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Research Article

Expansion of the North Pacific copepod *Eurytemora pacifica* Sato, 1913 (Copepoda: Calanoida: Temoridae) along the Atlantic coast of France

Jean-Michel Brylinski[1,*], Lucie Courcot[1], Valérie David[2] and Benoit Sautour[2]

[1]*Univ. Littoral Côte d'Opale, CNRS, Univ. Lille, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F. 62930 Wimereux, France*

[2]*Univ. Bordeaux, CNRS, UMR 5805, EPOC, Environnements et Paléoenvironnements Océaniques et Continentaux, F. 33405 Talence, France*

E-mail addresses: jean-michel.brylinski@univ-littoral.fr (JMB), v.david@epoc.u-bordeaux1.fr (VD), lucie.courcot@univ-littoral.fr (LC), b.sautour@epoc.u-bordeaux1.fr (BS)

**Corresponding author*

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Abstract

The North Pacific copepod *Eurytemora pacifica* Sato, 1913 was fortuitously found in a sea water tank of the Marinarium of Concarneau (Southern Brittany, France). The presence of ovigerous females in this tank as well as in some nearby ecosystems confirms the successful introduction of this species along the Atlantic coast of France. Morphology was examined using scanning electron microscopy and confirmed the species identity. The presence of two types of females resulting from cyclomorphosis was confirmed, as well as the production of two types of eggs. The French Atlantic coasts seem to be the only area in the world where the species has been reported as a non-indigenous species (NIS).

Key words: Non-Indigenous Species (NIS), invasive zooplankton, Crustacea, cyclomorphosis, *Crassostrea gigas*

Introduction

Eurytemora pacifica Sato, 1913 is a North Pacific Ocean species (Razouls et al. 2005–2016). It is distributed around the Bering Strait, both in Siberia (Brodsky 1950) and Alaska (Heron 1964) and also is distributed southward along the western Pacific coast. For instance, it was reported in south Sakhalin (Kos 1985), Japan (Chihara and Murano 1997), Korea (Myung et al. 1994), and China (Chen and Zhang 1965). Along the eastern Pacific coast, the species is limited southward to the Georgia Strait, British Columbia, Canada (DiBacco et al. 2012). So far, the only reports of this species as a Non-Indigenous Species (NIS) concerned the French Atlantic coasts, particularly the Bay of Marennes-Oléron (Sautour and Castel 1993, Audemard et al. 2004). The present paper confirms the previously unrecognized introduction of the species in this area.

Methods

Study Sampling took place four times in the Marinarium of Concarneau (Southern Brittany, Figure 1) and was designed to explore the zooplankton diversity in an inner artificial tank on 15 November 2010, 28 April and 9 September 2014, and 8 July 2015. This inner tank (1000 m^3) is filled, during high tide, with sea water pumped from outer ponds for 6 h twice daily. The water is intended for aquariums and thus is not filtered or treated.

Samples were collected through a trapdoor by means of several vertical hauls (3 m depth) of a plankton net. The net used was adapted to local technical constraints: it was conical, with a length of 40 cm and a mouth 40 cm in diameter. The mesh size was 200 µm. The plankton were fixed in 5% buffered formaldehyde (final concentration). Specimens of *Eurytemora pacifica* Sato, 1913 were only

collected on 9 September 2014 and 8 July 2015. Seawater temperature reached 18.5 °C and 19.5 °C, while salinity values were 35.5 and 35.0, respectively. Numbers per unit volume of zooplankton were not quantified but all the copepods were counted, and *Eurytemora pacifica* was identified and removed. Each sample was examined in it entirely to detect egg sacs disrupted by sampling. Specimens were cleared and dissected in lactic acid before microscopic observations. Body length was measured from the top of the prosome to the end of furca (excluding setae) with an ocular micrometer.

Eurytemora pacifica was identified following Brodsky (1950) and Johnson (1961). Several other papers describing the species are written in Japanese or Russian but pictures are presented in Razouls et al. (2005–2016). The female is easily identified based on the thoracic "wings" and particularly via the highly asymmetric fifth pair of legs.

