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### **Short Communication**

# First record of the Asian copepod *Pseudodiaptomus marinus* Sato, 1913 (Copepoda: Calanoida: Pseudodiaptomidae) in the southern bight of the North Sea along the coast of France

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#### Abstract

The presence of the demersal Asian copepod *Pseudodiaptomus marinus* is reported for the first time in the southern bight of the North Sea, in both Calais harbour and the coastal waters off Gravelines, France. This is the first record of *P. marinus* in the Atlantic Ocean sector and the North Sea area. The species was collected in January and October 2010, and in January, February and April 2011. The very low number of collected individuals (0.2 to 4.0 ind.m<sup>-3</sup>) and the capture of only two ovigerous females and of a few numbers of copepodid CV stages suggest that the species survives and actually reproduces in both sites but does not manage to develop an abundant population. The presence of *P. marinus* in Calais harbour and coastal waters of the southern North Sea supports recent observations of other Asian species in the same area and suggests a passive transport via ship's ballast waters.

Key words: Non Indigenous Species (NIS); ballast waters; Pseudodiaptomus marinus

## Introduction

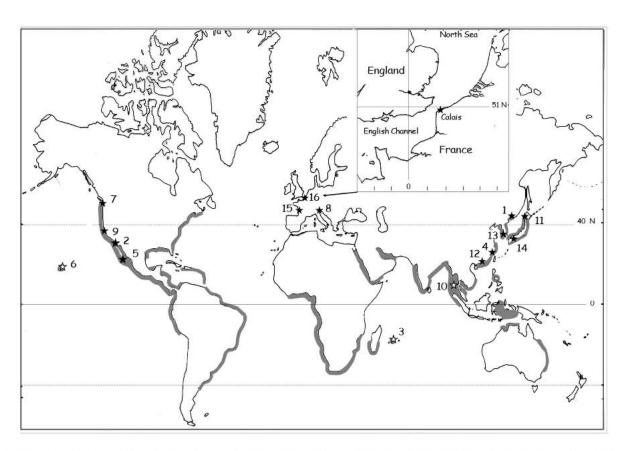
The calanoid copepod genus Pseudodiaptomus occurs worldwide in tropical and temperate fresh to hypersaline waters (Walter 1986a). Pseudodiaptomus marinus Sato, 1913 belongs to the Ramosus group characterized by a dominance of marine forms (Walter et al. 2006). P. marinus was described from the coast of Hokkaido, Northern Japan, and subsequently considered to be native to the Northwestern Pacific Ocean (Walter 1987). It occurs in Japan (Tanaka 1966; Nishida 1985), Russia (Brodsky 1950), South Korea (Soh et al. 2001) and China (Jiang et al. 2008), and has also been reported in the Andaman Islands, West-Thailand (Pillai 1976) and Mauritius (Grindley and Grice 1969). It has been recorded as an introduced species in Hawaii (Jones 1966), as well as along the western coast of North America in Puget Sound (Washington; Lawrence and Cordell 2010), Mission and San

Francisco bays, California (Fleminger and Kramer 1988, Orsi and Walter 1991), and Baja California (Jiménez-Pérez and Longoria 2006). Recently, P. marinus has been found in Southern Europe, in the Adriatic Sea (Mediterranean Sea; Olazabal and Tirelli 2011) (Figure 1). This species has not previously been reported in the Atlantic Ocean or the North Sea. There are geographical variations in the fifth leg (P5) of male P. marinus considering the number of points on the right endopod (Pillai 1976; Walter 1986b). In the original description from Northern Japan, they had 3 points (Sato 1913), while other Japanese specimens had 4 to 6 points (Tanaka 1966; Nishida 1985). Those from Mission Bay had 3 to 6 points (Fleminger and Kramer 1988), those from Hawaii had 4 points, and those from Mauritius had 2 points. In addition, Walter (1986b) considered the description of the specimens from the Andaman Islands (Pillai 1976) to be incomplete and proposed they

