribeondo, 2006). The study aims to report the introduction of *P. leniusculus* in the River Tambre basin (NW Spain) and to further investigate the potential implications of this introduction.

MATERIALS AND METHODS

The study was carried out in the River Tambre (Fig. 1), an oligotrophic river in north-western Iberian Peninsula (Galicia). The river, approximately 124 km long, drains a catchment area of 1530 km^2 (Río-Barja & Rodríguez-Lestegás, 1992). It is important to note that Galicia is drained by (i) a large basin, the River Miño-Sil basin, which covers the south of the region, and (ii) a group of small basins located at the west and north that jointly forms the so-called "Galicia Costa" district (Fig. 1). The studied basin is located within the Galicia Costa district.

On November 7, 2016, signal crayfish were captured by using hand nets in the Barrié de la Maza reservoir (42° 54' 4"N 8° 44' 2"W, NW Spain; Fig. 1). This reservoir was built in the main channel of the River Tambre in 1946 for hydropower generation, and has a length of 14 km, an area of 270 ha, and a capacity of 30.2 hm^3.

Each individual crayfish was weighed (± 1 g) and measured (± 1 mm, both total and carapace length). Using previously described methods (Capurro *et al*., 2015), the maturation stage of the gonads was noted as: I- undeveloped (white or light cream gonads), II- premature (cream), III- early mature or nearly mature (orange–brown), IV- ripe (black), V- ovigerous females. Fecundity was recorded as the number of pleopodal eggs for the ovigerous females. The disease condition of each

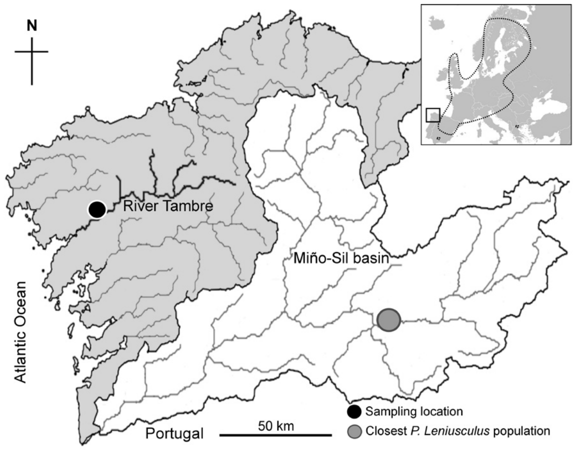

Figure 1. Map showing the sampling site (black dot) located in the Barrié de la Maza reservoir of the River Tambre basin (Galicia, NW Spain) and of the closest known *Pacifastacus leniusculus* population. The Miño-Sil basin is showed in white and the "Galicia Costa" district in grey. Top right corner: the previous distribution of the signal crayfish in Europe delimited by a dashed line and Galicia delimited by a square.
Figura 1. Mapa mostrando el punto de muestreo (círculo negro) ubicado en el embalse de Barrié de la Maza, en la cuenca del río Tambre (Galicia, NO España), y la población de *Pacifastacus leniusculus* más cercana conocida hasta el momento. La cuenca Miño-Sil se muestra en blanco y la región "Galicia Costa" en gris. En la esquina superior derecha: la distribución anterior del cangrejo señal en Europa delimitada por una línea de trazos y Galicia delimitada por un cuadrado.

individual was examined at a macroscopical level. Thus, presence of black spots in the appendices, usually caused by *A. astaci* (Diéguez-Uribeondo, 2006) and white spots usually caused by viruses (Souty-Grosset *et al.*, 2006) were recorded, as well as presence of ectosymbionts of the family Branchiobdellidae.

The carapace length frequency distribution at 2 mm length classes was used to determine the crayfish population structure (Petersen, 1892), following reference cohorts and data provided by Guan & Wiles (1996, 1999), Kirjavainen & Westman (1999) and Dana *et al.* (2010). Due to the small number of females captured ($n = 5$) both males and females were grouped together in this analysis. Spearman correlation tests were used to assess the relation between the number of eggs and the size of females. Mann Whitney *U* test was used to compare biometric parameters between males and females. These analyses were performed using IBM SPSS Statistics 20.0 software. A significance level of $p < 0.05$ was used in all analyses.