For scanning electron microscope analyses (S.E.M. LEO 438 VP), specimens were cleaned and sonicated for 2 min in ultra pure water (Millipore Synergy). Individuals were then dehydrated through graded alcohol series up to 100% ethanol (Merck PA) and subsequently immersed twice in HMDS (Hexamethyl disilazane, Molekula) for 30 min. After removing excess HMDS, the specimens were dried overnight under a fume hood. Finally, individuals were mounted on aluminium stubs (Agar Scientific) with double sticky carbon tabs (Agar Scientific) and sputter coated under Argon flow with Au/Pd Polaron SC 7620 for 90 seconds.

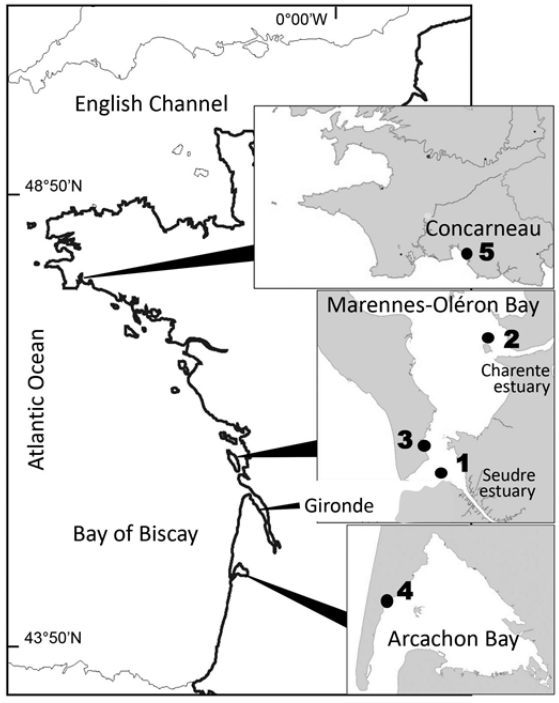

Figure 1. Distribution of *Eurytemora pacifica* along the Atlantic French coast. 1) Seudre estuary (45º48′19″N; 1º09′23″W); 2) Charente estuary (45º58′06″N; 1º06′40″W); 3) Oléron island (45º51′46″N; 1º12′10″W); 4) Arcachon Bay – Piraillan's pond (44º42′39″N; 1º13′23″W); 5) Marinarium of Concarneau (47º52′06″N; 03º55′00″W).

Results

Morphology

Female: Length 1.07–1.27 mm. Rostrum bifurcate with 2 filaments. Metasome segments not fused. Back corners of the last metasome segment with wing-like growth, which reach almost to the end of the genital segment ("winged" females; Figure 2A, C). Some female specimens without wings ("wingless" females; Figure 2B). Urosome 3-segmented. Genital double-segment widen in the sides, as long as either of the next 2 segments. Genital antrum armed with 3 thorns and a gutter for spermatophore placement (Figure 2D), covered by a central mobile triangular operculum. Furcal rami not longer than the third abdominal segment, slightly shorter left. P5 asymmetrical, uniramous, 3-segmented (Figure 2E). Segment 2 with two lateral spines and inner stout serrated process directed distally, parallel to leg axis. This process as long as segment 3 and forming pliers with this one. Distal segment armed with one subterminal and one apical spine. Inner side with numerous setules. Asymmetry is evident in the length of the distal segment and the inner process of the segment 2. In the right leg, the process is shorter with 5–6 teeth, the distal segment is shorter, and the terminal spine almost as long as the last two segments. In the left leg, the process is longer with 7–8 teeth, the terminal segment is bigger and the terminal spine is shorter than the terminal segment.