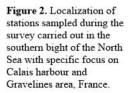
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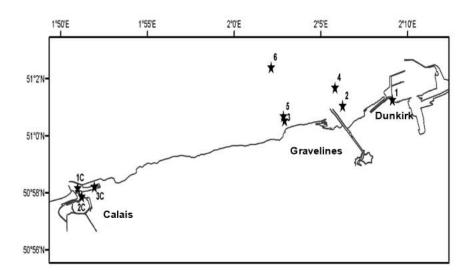
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**Figure 1.** Worldwide distribution of the genus *Pseudodiaptomus* (grey lines) and of the species *P. marimus* (Adapted from Walter, 1986a). Black stars = *P. marimus sensu* stricto and white stars = *Pseudodiaptomus* cf. *P. marimus* (see introduction section and Appendix 1 for further details on 1-15), 16 – present study (see Figure 2 and Table 1 for details).





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**Table 1.** Environmental parameters (at 5 m depth) and characteristics of collected *Pseudodiaptomus marinus* in the southern bight of the North Sea. Depicted are the number of individuals per sample or sub-samples (N), sex or stage (M: male; F: female; C: Copepodite; OF: ovigerous female), size (Total length, mm) and water temperature (T). n.d.: no data.

Site	Station	Coordinates		ъ.	T (0.00)	a 1: ::	Max		a .a.	Size (TL,
		Latitude, N	Longitude, E	- Date	T (°C)	Salinity	depth (m)	N	Sex/Stage	mm)
CALAIS	1C	50°58′09″	1°51′12″	21/01/2010	5.6	34.1	8.0	2	M/F	n.d.
	2C	50°57′51″	1°51′13″	22/10/2010	n.d.	n.d.	n.d.	1/2	F/CV	1.5 / n.d.
	3C	50°58′12"	1°51′57″	22/10/2010	n.d.	n.d.	n.d.	1	F	1.3
	3C	50°58′12"	1°51′57″	21/04/2011	12.5	33.7	14.0	1	OF	1.4
GRAVELINES	1	51°01′15″	2°09′07"	16/01/2011	5.8	33.1	7.0	1	M	1.3
	1	51°01′15″	2°09′07"	16/02/2011	6.8	33.6	7.0	2	F	1.4 - 1.5
	2	51°01′02″	2°06′15″	17/02/2011	8.2	34.0	2.0	3	M	1.3 - 1.5
	2	51°01′02"	2°06′15″	15/09/2011	19.0	34.2	1.9	1	OF	1.4
	3	51°00′31″	2°05′55"	18/02/2011	9.0	33.9	3.5	1	F	1.7
	4	51°01′40″	2°05′49"	15/09/2011	18.0	34.1	14.0	1	F	1.8
	5	51°00′40″	2°02′50″	16/09/2011	18.2	34.2	6.8	1	CV	1.2
	6	51°02′23″	2°02′08″	16/09/2011	17.7	34.2	24.0	1	CV	1.2

should be referred to as *Pseudodiaptomus* cf. *P. marinus*. In this article, we detail the occurrence of *P. marinus* from French coastal waters of the southern bight of the North Sea that fit the original species description and discuss possible mechanisms for its introduction there.

### Methods

Zooplankton samples were collected every month during routine surveys in stations located in Calais harbour (From January to October) and in coastal waters off Gravelines (From January to December; Figure 2; Table 1). Surface water temperature and salinity were measured using a Seabird SBE 25 Sealogger CTD and a WTW 340i Multi-meter in Calais and Gravelines. Zooplankton was collected with a WP2 net (200 μm mesh size) fitted with a flowmeter. Sampling consisted in oblique tows run at 1 m s<sup>-1</sup> and the net reached a maximum depth of 2 meters above the bottom (Table 1). The filtered volume ranged from 2 to 20 m<sup>3</sup> per haul. Collected zooplankton samples were immediately preserved in formalin solution (5%) and identified under a dissecting microscope.

