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Quick spreading of the exotic amphipod *Laticorophium baconi* (Shoemaker, 1934): another small stowaway overlooked?

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Text S1. Detailed information on sampling surveys.

Portugal, Spain and Morocco

Fouling communities of three marinas of Southern Spain localities (El Rompido, Puerto América and La Línea marina) were collected in 2010 as part of a sampling programme to characterise seasonal fluctuations of amphipods (Ros *et al*., 2013; Guerra-García *et al*., 2015). During this survey, samples growing on pontoons, ropes, buoys, and ship hulls were also collected, including the bryozoans *Amathia verticillata* (delle Chiaje, 1822) and *Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985, the hydroid *Eudendrium racemosum* (Cavolini, 1785); and the seaweed *Ellisolandia elongata* (J. Ellis et Solander) K. R. Hind et G.W. Saunders. During the late spring-summer of 2011, a total of 88 marinas were surveyed along the whole Iberian Peninsula and North Africa coasts (see Ros *et al*., 2014 for details). In 42 out of the 88 investigated marinas (see Ros *et al*., 2015), three colonies of the bryozoan *Bugula neritina* (Linnaeus, 1758) were hand-collected from the submerged portion of pontoons close to the surface. In April-May 2019, three replicate samples of the bryozoan *B. neritina* and the polychaete *Sabella spallanzanii* (Gmelin, 1791), were collected from the marinas Sines, Fuengirola, Benalmádena, Caleta de Vélez, Motril and Adra. In September 2021, an additional sampling of *B. neritina* (three replicates per marina) was conducted in ten selected marinas along the Andalusian coast (Isla Canela, El Rompido, Chipiona, Puerto América, Sancti Petri, La Línea, Fuengirola, Caleta Vélez, Marina del Este, and Adra). In March-June 2023, floating ropes (marine debris) were collected in the marina of Mazagón, Huelva. All samples obtained were fixed in ethanol 96% and sieved using a mesh size of 0.5 mm.

As part of a sampling programme in offshore aquaculture facilities (Fernandez-Gonzalez & Sanchez-Jerez, 2017), a fish farming site off the coast of Guardamar del Segura, Alicante, was sampled in July 2010 and July 2011. The fish farm (growing European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) and gilthead sea bream *Sparus aurata* (Linnaeus, 1758)) consisted of 18 offshore aquaculture cages with a diameter of 19-25 m and net cage reaching depths from 12 to 15 m, set over a seabed at ca. 25 m depth. Fouling samples were collected by scraping fouling organisms from shallow ropes of the mooring lines (1-10 m depth). Samples were sieved through a 0.5 mm mesh with seawater and subsequently preserved in 4% formalin seawater solution (see more details in Fernandez-Gonzalez & Sanchez-Jerez, 2017; Fernandez-Gonzalez *et al*., 2021). Additional samples were collected using artificial collectors with a volume of 250 ml suspended from a submerged buoy anchored by a rope at approximately 15 m from the sea bottom and between 5 and 8 m below the surface. Collectors were deployed in July 2011, at 50 m and 2 km from the fish farm. After 15 days, they were collected, preserved in 4% formalin seawater solution and sieved through a 0.25 mm mesh. Nocturnal plankton hauls were also conducted to detect amphipods drifting in the water column. Vertical plankton hauls were performed using a conical 0.25 mm-mesh plankton net 0.6 m in diameter from the maximum depth to the surface. Samples were collected in the same area of the fish farm where the collectors were placed and preserved in 4% formalin seawater solution (Fernandez-Gonzalez *et al*., 2021).

During a general study of the substrate type influence in non-indigenous species (NIS) abundance in Calheta marina, Madeira (Sempere-Valverde *et al*., 2023), fouling communities were scrapped from experimental structures, fixed in 96% ethanol and sieved with a mesh size of 0.5 mm.

