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Report of the Workshop entitled “Towards a European observatory of the invasive calanoid copepod *Pseudodiaptomus marinus*” (WKEUROBUS)

29–30 January 2018

Naples, Italy



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Contents

Executive summary	2
1 Background.....	4
2 WKEUROBUS Terms of Reference.....	4
3 Summary of contributions	5
4 Response to the ToRs and definition of a roadmap.....	7
5 References	8
Annex 1: List of participants.....	10
Annex 2: Agenda.....	12
Annex 3: Draft resolution for a new Working Group	14

Executive summary

The workshop entitled “Towards an EUropean OBservatory of the invasive calanoid copepod *Pseudodiaptomus marinus* US” (WKEUROBUS) was held at the Stazione Zoologica Anton Dohrn in Naples, Italy, 29–30 January 2018. The WKEUROBUS, chaired by Marco Uttieri, Italy, gathered 29 participants from 9 countries.

The workshop aimed at establishing a network of European scientists and institutions working on the different aspects related to the biology and ecology of *Pseudodiaptomus marinus* Sato, 1913. This species, native to the Indo-Pacific region, is well known for its invasive ability, as evidenced along the USA Pacific coasts. Since 2007, *P. marinus* has established in several European sites and has spread at an unexpectedly fast pace over the last ten years. The high invasive potential of *P. marinus* is supported by specific biological, ecological and behavioural traits, including wide temperature and salinity tolerance, swimming plasticity, tolerance to stressful conditions and presence of different ecophenotypes. Despite its ability to colonise several environments (transitional, coastal and neritic areas), to our present knowledge, this species never becomes dominant in the water column. Consequently, no negative impacts have been reported so far on the occurrence of indigenous planktonic copepods. No information, however, is available about potential detrimental effects on the benthic and hyperbenthic zooplankton communities. In addition, *P. marinus* has often been indicated as suitable for mass rearing as food in aquaculture farms, and it is also eligible as a target species in ecotoxicological studies. All these aspects make this species of peculiar interest for ecological and applied research topics.

The workshop was structured as an open forum where each participant could share his/her experience and know-how. In particular, on the first day of the meeting, each rapporteur presented his/her research, highlighting present advances in the knowledge on the distribution, ecology and biology of *P. marinus*, and provided an outline of future research. The limited molecular results gathered on this species were shared as well among the participants. On the second day, an open discussion promoted the exchange of ideas for potential comparative studies between the different research groups; in particular, an outline for future joint initiatives was considered. Furthermore, the participants supported the initiative of establishing an ICES Expert Group.

The results presented at the workshop, together with a description of the priority lines of research, will be collected in a position paper, which will be submitted to an international journal by mid-June 2018.

The workshop report provides a background of the biological and ecological information at the base of this initiative, a summary of the main outcomes and the key issues emerged from the open discussion session.

In addition to ICES endorsement, the workshop was financially and logistically supported by Stazione Zoologica Anton Dohrn. Financial support was also provided by Italian Society of Marine Biology (SIBM) and National Interuniversity Consortium for Marine Sciences (CoNISMa). The workshop was also additionally endorsed by the World Association of Copepodologists (WAC), Sir Alister Hardy Foundation for Ocean Science (SAHFOS) and MOTax (Marine Organism Taxonomy) Service (from Stazione Zoologica

Anton Dohrn). The role of all endorsers was explicitly declared on the workshop website (<http://eurobuswg.wordpress.com>) as well as on the workshop material.