# Results

Environmental context of P. marinus records

In Calais and Gravelines waters, seawater temperatures ranged from 5.6 to 19.0°C whereas salinity varied between 33.1 and 34.2 (Table 1). Adult stages of *P. marinus* (male and female) were first collected in January 2010 at the

entrance of Calais harbour (Station 1C; Figure 2; Table 1). One female and two copepodites C5 were also collected in October 2010 (Station 1C and 2C). Only one ovigerous female (1.4 mm) was observed in this area in April 2011 (Station 3C). Captures of *P. marinus* males and females in Gravelines waters occurred in January and February 2011 (Stations 1 to 3) with copepodites stages (CIV-CV) observed in September 2011 (Stations 4 to 6). This low number of individuals per sample or sub-samples resulted in markedly low abundance values ranging from 0.2 to 4.0 ind.m<sup>-3</sup>.

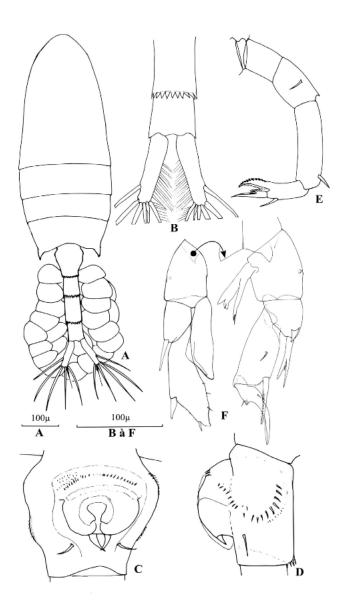
## Description of P. marinus from Calais

The general description of *Pseudodiaptomus* genus was given by Walter (1986b). The specific determination requires the examination of the fifth pair of legs (P5), particularly for males.

### Female (Figure 3A-E)

Length 1.3-1.8 mm. Anterior and posterior regions in following length proportions: 65% and 35%. Last thoracic segment with two terminal corners symmetrically directed backwards (Figure 3A). Genital double-somite slightly asymmetrical, i.e. lateral inflation pronounced in the proximal part of the left side (Figure 3C). This one with a curved row of spines (Figure 3D). Very protuberant ventral face falling into two convergent points directed backwards as shown by Soh et al. (2001). Distal margins of the genital double-somite and of the two following

Figure 3. Pseudodiaptomus marinus Sato, 1913. Female: habitus, dorsal (A); 4th abdominal segment and furca, dorsal (B); genital double-somite, ventral (C); genital segment, left lateral (D); Right fifth leg, posterior (E); Male: fifth pair of legs, posterior (F).



somites lined dorsally with triangular spinules (Figure 3A-B). Furcal rami almost 4 times as long as wide with long fine setae on the inner side. P5 uniramous, five segmented and symmetrical (Figure 3E). Fifth segment implanted in the middle of the 4th one and both forming a so-called pliers.

## Male (Figure 3F)

Length 1.3-1.5 mm. Similar shape than female with a more regular genital segment. Right geniculated antenna. 18th segment with serrated margin on its anterior face. P5 biramous on each

side. 2-segmented protopods. The right leg with 3-segmented exopod (Re1, Re2 and Re3): Re1 with a large forked spine distally to a thickness spine. Re2 with a long straight spinulose spine on the distal part of its outer margin. Re3 sickle-shaped with three setae and a striated process. Endopod with two branches. Thickest one ended with 3 points. Second branch longer and thinner, extended by a sub-terminal spine. Left leg with 2-segmented exopod and 1-segmented endopod. Re1 with a large external spine. Re2 with a thick spine and a smaller sub-terminal spine. The space between these two spines fringed by a row of spinules.