Tunisia and Greece

As part of NIS Rapid Assessment Surveys (RAS) (Campbell *et al*., 2007; Chebaane *et al*., 2019) in the Mediterranean Sea, fouling samples were taken from three Tunisian marinas (Bizerte, El Kantaoui and Monastir) in August 2021, and two Greek marinas (Zakynthos harbour in the Ionian Sea and Nafplio in South Aegean Sea) in November 2022. Samples included a variety of biogenic substrate types (mainly seaweeds, hydroids and bryozoans) and were collected from the sea surface to 1 m depth. Samples were preserved in ethanol 96% and sieved through a 0.5 mm mesh.

Italy and France

In May 2010, vertical artificial hard substrates of Olbia port, Sardinia, were sampled with a scrapping hand-held rigid net (1 mm mesh size) in concrete docks. In June 2010 and July 2013, the same methodology was used in La Spezia port (Ligurian Sea). In October 2018, an analysis was performed on fouling communities associated to polyvinyl chloride (PVC) plates (Tamburini *et al*., 2021) deployed in the Gulf of La Spezia. In June 2019, fouling assemblages were collected from the touristic harbour of the Port of Livorno by scraping the submerged vertical part of concrete docks (Tempesti *et al*., 2022a). In August 2021 and February 2022, as part of a study to explore the effects of antifouling treatments in fouling communities on PVC plates (Santos-Simón *et al*., unpublished data), samples of associated macrofauna were collected from settlement plates located in Fezzano and Le Grazie marinas. Samples from PVC plates were also collected in the Catania port, Sicily (July 2022) in the framework of the programme conducted by the Regional Agency for Environmental Protection (ARPA) for the monitoring of algae. In March-April 2023, fouling organisms were collected in the port of Livorno through the colonisation on plastic experimental substrates attached to the vertically submerged part of docks, and on mid-water experimental ropes (Tempesti *et al*., 2022b, 2023) surface. Also in March 2023, fouling communities of a fish farm located in Lagoon of Orbetello, Tuscany, were collected from the vertical surfaces of two floating docks (0.2-0.5 m depth); a total of six replicates of biogenic substrate were scrapped using a chisel and a hand net (1 mm mesh size). In May-June 2023, samples were collected from mooring ropes and buoys of fishing vessels and recreational boats moored in the commercial port of Civitavecchia (0.5-1.5 m depth); benthic macrofauna was collected by scraping the surface of artificial substrates with a steel blade. Additionally, fouling communities associated with mooring ropes (0.4-0.5 m depth) were also collected from the marinas of Riposto port (8 July 2023) and Siracusa harbour (15 July 2023), Sicily. In August 2023, colonies of the bryozoan *Amathia verticillata* (delle Chiaje, 1822) were collected from the Port of Brindisi (Apulian coast, Southern Adriatic Sea). In November 2023, fouling communities associated to mooring ropes were scratched (0.5-1 m depth) from Port of Ortona (Central Adriatic Sea). All samples were preserved in 70% ethanol.

In 2020, within the expedition 'Our planet reviewed in Corsica', organised by the Muséum national d'Histoire naturelle (MNHN, Paris), fouling samples were collected from marinas of Porto-Vecchio (with brushing method) and Bonifacio (hand Hydrozoa collection), Corsica (France).

Egypt

In September 2022, artificial hard substrates of the military port of Abu-Qir, Egypt were explored and a modified RAS was conducted following the methodology proposed by Ulman *et al.* (2017). Fouling biota were collected from different hard artificial substrates observed inside the port (i.e., buoys, piers, tiers, ladders, pontoons, pilings, seawalls and ropes), within arms-reach, up to maximum of ca. 1 m depth, using a 4-inch paint scraper and a 1 mm mesh aquatic net. Collected material was immediately preserved in plastic bags containing 75% ethanol and transferred to the laboratory. A 1 mm mesh was used to clean the samples.