1 Background

Since the 1950s, the copepod *P. marinus*, native to the Indo-Pacific region (Sato, 1913), has widened its distributional range by colonising several coastal and transitional areas of the Northern and Southern Pacific Ocean as reviewed in Sabia *et al.* (2015). However, since 2007, *P. marinus* appears to have jumped ocean basins and is now present in European waters. Confirmed occurrences have been documented for the western Mediterranean Sea, the Adriatic Sea, the European Atlantic coast and the Southern North Sea (Brylinski *et al.*, 2012; de Olazabal and Tirelli, 2011; Delpy *et al.*, 2012; Jha *et al.*, 2013; Lučić *et al.*, 2015; Sabia *et al.*, 2014; Sabia *et al.*, 2015; Uriarte *et al.*, 2016), and most recently the Black Sea (Garbazey *et al.*, 2016). The number of recordings is increasing, often showing a stable settlement in the newly colonised environments. Ballast waters are considered the primary means of introduction, as this species has been observed in ballast water samples (Cabrini *et al.*, in press; Choi *et al.*, 2005; Cordell *et al.*, 2008; Lawrence and Cordell, 2010); however, aquaculture also seems to be a plausible means of introduction, specifically for those invaded environments that are close to ship traffic (Fleminger and Hendrix Kramer, 1988; de Olazabal and Tirelli, 2011; Sabia *et al.*, 2015). Secondary introduction through coastal circulation has also been considered applicable in the North Sea (Jha *et al.*, 2013).

The high invasive potential of *P. marinus* is supported by specific biological, ecological and behavioural traits, including swimming plasticity, tolerance to stressful conditions, presence of different ecophenotypes and possible occurrences of different morphs (Sabia *et al.*, 2014; Sabia *et al.*, 2015; Sabia *et al.*, 2017; Tlili *et al.*, 2015).

In addition to its invasive attitude, *P. marinus* has often been indicated in the literature as suitable for mass rearing as food in aquaculture farms (Mauchline, 1998). Moreover, this calanoid can also be used as a target species in ecotoxicological studies (Arias *et al.*, 2016; Tlili *et al.*, 2015).

2 WKEUROBUS Terms of Reference

- a) Define the present status of the European invasion by *P. marinus* and identify the potential means of primary and secondary introduction (e.g., ballast waters, aquaculture);
- b) Provide a forum for real-time monitoring of the distributional range, abundance and/or biomass of this species in European waters; and the environmental conditions in the invaded environment;
- c) Establish a group dedicated to promoting knowledge sharing and sustaining international collaborations;
- d) Make country-level introduction events information of *P. marinus* available through AquaNIS;
- e) Identify future research perspectives of this species, including ecosystem impact, systematics and taxonomy.

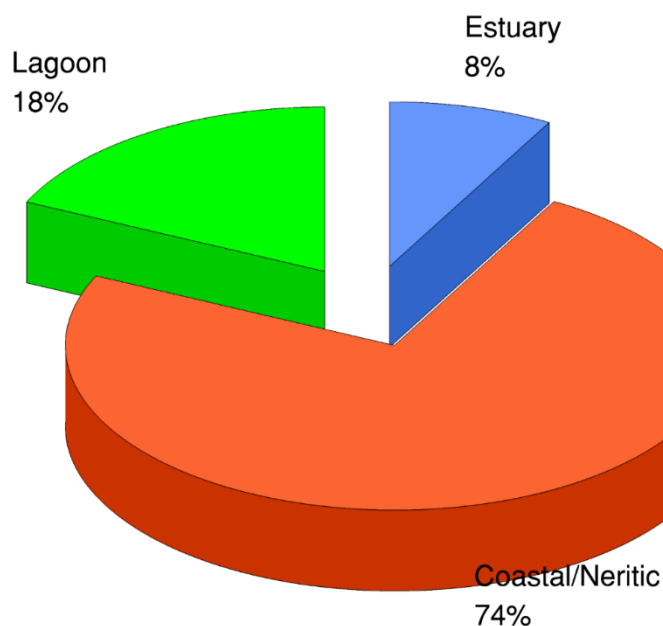
3 Summary of contributions

Based on the agenda (Annex 2), 12 talks were presented from 37 authors.