Male: Length 0.90–0.97 mm. Body slender more than in female (Figure 2H). Back corners of the last metasome segment without wing like processes. Right An1 geniculate. One spine on the segments 8, 9 and 12. Urosome 5-segmented. Furcal rami equal in length to the last 2 segments combined. P5 asymmetrical, uniramous, 4-segmented (Figure 2I). Distal segment elongated and curved on the right leg. Segment 2 of left leg with inner enlargement. Distal segment usually presented as triangular with a hook. SEM observation showed a concave triangular pallet stocked with bundles of fine setae and with a thorny bludgeon process (Figure 2J).

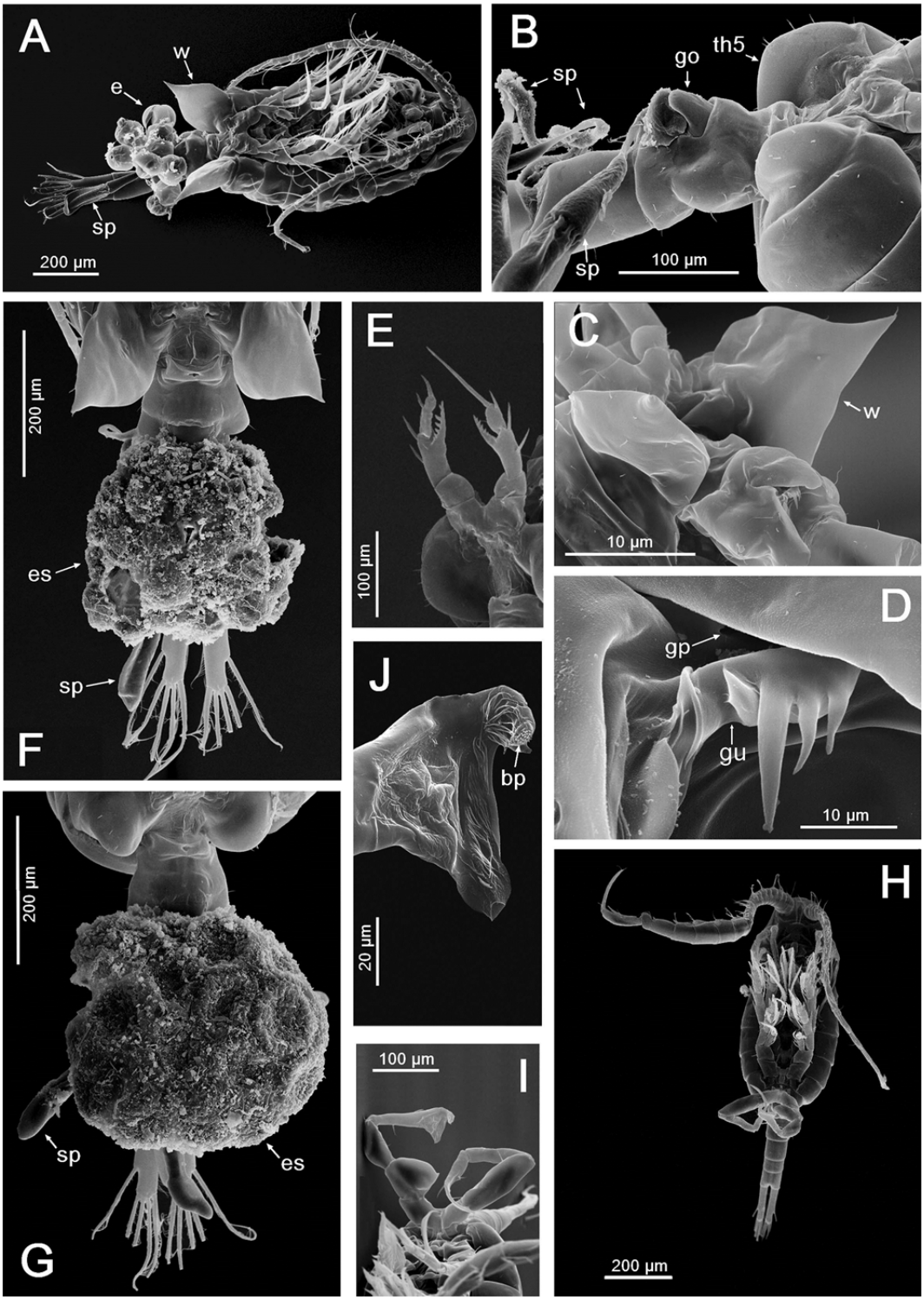

Figure 2. *Eurytemora pacifica*, Sato 1913, morphological specifications (SEM). Winged female, ventral view (A) and wingless female (B); winged female, genital double-somite (C); genital segment, detail of genital antrum (D); female P5, posterior view (E); eggs-sac of winged female (F); eggs-sac of winless female (G); male, ventral view (H); male P5, anterior face (I); male P5, extremity of left fifth leg, anterior view (J). bp, bludgeon process; e, egg; es, eggs-sac; go, genital operculum; gp, gonopore; gu, gutter; sp, spermatophore; th5, last thoracic somite; w, "wing".

Table 1. Presence of *Eurytemora pacifica,* incidence of winged females, ovigerous females, sex ratio (% males), and incidence of females carrying spermatophores in zooplankton samples collected in the Concarneau Marinarium in 2014 and 2015.

Date	% / total copepods	% winged females / total females	% ovigerous females / total females	Sex ratio (% males)	% females with spermatophores / total females
September 2014	42.0%/121	100%/11	0%/11	69	0
July 2015	17.6%/847	80%/40	25.7%/40	75	82.5%/40

Biological data

Eurytemora pacifica was the main copepod (including numerous Harpacticoida) present in September 2014 and July 2015 (Table 1). Only winged forms were observed in September 2014, whereas 20% of females were wingless in July 2015. Several females were ovigerous in July (25.7%). The sex-ratio indicated a clear preponderance of males.

Spermatophores were found on 33 females (82.5%) in July 2015. The number mostly varied between 1 and 7 spermatophores female^{-1} but reached up to 18 spermatophores female^{-1}. Not all spermatophores were fixed on the genital antrum; some were observed on the urosome (Figure 2B) or on a leg base.