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### Discussion

Transoceanic introductions into new habitats through ballast water discharges from international shipping has dramatically increased over the past century (Ruiz et al. 2000; Goulletquer et al. 2002; Bollens et al. 2002) and ultimately contributed to the establishment of invasion corridors (Ricciardi and MacIsaac 2000; Drake and Lodge 2004). For example, records of *P. marinus* outside its native Northwestern Pacific habitat have been reported a number of times, particularly along the west coast of North-America (e.g. Lawrence and Cordell 2010) and more recently in the Mediterranean Sea (Olazabal and Tirelli 2011).

The detailed morphological analyses presented here is the first confirmed report of *P. marinus sensu stricto* in the Atlantic Ocean sector. The three points on the right endopod of the P5 (Figure 3F) are clearly visible and is shown for specimens identified from the Northwest Pacific Ocean (Sato 1913) and the coastal waters of the western North American coast (Fleminger and Kramer 1988). *P. marinus* therefore appears as the fourth Non Indigenous Species (NIS) of copepods identified in the coastal waters of the southern North Sea and the second Asian copepod species with *Acartia omorii* (Seuront 2005) found in Calais harbour.

We cannot exactly specify the date of *P. marinus* introduction in Calais, since only sparse surveys were carried out in the harbour before 2010. However, this introduction is certainly recent as *P. marinus* was only collected since 2011, although the long-term plankton survey off Gravelines started in the 1970s (Antajan 2012).

The introduction of *P. marinus* in the southern North Sea could result from various processes. Three hypotheses can be suggested to explain its presence. Although suggested along the western coast of America (Orsi and Walter 1991; Jiménez-Pérez and Longoria 2006), natural importation by ocean currents in the North Sea is incompatible with involved distances and global ocean circulation.

Aquaculture of oyster and mussels from Japanese coastal waters has already been considered as a vector of introduction of *P. marinus* in California embayments (Fleminger and Kramer 1988). This hypothesis should also be dismissed as regional aquaculture only involves European species of mussels (*Mytilus*)

edulis) and fish (Sparus aurata, Dicentrarchus labrax, Psetta maxima).

It is suggested, instead, that the introduction of *P. marinus* in the southern North Sea is related to ballast water discharge. The North Sea has indeed received a large number of invasive and non-invasive non-indigenous species (e.g. Reise et al. 1999; Wolff 2005) and is considered the second-most invaded water body in Europe behind the Mediterranean Sea (Gollasch 2006). In a recent review, Dewarumez et al. (2012) identified 40 NIS in the eastern English Channel and the southern bight of the North Sea, among which 30 were Asian.

This introduction pathway of P. marinus is congruent with hypotheses suggested for two east-American calanoid copepod species observed in Dunkirk harbour (Figure 2; Acartia tonsa and Eurytemora americana; Brylinski 1981, 2009) and in the coastal waters off Calais harbour for the Asian species Acartia omorii (Seuront 2005). One of the greatest factories of submarine cable network in the world is located in Calais harbour. Cable ships are filled with seawater (1000 to 1500 m<sup>3</sup>) during cable deployment worldwide including Pacific waters. Ballast waters are kept 45 days (without reballasting) on the ship's way back to Calais, where they are discharged during the next cable loading. Ships can also use the Northwest route (Arctic ocean) during summer resulting in a transit time of 25 days. If P. marinus has never been recorded along the eastern American coasts (Figure 1), its introduction from Californian coasts to Calais via the Panama Canal is possible as travel duration lasts 25 days. A small proportion of P. marinus may have survived these different trips and got into Calais harbour. However, specific studies of ballast water contents are needed to confirm this hypothesis.