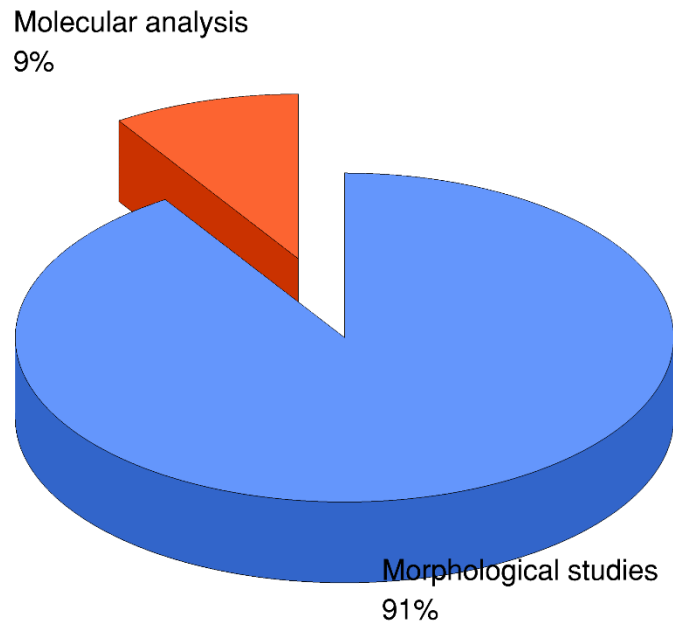
An introductory session was scheduled to delineate the aims of the workshop and to highlight the ToR agreed with ICES. The vast majority of the presentations dealt with the distribution of *P. marinus* in different regions: English Channel and Southern North Sea; estuaries along the Atlantic coast of Spain and Portugal; transitional and coastal areas in the Western and northernmost Mediterranean Sea (Tyrrhenian and Adriatic Sea) and in the Black Sea. These contributions underlined an ongoing colonising process, developing also in deeper neritic waters to which this species was considered not to be adapted, over a wide range of temperatures and salinities. The results also indicated that *P. marinus* can enter new environments both by ballast waters and by aquaculture/mariculture, and that – at least at present knowledge – its establishment does not determine processes of niche exclusion or competition with indigenous pelagic zooplankton organisms.

In addition to those listed above, one presentation showed behavioural and biological traits sustaining the invasiveness of *P. marinus*. In particular, plasticity in swimming behaviour and tolerance to wide ranges of temperature and salinity turned out as factors allowing this species to be able to successfully adapt to diverse environments. A last contribution summarised the present molecular identification of *P. marinus* from European waters, and provided a view on potential uses and applications of next-generation sequencing (NGS) techniques.

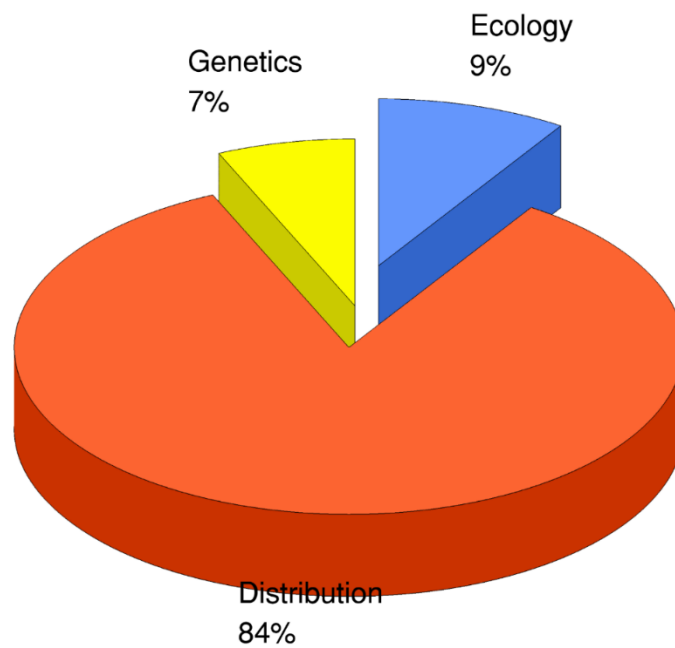
EUROBUS Survey Site Typology



EUROBUS Survey Taxonomic Identification



EUROBUS Survey Lines of Investigation



4 Response to the ToRs and definition of a roadmap

The contributions presented at the meeting provided an updated summary of the distribution of *P. marinus* in European waters and identified the main vectors of primary and secondary introduction (see ToR a). In addition, the establishment of a brand new Working Group (WKEUROBUS) set the base for a real-time monitoring network of the settlement and expansion of this species in European areas, focusing on the environmental characteristics to which *P. marinus* can adapt (in compliance with the ToR b). The WK also provided an excellent forum through which to exchange information and promote knowledge sharing; communication has been further encouraged by subsequent mail exchanges among the participants (as requested in ToR c).