Saudi Arabia

During a sampling programme to investigate the presence of NIS in natural and artificial habitats in the central Red Sea coast of Saudi Arabia, panels (14 x 14 cm, grey 4 mm PVC) were deployed in a marina close to the Jeddah Islamic port, in the KAUST marina and two nearby coral reefs (Shark reef and Al Fahal reef). Panels were deployed in individual vertical suspended arrays for one week (short-deployments) during three different sampling events in boreal winter of 2020 and for one year (retrieved in the winter of 2021; long-deployments). Identification was conducted based on molecular analysis of COI sequences (no material is available for morphological confirmation). Upon retrieval, panels were placed into individually sealed plastic bags, transported on ice to the laboratory and stored at -80°C until further analysis. Individually packed sterilised swabs (Deltalab) were used to collect biofouling of short-deployment panels by thoroughly and consistently rubbing material from the surface of each thawed plate (first, with a swab with nucle-

ase-free water, and later with a dry swab to maximise the amount of sample retrieved and standardise yield) (Pang & Cheung, 2007; Brownlow *et al.*, 2012). Long-deployment panels were scrapped. DNA extraction was conducted immediately after sample collection. DNA was isolated using the DNeasy Blood and Tissue Kit (QIAGEN) and DNA concentration was measured with the Qubit dsDNA High Sensitivity assay Kit (Invitrogen, Thermo Fisher Scientific) using the Qubit 4 Fluorometer. DNA integrity was visualised using gel electrophoresis (1% agarose). Each DNA sample was normalised to 5 ng/µL using nuclease-free water and stored at -20°C until further processing. PCR procedure involved an initial step (amplicon) where PCR was performed in triplicate, then pooled. The universal primer mlCOIintF/jgHCO2198 (Geller *et al*., 2013; Leray *et al*., 2013) was used to amplify a 313 bp fragment of the COI gene with attached Illumina overhang adapters. COI taxonomic assignment was undertaken using DADA2 with a cut-off of 0.5 against a custom database that was constructed using the CRABS algorithm (Jeunen *et al*., 2022) with sequences obtained from NCBI (NCBI, 2016) and Barcode of Life Data (BOLD) Systems (Ratnasingham & Hebert, 2007).

Australia and New Caledonia

Samples were collected from Western Australian port infrastructure in Bunbury (1996), Fremantle and Cockburn Sound (1999) during surveys for NIS led by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). More recent samples (2011–12) were collected within a NIS programme monitoring hull fouling communities on vessels travelling to Barrow Island, Western Australia as part of quarantine efforts to protect the Barrow Island Conservation Reserves.

During a mission in 2019 within the expedition 'Our planet reviewed in New Caledonia', organised by the MNHN, fouling samples were collected from Koumac marina (with vacuum cleaner, station code KS). Additionally, samples from the Grand récif de Koumac were collected using the *lumun* technique, which consists of the use of a fish net, with an extremity hooked on the barrier reef at ca. 10 m depth and the other extremity deployed offshore (ca. 150 m) and maintained with a ballast. The fish net was left in the water for a year.

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Table S1. Detailed information on voucher material of *Laticorophium baconi* deposited in museums, including Museum Catalogue numbers, sampling locations, coordinates, collection date, substrates and numbers for the specimens. MNCN: Museo Nacional de Ciencias Naturales de Madrid, Spain; MNHN: Muséum National d'Histoire Naturelle, France; MSNPV: Museo di Storia Naturale dell'Università di Pavia, Italy; MZPA: Museum of Zoology of the University of Palermo, Italy; MBMCA: Museo di Biologia Marina "Pietro Parenzan" at the University of Salento, Italy; WAM: Western Australia Museum, Perth, Australia; *Av=Amathia verticillata*; *Bn=Bugula neritina*; *Er=Eudendrium racemosum*, Fc=Fouling communities. RAS: Rapid Assessment Survey.