Lawrence and Cordell (2010) recently highlighted the need to scrutinize intra-coastal traffic since it could be a good predictor of NIS propagule. The English Channel area concerns 20% of the world ocean traffic. In 2010, more than 41000 ships crossed the Strait of Dover towards the North Sea travelling along the French coasts (Anonymous 2011). These ships regularly serve Calais and Dunkirk harbours as well as main European harbours (Rotterdam, Anvers, Zeebrugge, Hambourg). The situation observed in our studied area may be analogous to that along the Pacific American coasts (Lawrence and Cordell 2010). In other words, trans-oceanic routes may have resulted in

P. marinus primary introduction in Calais harbour whereas intra-coastal traffic could represent a high risk of regular P. marinus introduction. This hypothesis is congruent with the occurrence of P. marinus in the Mediterranean Sea (Olazabal and Tirelli 2011) as well as in the Gironde estuary (Sautour and Dessier pers. com.). The Mediterranean basin is indeed the shipping route of vessels transiting from Asia to Europe via the Suez Canal. These European records emphasize the need to better acknowledge P. marinus presence along intra-coastal and trans-oceanic shipping routes.

P. marinus spreading outside Calais harbour cannot be inferred from our study. However, a long-term survey (SOMLIT national survey network) carried out in the eastern English Channel (60 km southward) since 1997 never identified P. marinus (Brylinski 2009). The northward drift of nearshore coastal waters (Brylinski et al. 1991) in the area can explain their absence southward and their spreading northward, which is confirmed by the first record of P. marinus within the power plant ecological survey near Gravelines in 2011 (Antajan 2012). Given this general circulation pattern, natural transport from nearby northern estuaries such as the Scheldt seems also unlikely.

Our records of Pseudodiaptomus marinus in Calais harbour and Gravelines are, to our knowledge, the highest latitude records for this species, indicating that North Sea waters are compatible with their development. The survival of P. marinus in Calais and Gravelines is also compatible with the temperature-dependent model distribution proposed by Rajakaruna et al. (2011; their Figure 6). Pseudodiaptomus species are defined as demersal species reported to remain in, on or near the bottom during daylight hours (Walter 1986a; Jacoby and Greenwood 1991) and to migrate to the water column at dusk. The fact that samplings were performed during daylight hours and the net's mouth was always lowered at least 2 m from the bottom may abundance underestimation. have led to However, sustained strong tidal currents in Gravelines and frequent ferry traffic (3 to 4 per hour) in Calais harbour trigger a permanent homogenization of the water column, hence allowing constant resuspension of suprabenthic species such as Pseudodiaptomus. Finally, the capture of 2 ovigerous females (24 eggs at Calais and 28 eggs at Gravelines) and copepodid stages in both sites suggests the presence of reproducing populations. Stating whether this population becomes invasive in Calais and northward cannot be inferred from our short study period. Therefore systematic and synoptic sampling of the area along with specific estimations of NIS inoculation size and frequency (i.e. propagule pressure) should be undertaken.

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#### Addendum

After the manuscript submission, zooplankton sampling in Calais harbour (Station 1C; March 19th, 2012) revealed that *P. marinus* abundance increased up to 120 ind.m<sup>-3</sup>. Numerous females were ovigerous carrying on average 14.6 eggs per sac (10-17 eggs for 26 individuals). This record at a seawater temperature of 7.6°C is consistent with the published study of Liang and Uye (1977) on *P. marinus* population dynamics.

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Appendix 1. Published records of Pseudodiaptomus marinus.