Discussion

In France, *E. pacifica* was observed for the first time as a NIS in 1986 by Sautour (1991) at the mouth of the Seudre river in the Marennes-Oléron Bay (Figure 1). Abundance was 15 individuals m^{-3} in surface water masses on 29 April. This species was observed thereafter at the mouth of the Charente estuary (Sautour and Castel 1993) as well as in oyster ponds along the eastern coast of Oléron Island (Audemard et al. 2004). The species was not specifically studied and was assumed to be an accidental occurrence (< 10% of samples). Goulletquer et al. (2002) attributed this introduction to ships' ballast water or to be linked to the importation of Pacific cupped oysters (Grizel and Héral 1991). Indeed, following an epizootic (viral disease) in the late 1960's, the farmed oysters *Crassostrea angulata* (Lamarck, 1819) were gradually replaced in Oléron Bay and Arcachon Bay with the non-native oyster *Crassostrea gigas* (Thunberg, 1793). From 1971 to 1977 the quantities of non-native oysters imported to Oléron Bay alone consisted of 256 t of adults from British Columbia and 4,574 t as spat on spat collectors from Japan (Grizel and Héral 1991).

The massive importation of *C. gigas* has resulted in the introduction of many non-native organisms (Carlton 1992). It can be hypothesized, for example, that diapaused eggs of *E. pacifica* were present within intervalve water of cupped oysters or were associated with detritus or sediment on the shells (Panov et al. 2004). Moreover, the importing of *C. gigas* has been implicated in the introduction of many non-native species to eastern Atlantic waters and the Mediterranean Sea (Grizel and Héral 1991; Wolff and Reise 2002; Verlaque et al. 2007).

The first record of *E. pacifica* in the Arcachon Bay was unfortunately not published but its presence in Piraillan's pond (Figure 1) was recently confirmed by V. David (> 98% of 3000 copepods, 14 March 2014; unpublished data). This additional observation points out the capacity of the species to colonize areas independently of oyster farming. So, an overview of the distribution all along the whole French Atlantic coasts would require a more intensive survey program.