Catalogue number	Location	Coordinates	Date	Substrates	Number of specimens
MNCN 20.04/20801	Sines marina, Portugal	37.95053º N, 8.86511º W	9 May 2011	*Bn* (pontoons)	7 males, 3 females
MNCN 20.04/20802	La Línea (Puerto Chico) marina, Spain	36.15989º N, 5.35756º W	7 Sep 2021	*Bn* (pontoons)	4 males, 4 females
MNCN 20.04/20803	Caleta Vélez marina, Spain	36.74883º N, 4.06731º W	8 Sep 2021	*Bn* (pontoons)	5 males, 5 females
MNCN 20.04/20804	Alicante marina, Spain	38.33964º N, 0.48631º W	29 Jun 2011	*Bn* (pontoons)	7 males, 2 females
MNCN 20.04/20805	Barcelona marina, Spain	41.37725º N, 2.18292º E	26 Jun 2011	*Bn* (pontoons)	5 males, 5 females
MNCN 20.04/20806	Palma marina, Baleares, Spain	39.56272º N, 2.62836º E	Jun 2012	*Er* (pontoons)	2 males, 2 females
MNCN 20.04/20807	Kabila marina, Morocco	35.71986º N, 5.33550º W	30 May 2011	*Bn* (pontoons)	5 males, 5 females
MNCN 20.04/20808	Monastir marina, Tunisia	35.77969º N, 10.83325º E	Aug 2021	Fc (RAS: pontoons, buoys, mooring ropes, etc)	2 males, 1 female
MNHN-IU-2021-8810	Bonifacio marina, Corsica, France	41.38945º N, 9.15988º E	20 Oct 2020	Fc (Hydrozoa, hand collection)	2 males, 1 juvenile
MNHN-IU-2021-8811	Porto-Vecchio marina, Corsica, France	41.58788º N, 9.29034º E	15 Oct 2020	Fc (brushing technique)	2 males, 5 females
MNHN-IU-2021-8814	Porto-Vecchio marina, Corsica, France	41.58794º N, 9.28983º E	27 Oct 2020	Fc (brushing technique)	1 male, 13 females
MSNPV CCR0803	Fezzano marina, Italy	44.08028º N, 9.82806º E	Aug 2021	Fc (plates)	10 males, 10 females
MBMCA-ORB0323-LB03	Orbetello lagoon (fish farm), Italy	42.43200º N, 11.16306º E	2 Mar 2023	Fc (floating dock)	167 specimens
MBM-CA-CIVP0523-LB02	Civitavecchia port (marina), Italy	42.09400º N, 11.78919º E	15 May 2023	Fc (mooring ropes and buoys)	45 specimens
MBM-CA-CIVP0623-LB01	Civitavecchia port (fishing dock), Italy	42.09561º N, 11.78836º E	25 Jun 23	Fc (mooring ropes)	103 specimens
MZPA IM 561-563	Catania Port, Sicily, Italy	37.49906º N, 15.09853º E	Jul 2022	Fc (plates	3 specimens
MBMCA-BRP0823-LB04	Port of Brindisi, Italy	40.65967º N, 17.96383º E	7 Aug 2022	*Av*	11 males, 26 females
MBM-CA-ORP01123-LB05	Port of Ortona, Italy	42.34667º N, 14.41533º E	19 Nov 2023	Fc (mooring ropes)	1 male, 5 females
MNCN 20.04/20809	Zakynthos harbour, Greece	37.78406º N, 20.90233º E	Nov 2022	Fc (RAS: pontoons, buoys, mooring ropes, etc)	4 males, 3 females
WAM C49812	Dampier, WA	20.66199º S, 116.70087º E	27 Mar 2011	Fc (hull fouling)	2 males, 4 females
WAM C49003	Dampier, WA	20.58333º S, 116.63333º E	4 Jul 2011	Fc (hull fouling)	3 males, 1 female
WAM C49883	Fremantle, Inner Harbour, Fremantle Traffic Bridge, WA, Australia	32.05000º S, 115.73330º E	Apr 1999	Fc (scraping)	1 female