Map	Location and Site	Coor	dinates	- Record Date	Temperature	Salinity	Abundance (number	Reference	
ref.	Location and Site	Latitude Longitude		Record Date	(°C)	Sammy	collected)	ACTORICE	
1	Eastern coasts of Russia								
	Peter the Great Bay	42°40′N	141°43′E	-	-	-	-	Brodsky (1950)	
	Amur Bay	43°03'N	131°42′E						
2	Western coasts of U.S.								
	California								
	Mission Bay	32°46′N	117°14′W	Dec. 1986	14.0	33.0	< 100*		
				Jan. 1987	15.2	34.0	250-300* <30*	Fleminger and	
				Feb. 1987 Apr. 1987	16.9 22.0	33.0	50-100*	Kramer (1988)	
				May 1987	21.6	-	300"		
				Jun. 1987	19.2	33.0	<30*		
	Agua Hedionda Lagoon	33°08'N	117°19′W	May 1987	19.7	-	-		
3	Indian Ocean								
	Mauritius Island							Grindley and	
	Port Louis Harbour	20°09′S	57°29′E	Jun. 1964	-	-	13 ind.	Grice (1969)	
4	China								
	Zhejiang Province							Jiang et al.	
	Yueqing Bay	28°19′N	121°09′E	Apr. 2007	16.0	-	-	(2008)	
5	Western coasts of U.S.			Aug. 2007	28.0				
,	Baja California, Mexico	From	From	Oct. 1998	14.0 to 23.0	33.0 to	3 ind.		
	Todos Santos Bay	31°43′ to	116°36′ to	Feb. 1999	2110 10 2510	34.0	7 to 68 ind.	Jiménez-Pérez	
	, and the second	31°54′N	116°49′W	Mar. 1999			$\mathrm{m}^{-3}$	and Castro-	
				Jul. 1999-2000			1 ind. m <sup>-3</sup>	Longoria	
				Nov. 1999-2000			20 ind. m <sup>-3</sup>	(2006)	
				Jan. and Oct. 2002			34 ind. m <sup>-3</sup> < 1 ind.m <sup>-3</sup>		
6	Hawaii, U.S.	21°17′N	157°49′W	1964 and 1967		18.0	11 ind.	Ionas (1066)	
7	Ala Wai Canal, Oahu	21°17′N	137 49 W	1904 and 1907		18.0		Jones (1966)	
7	Western coasts of U.S. Washington						375 to 4326 ind. m <sup>-3</sup>	Lawrence and	
	Puget Sound	47°56′05″N	122°29′51″W	2001 to 2007			(11 to 26	Cordell (2010)	
	1 aget sound	., 50 05 11	122 27 71 11	2001 to 2007			ind.)	0010011 (2010)	
8	Mediterranean Sea						1.6 ind. m <sup>-3</sup>	De Olazabal	
	North Adriatic Sea	44°17′33′′N	12°42′07″E	Nov. 2007	15.98	37.47	(11 ind.)	and Tirelli	
	Italy	45°47′39′N	13°34′28″E	May 2009	25.30	29.99	3.2 ind. m <sup>-3</sup>	(2011)	
9	Western seeds of U.S.						(4 ind.)	(2011)	
9	Western coasts of U.S. California								
	Sacramento-San Joaquin	38°04'N	122°02′W	Oct Nov. 1986		6.1 to		Orsi and Walter	
	Estuary			Mar. 1987		7.8		(1991)	
				Jun. 1987					
10	Andaman Sea Indian			Apr. 1988					
10	Ocean	11°25′N	92°44'E	Apr. 1968	-	_	19 ind.	Pillai (1976)	
	Isle of Andaman	2511	22 112	14p1. 1500			22 1110.	1 11111 (1770)	
11	Japanese coastal waters	43°N	140°E	1913	_		_	Sato (1913)	
12	Oshoro - Takashima	12.11	1.0 L	1713				5410 (1913)	
12	Leizhou peninsula China			Apr.1961 -	-	-		Shen and Lee	
	Zaikong river	20°53′N	110°12′	March 1962			28 ind.	(1963)	
	Chiekong river	20 33 14	110 12	March 1902			20 ma.	(1905)	
13	Korean coastal waters	From	From	-	-	-	-	Soh et al.	
		33°00′ to	126°00′ to					(2001)	
1.4	T	38°00'N	130°00′E					(2001)	
14	Japanese coastal waters North West coast of	33°47′N	130°27/E	-	-	-	-	Tanaka (1966)	
	North West coast of Kyushu	33 4/ N	130°27′E					тапака (1900)	
15	Bay of Biscay	From	From 0°38′	-	-	-	-	Sautour and	
-	Southwest of France	45°04′ to	to 0°48'W					Dessier (pers.	
	Southwest of France	75 07 10	10 0 10 11						