Unfortunately, there is no information concerning the zooplankton species make-up of Concarneau Bay. However, less than half of the volume of the inner tank is transferred towards the aquarium every day and, as observed in another similar inner tank, the zooplankton in such a system does not necessarily reflect the populations of the surrounding waters (Brylinski, personal observation).

The dispersion of *E. pacifica* from Marennes-Oléron Bay to nearby coastal areas may be facilitated by natural transport in river plumes and currents on the continental shelf of the Bay of Biscay. Freshwater inputs from estuaries, inducing buoyant river plumes on the continental shelf, exhibit strong seasonal variability induced by runoff and wind regime (Lazure and Jegou 1998). In autumn and winter, the river plumes remain near the coast and drift northward to Brittany (Lazure et al. 2009). At the present time, surprisingly, the French Atlantic coast (approximately along 5º of latitude) seems to be the only area where *E. pacifica* has been reported as a NIS within the entire Atlantic Ocean for the last 40 years. This contrasts with the recent and rapid invasion of another other Asian copepod *Pseudodiaptomus marinus* (Sato, 1913) that was reported almost simultaneously in the southern part of the North Sea (Brylinski et al. 2012; Jha et al. 2013), in the European Atlantic Ocean (Brylinski et al. 2012; Sabia et al. 2015; Uriarte et al. 2015), and

in the Mediterranean Sea (Olazabal and Tirelli 2011; Delpy et al. 2012; Sabia et al. 2014).

The native range of *E. pacifica* is not well documented. While Fulton (1968 and 1972) listed *E. pacifica* in the Georgia Strait (British Columbia, Canada), he specified the species had been observed by previous workers but were not found in the author's own collections. Nevertheless, the species was assumed to be "indigenous" as opposed to naturalized (DiBacco et al. 2012) in British Columbia waters where *C. gigas* was introduced in the early 1900s and extensively farmed since that time (DFO 2014). British Columbia seems to be the most southern location in the East Pacific for *E. pacifica*, while *Pseudodiaptomus marinus*, in contrast, was able to propagate down to the Mexican coasts (Jimenez-Perez and Castro-Longoria (2006).

We can hypothesize that the pelagic copepod *E. pacifica* (adult) poorly withstands the ship transport compared to the demersal copepod *P. marinus*. However, the life cycle of *E. pacifica* should be investigated to assess the contribution of resting egg stages in the processes of scattering. Indeed, cupped oysters have been also imported into Brittany and possibly stored in the external flood tanks of the Marinarium along with resting eggs of *E. pacifica*. These external tanks were private at that time and this storage is unfortunately not documented.

In Kivalina lagoon (Alaska; 67º43′48″N; 164º31′45″W), Heron (1964) found only egg sacs on winged females. In Gladkovskaya lagoon (Commander's islands; 54º44′03″N; 167º43′31″E). Solokhina (1992) observed ovigerous winged and wingless females at different times of the year and suggested specific cyclomorphosis of *E. pacifica*: "The diapause eggs produce the females with appendages which later lay subitaneous eggs. The females without appendages hatch from the subitaneous eggs and lay diapause eggs" (Solokhina 1992). Subitaneous eggs (from winged females) were smaller than resting eggs (from wingless female) (Figure 2F, G) and were described in Solokhina (1992). In contrast, winged and wingless females were simultaneously ovigerous in our Concarneau sample of July. The temperate climate in Brittany may have modified the alternate reproduction cycle observed in the North Western Pacific where waters are free of ice only for 3 months (Solokhina 1996). The cyclomorphosis in the *E. pacifica* life cycle, an exceptional case for the copepods, should be studied further in the temperate climate of our study area.

The existence of ovigerous winged and wingless forms in Concarneau and in Marennes-Oléron Bay, and the presence of juveniles, confirms the establishment of the species in the French Atlantic coast. Finally, the colonization by *E. pacifica* is progressing slowly, and ongoing monitoring surveys along the western English Channel should allow to better define its potential northward spread.

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