Continued

Table S1 continued

Catalogue number	Location	Coordinates	Date	Substrates	Number of specimens
WAM C49880	Fremantle, Rous Head Harbour 2, WA, Australia	32.05000º S, 115.73330º E	20 Apr 1999	Fc (scraping)	1 female
WAM C49881	Fremantle, Rous Head Harbour 2, WA, Australia	32.05000º S, 115.73330º E	20 Apr 1999	Fc (scraping)	1 female
WAM C49878	Fremantle, Inner Harbour, south Victoria Quay, Berth D, WA, Australia	32.05000º S, 115.73330º E	23 Apr 1999	Fc (scraping)	1 female
WAM C49882	Fremantle, North Mole, Channel Marker 3, WA, Australia	32.05000º S, 115.75000º E	23 Apr 1999	Fc (scraping)	1 female
WAM C49879	Fremantle, Swan River. Rocky Bay (Water Police Jetty), WA, Australia	32.03019º S, 115.75978º E	14 May1999	Fc (scraping)	1 male
WAM C49884	Fremantle, Swan River. Rocky Bay (Water Police Jetty), WA, Australia	32.03019º S, 115.75978º E	14 May 1999	Fc (scraping)	1 male
WAM C49000	Fremantle, operating between Dampier and Barrow Island, Western Australia	32.04836º S, 115.74322º E	12 Sep 2011	Fc (hull fouling)	5 males, 6 females
WAM C36050	Henderson, WA, Australia	32.25411º S, 115.74272º E	25 Apr 1999	Fc (scraping)	1 female
WAM C48440	Henderson, WA, Australia	32.15509º S, 115.76319º E	23 Sep 2011	Fc (hull fouling)	3 males, 2 females
WAM C49043	Henderson, WA, Australia	32.15509º S, 115.76319º E	6 Oct 2011	Fc (hull fouling)	1 male, 1 female
WAM C49046	Henderson, WA, Australia	32.15509º S, 115.76319º E	6 Oct 2011	Fc (hull fouling)	3 males, 1 female
WAM C49712	Henderson, WA, Australia	32.15509º S, 115.76319º E	9 Feb 2012	Fc (hull fouling)	3 females
WAM C49713	Henderson, WA, Australia	32.15509º S, 115.76319º E	9 Feb 2012	Fc (hull fouling)	4 males, 4 females
WAM C49714	Henderson, WA, Australia	32.15509º S, 115.76319º E	9 Feb 2012	Fc (hull fouling)	1 male, 7 females
WAM C49705	Henderson, WA, Australia	32.15509º S, 115.76319º E	20 Feb 2012	Fc (hull fouling)	1 female
WAM C49813	Henderson, WA, Australia	32.15509º S, 115.76319º E	26 Mar2012	Fc (hull fouling)	5 females
WAM C49876	Bunbury, Inner Harbour. Alcoa Berth, WA, Australia	33.32000º S, 115.66000º E	Mar 1996	Fc (pylons)	1 male, 1 female
WAM C49877	Bunbury, Inner Harbour, Woodchip Berth, WA, Australia	33.32000º S, 115.66000º E	Mar 1996	Fc (pylons)	2 males, 2 females
MNHN-IU-2021-1758	Koumac marina, New Caledonia, France	20.57994º S, 164.27481º E	18 Nov 2019	Fc	1 male, 10 females
MNHN-IU-2016-3361	Koumac marina, New Caledonia, France	20.58086º S, 164.27469º E	19 Nov 2019	Fc	1 male, 1 female
MNHN-IU-2021-4012	Koumac marina, New Caledonia, France	20.58136º S, 164.27383º E	18 Nov 2019	Fc	1 female, 1 juvenile
MNHN-IU-2021-3473	Grand récif de Koumac, New Caledonia, France	20.83092º S, 164.27808º E	18 Nov 19	Natural habitats on the reef	1 juvenile

Table S2. Monthly presence/absence of *Laticorophium baconi* in Puerto América, Cádiz and Palma, Balearic Islands throughout the year 2012. *Substrates absent.

	2012											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Puerto América Marina, Cádiz (*Amathia verticillata* and *Eudendrium racemosum*)	✓	*	*	✓	✓	✓	✓	✓	✓	✓	✓	✓
Palma Marina, Baleares (*Eudendrium racemosum*)			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