Discussion among WK members also focused on the opportunity of providing information about *P. marinus* expansion through the AquaNIS portal (ToR d). This platform will be evaluated by each EUROBUS participant, allowing each researcher to decide on this issue.

The open discussion sessions (see Annex 2) provided food for thought on several aspects related to the study of *P. marinus*, in line with what was requested by point e) in the ToR. The first topic discussed was related to the sampling strategies employed. None of the participants is/was involved in a project dedicated to *P. marinus*, and the data presented were obtained from zooplankton samples collected in several different projects. Depending on the site and on the research programme, different methods were employed; nonetheless the majority of the samplings were carried out with plankton nets towed by vertical/oblique/horizontal hauls. In addition, samplings were not performed at the same time of the day and were mainly concentrated during daylight hours. Most of these procedures do not allow for sampling in the first meters above the bottom, where probably *P. marinus* is more abundant during the daytime. Such discrepancies cannot be easily addressed, as they would require the redesign of ongoing activities. Heterogeneity was reported also for environmental parameters (e.g., temperature and salinity): vertical integration of the variables, surface values or measurements at the same depth of zooplankton collection. It was suggested to emphasize these differences in order to highlight potential sources of inhomogeneity among sites. A starting idea in future project calls is the standardization of the sampling protocol for *P. marinus* in different areas, at least in some key moments of the year. It was also suggested that the existing table used to screen the occurrence of *P. marinus* in the EUROBUS framework should be improved by specifying the sampling strategy used, so as to make the information more complete.

The discussion then focused on the possible uses of *P. marinus* in applied research. This species has a demonstrated ability to grow and reproduce in the laboratory, making it eligible as a target species in ecotoxicological studies. In addition, *P. marinus* has the potential to be used as food in aquaculture farms. As such, ideas on possible rearing experiments and on analysis of the nutritional value of *P. marinus* were put forward.

A general consensus on the need for submitting proposals to project calls was underlined by all participants. Some possible calls have been identified in the framework of H2020, particularly in consideration of new openings due in forthcoming months, but the necessity to investigate other levels of funding (e.g., national projects, transnational/bilateral

calls, etc.) was also emphasised. The participants agreed to share information about calls of potential interest within EUROBUS mailing list.

The presentations revealed that, based on present knowledge, *P. marinus* does not impact the structure of the pelagic trophic web in invaded sites, as no competitive exclusion has been reported yet. The participants suggested focusing on possible effects on the benthic community. Since at present this topic is not addressed by any of the participants, it might be a possible focus for future project calls.

To further promote interaction among participants, joint initiatives between already existing programmes, such as EMBRC-ERIC, were highlighted. In addition, the creation of a *P. marinus* repository, containing voucher specimens collected from different regions, was proposed. Within MOTax (Marine Organism Taxonomy) Service, the Stazione Zoologica could work as hub for the conservation of ethanol preserved specimens, to be used for taxonomic and molecular analyses. Any work carried out on such voucher specimens would be a joint project between the host organisation (Stazione Zoologica) and the researcher who collected the specimen. At present, discussion within MOTax is in course to define the modalities of networking.

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Annex 2: Agenda

29 January

10:00 – Registration

11:00 – Opening session “The EUROBUS Working Group” (M. Uttieri)

11:30 – Session topic “*Pseudodiaptomus marinus* – The ongoing invasion” (Part I)

11:35 – “*Pseudodiaptomus* in the CPR survey” (M. Brunetta, M. Wootton)

11:50 – “The occurrence of *Pseudodiaptomus marinus* in the estuaries of the Basque coast (Bay of Biscay)” (A. Iriarte, I. Uriarte, Z. Barroeta, F. Villate)

12:05 – “Insights on the occurrence of the invasive copepod *Pseudodiaptomus marinus* in the Mondego estuary” (S. Cotrim, A. L. Primo, J. Falcão)

12:30 – Lunch break (SZN canteen)

14:00 – Session topic “*Pseudodiaptomus marinus* – The ongoing invasion” (Part II)

14:05 – “First record of *Pseudodiaptomus marinus* Sato, 1913 in Berre Lagoon (NW Mediterranean, France)” (F. Delpy, M. Pagano – presented by M. Uttieri on behalf of the authors)