Table I. Total body length, carapace length (CL) and weight of signal crayfish *Pacifastacus leniusculus* captured in the Barrié de la Maza reservoir (River Tambre basin). Mean ± standard error and range in parenthesis.
Tabla I. Longitud total, longitud cefalotorácica (CL) y peso total de los cangrejos señal *Pacifastacus leniusculus* capturados en el embalse de Barrié de la Maza (cuenca del río Tambre). Media ± error estándar y el rango en paréntesis.

	n	Total length (mm)	Carapace length (mm)	Weight (g)
Males	66	85.2 ± 1.7 (38.0-114.0)	49.8 ± 1.1 (21.0-69.0)	38.2 ± 2.3 (2.5-95.4)
Females	5	92.2 ± 4.6 (78.0-102.0)	52.4 ± 2.3 (45.0-57.0)	40.0 ± 6.0 (21.0-51.4)
All	71	85.7 ± 1.7 (38.0-114.0)	50.0 ± 1.0 (21.0-69.0)	38.3 ± 2.2 (2.5-95.4)
By CL intervals *(mm)*				
21-25	3	41.0 ± 2.5 (38-46)	22.7 ± 1.2 (21-25)	3.2 ± 0.6 (2.5-4.4)
26-30	0	-	-	-
31-35	0	-	-	-
36-40	3	66.7 ± 1.8 (64-70)	38.0 ± 1.2 (36-40)	14.7 ± 2.0 (11.0-17.8)
41-45	10	74.7 ± 0.5 (73-78)	43.5 ± 0.3 (42-45)	21.4 ± 0.9 (17.5-26.8)
46-50	20	83.6 ± 0.4 (80-87)	48.7 ± 0.2 (47-50)	31.4 ± 0.9 (22.3-39.6)
51-55	19	90.8 ± 0.7 (87-97)	52.9 ± 0.3 (51-55)	42.8 ± 1.3 (32.9-56.3)
56-60	10	98.3 ± 0.9 (95-102)	57.1 ± 0.4 (56-60)	56.7 ± 2.5 (45.2-68.3)
61-65	4	103.8 ± 1.4 (101-107)	62.3 ± 0.8 (61-64)	65.5 ± 5.4 (51.1-76.4)
66-70	2	111.5 ± 2.5 (109-114)	68.0 ± 1.0 (67-69)	90.9 ± 4.6 (86.3-95.4)

RESULTS

A total of 71 crayfish were captured, 66 males and five females (sex ratio 13:1). Total length-weight relationship followed a potential trend ($y = 0.0002x^{3.1367}$; $R^2 = 0.97$). No differences were recorded for any biometric parameter between males and females (Mann Whitney *U* test, $p > 0.05$) (Table I). Different cohorts were observed in the studied population (Fig. 2).

Four of the five captured females had pleopodal eggs (stage V). Fecundity was, on average (± *SE*),

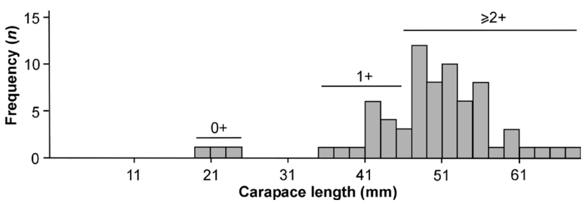

Figure 2. Carapace length frequency distribution and estimated age classes of signal crayfish, *Pacifastacus leniusculus*, from the River Tambre.
Figura 2. Distribución de las frecuencias de las longitudes cefalotorácicas y clases de edad estimadas de cangrejo señal, *Pacifastacus leniusculus*, en el río Tambre.