14:20 – “Occurrence of *Pseudodiaptomus marinus* in the Southern Tyrrhenian Sea” (M. G. Mazzocchi, I. Di Capua)

14:35 – “Seasonal dynamics of the invasive copepod *Pseudodiaptomus marinus* in the coastal Lake Faro and its recent expansion in Lake Ganzirri” (G. Zagami)

14:50 – “The new invasive copepod *Pseudodiaptomus marinus* in the Lagoon and the Gulf of Venice” (E. Camatti, M. Pansera)

15:05 – “Update of the presence of *Pseudodiaptomus marinus* in the Adriatic Sea” (V. Tirelli, A. de Olazabal, A. Goruppi)

15:20 – “*Pseudodiaptomus marinus* – Snapshots from the Bay of Šibenik (central Adriatic Sea)” (O. Vidjak, S. Rožić, N. Bojanić)

15:35 – “*Pseudodiaptomus marinus* in Sevastopol Bay: September, 2016 – December, 2017” (A. D. Gubanova – presented by M. G. Mazzocchi on behalf of the author)

15:50 – “Biological and behavioural traits sustaining the invasiveness of *Pseudodiaptomus marinus*” (M. Uttieri, L. Svetlichny, E. Hubareva, A. Khanaychenko)

16:05 – “Alien species and molecular approaches: a case study” (A. Amato, R. Aiese Cigliano)

16:30 – Coffee break

17:00 – Open discussion

this section is intended as an open forum, within which sharing experience and knowhow, exchanging and commenting on the results presented, with the aim of building an international network of collaborations

19:00 – Closure of activities

20:15 – Social dinner

30 January

09:45 – Session topic “Defining a roadmap for the EUROBUS Working Group” (Part I)

this section is intended as an open forum, focusing (but not limiting) to the following topics:

- synthesis of the contributions presented
- future perspectives in the studies on *Pseudodiaptomus marinus*
- involvement as ICES Expert Group
- identification of possible project calls (national, international and transnational)
- outline of a position paper

11:00 – Coffee break

11:30 – Session topic “Defining a roadmap for the EUROBUS Working Group” (Part II)

12:30 – Lunch break (SZN canteen)

14:00 – Open discussion

15:00 – Closure of works

Annex 3: Draft resolution for a new Working Group

A Working Group entitled “Towards an EUROpean OBservatory of the invasive calanoid copepod *Pseudodiaptomus marinus*” (WGEUROBUS), co-chaired by Marco Utter, Italy, and Arantza Iriarte, Spain, will be established and will work on ToRs and generate deliverables as listed in the Table below.

	MEETING DATES	VENUE	REPORTING DETAILS	COMMENTS (CHANGE IN CHAIR, ETC.)
Year 2019	to be decided	to be decided		
Year 2020				
Year 2021				

ToR descriptors

TO R	DESCRIPTION	BACKGROUND	SCIENCE PLAN TOPICS ADDRESSED	DURATION	EXPECTED DELIVERABLES
a	Compile and analyse data on the geographical distribution, seasonal patterns and interannual variations of <i>Pseudodiaptomus marinus</i> in European waters.	a) <i>Pseudodiaptomus marinus</i> was first observed in European waters in 2007, and it has since been expanding relatively rapidly in European waters, reaching transitional, coastal, as well as oceanic environments. Much of the information on its occurrence, though, is still unpublished and a clear view of the spatial and temporal distribution of this species in Europe is lacking. Thus a need to compile data and update the geographical distribution, seasonality and interannual variations of this species in European waters has been identified. Furthermore, the analysis of the spatial and temporal variations will be very useful to understand which are the environmental conditions that favor the establishment of this alien species.	The proposed activities align with the “Proposed science priorities and flagship science activities” published by the Science Committee and are therefore expected to align with priorities in the new ICES Strategic Plan’.	Years 1-2	An updated review of the knowledge about the spatial and temporal distribution patterns of <i>P. marinus</i> in European waters. Write up of joint project proposals to be submitted to various project calls. Peer-reviewed publication (jointly with outcomes from ToR b) Yr 2.
b	Identify priority lines of research on <i>Pseudodiaptomus marinus</i> , including main traits, ecosystem impact, systematics and taxonomy.	a) The presence of <i>Pseudodiaptomus marinus</i> in European waters is now prompting many questions such as those related to the means of introduction, impact on the native biota and ecosystem services, the common/diverse origin of populations established in different geographic locations	The proposed activities align with the “Proposed science priorities and flagship science activities”	Years 1-2	Assessment of future perspectives of research on <i>P. marinus</i> . Write up of joint