319 ± 11.5 eggs per female (range: 300-352). Those females had a carapace length of 54.3 ± 1.8 mm (range: 49.0-57.0) and a weight of 44.8 ± 4.7 g (range: 31.1-51.4). The female without eggs (stage I) had a carapace length of 45.0 mm and a weight of 21.0 g. The number of eggs were not significantly correlated with the total length, carapace length or weight of the female (Spearman correlation test; $p > 0.05$).

Presence of branchiobdellidans was recorded in 96% of the captured crayfish. Only two individuals from the cohort 0+ and one from the cohort ≥ 2+ did not present these ectosymbionts. White spots were observed in three individuals (4%) while no melanised spots were found in any crayfish.

DISCUSSION

This study provides the first report of the introduction of *P. leniusculus* and ectosymbionts of the family Branchiobdellidae in the Galicia Costa district (north-western Iberian Peninsula). Our findings support the view that this new crayfish population is viable and capable to be successful, as we found different age classes, including young of the year individuals and ovigerous females. In addition, this population is located *ca*. 150 km away in straight line from the closest known signal crayfish population (Fig. 1), which belongs to another basin (Miño-Sil basin) (Mouriño, 2005). Therefore, the described population is caused by an anthropogenic introduction and there is a high risk for having similar introductions in this and other basins of the region in the future, which has previously occurred in other Iberian regions (Bernardo *et al*., 2011). In fact, the River Tambre signal crayfish population is a potential source of individuals for new introductions of this species. Additionally, due to the absence of upstream dams within the River Tambre and the capacity of dispersion of this species (Bubb *et al*., 2005; Bernardo *et al*., 2011; Dunn, 2012), this population is expected to progressively increase its distribution within this river basin. Due to this increase, management actions should include the limitation of the dispersion and growth of this signal crayfish population. Furthermore, one should protect free-signal crayfish watersheds in order to avoid translocations.

There are no native crayfish populations [*Austropotamobius pallipes* (Lereboullet, 1858)] in the studied basin. The closest populations of this endangered species are located in the east of Galicia (Alonso, 2012). Therefore, the described non-native crayfish population is not expected to have a direct impact on native crayfish populations in the short term. However, there is a risk of new introductions in areas inhabited by *A. pallipes* in the medium-long term.

Although the presence of branchiobdellidans is common in signal crayfish populations of the centre and the east of Europe (Souty-Grosset *et al*., 2006), its presence in Spain has rarely been reported. So far, it has been discovered in the Ebro, Jucar and Nervión basins (Oscoz *et al*., 2010; Vedia *et al*., 2014, 2016) as well as in the River Porma (Cobo, unpublished data) and the River Adaja (Sánchez-Hernández unpublished data). This suggests that one of these populations may be the origin of individuals introduced in the River Tambre (Ohtaka *et al*., 2005), although they are located more than 250 km away in straight line from the River Tambre population.

Although virus infections are considered to probably impact native crayfish populations in Spain (Alonso, 2011), the incidence and impact of viruses on Spanish crayfish populations is unknown. That said, future crayfish monitoring programmes still need to take into account virus protocols in order to improve the knowledge about disease threats and epidemiology in nature.

The high deviation of the sex ratio observed in this population is expected to be caused by the more cryptic behaviour of ovigerous females after the reproduction season (Bubb *et al*., 2005). Our findings about size and age (≥ 2+) of ovigerous females as well as the number of pleopodal eggs per female are in accordance with results observed in previous studies (Guan & Wiles, 1999; Souty-Grosset *et al*., 2006; Capurro *et al*., 2015). However, in contrast to what was observed in an Italian population (Capurro *et al*., 2015), the number of eggs were not correlated with crayfish size, most likely due to the small sample of females available in this study.

CONCLUSIONS

This study describes the anthropogenic introduction of *P. leniusculus* and branchiobdellidans in a reservoir of the River Tambre (NW Spain). In the upcoming years, this population is expected to disperse through this basin and may be a source of individuals for future introductions in neighbouring basins. That said, management actions should be pursued to avoid the dispersion of this alien species in the Iberian Peninsula such as eradication and control programmes (reviewed in Gherardi *et al*., 2011), as well as changes in the current fishing law (e.g. ban of crayfish fishing).

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