		and types of environments, or its potential applied uses (e.g., food for aquaculture). The establishment of priority lines of research is necessary as a first step to design and undertake future coordinated and collaborative research activities.	published by the Science Committee and are therefore expected to align with priorities in the new ICES Strategic Plan’.		project proposals to be submitted to various project calls. Peer-reviewed publication (jointly with outcomes from ToR a) Yr. 2.
c	Compile data and provide expertise for the identification of key ecological, biological and behavioural traits of <i>Pseudodiaptomus marinus</i> .	a) The identification of the ecological, biological and behavioural traits of this species will help to understand its successful colonization of different types of environments and will provide vital information to establish its potential uses.	The proposed activities align with the “Proposed science priorities and flagship science activities” published by the Science Committee and are therefore expected to align with priorities in the new ICES Strategic Plan’.	Years 1-3	A database compiling known traits for <i>P. marinus</i> in different environments in European waters. A list of traits that are still unavailable for this species.
d	Design and promote coordinated and collaborative activities for the molecular identification of <i>Pseudodiaptomus marinus</i> strains occurring in different environments in European waters.	a) Molecular characterization is a useful tool to identify the geographic origin of <i>Pseudodiaptomus marinus</i> genotypes present in European waters. Genomics and transcriptomics analyses may help to understand the apparent versatility regarding the environmental conditions in which it can live.	The proposed activities align with the “Proposed science priorities and flagship science activities” published by the Science Committee and are therefore expected to align with priorities in the new ICES Strategic Plan’.	Years 1-3	Plan of collaborative initiatives . Write up of joint project proposals to be submitted to various project calls. Establishment of a repository of European voucher specimens preserved according to a commonly agreed protocol, to be used for comparative studies.
e	Provide expert knowledge and guidance to test for possible dormancy strategies of <i>Pseudodiaptomus marinus</i> .	a) <i>Pseudodiaptomus marinus</i> has no documented resting stages, however recent data point at the potential adoption of dormancy strategies to overcome unfavourable conditions. The exploration of this topic will shed light on possible biological adaptations used to increase the	The proposed activities align with the “Proposed science priorities and flagship science activities”	Years 1-3	Plan of collaborative initiatives . Write up of joint project proposals to be submitted to

invasiveness of this species.	published by the Science Committee and are therefore expected to align with priorities in the new ICES Strategic Plan'.	various project calls.
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Summary of the Work Plan

Year 1	The group will deal with all of the ToRs during the Year 1 (with various degrees of intensity).
Year 2	The group will continue with all of the ToRs and we expect that two of those will be completed during Year 2: geographical, seasonal, interannual distribution (a) and identification of priority lines of research (b)
Year 3	The group will focus on completion of the remaining ToRs (c, d and e)

Supporting information

Priority	Biological invasions represent a serious threat to aquatic ecosystems, and are presently a major issue in the scientific community. Among invasive copepods, the calanoid copepod <i>Pseudodiaptomus marinus</i> , native to the Indo-Pacific, has been increasingly reported in European waters since 2007. This species is particularly well-suited to serve as a model organism for ecotoxicological studies, and is amenable to experimental rearing. The participants will constitute a network to explore joint initiatives to study the different aspects of the biology and ecology of <i>P. marinus</i> .
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants	Approximately 30 participants expected
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	There are no obvious direct linkages.
Linkages to other committees or groups	This workshop is directly related to research and advisory goals of several EPDSG EGs, including the Working Group on Integrative Morphological and Molecular Taxonomy (WGIMT) and Working Group on Zooplankton Ecology (WGZE). There are also direct linkages with HAPISG EGs, including the Working Group on Introductions and Transfers of Marine Organisms (WGITMO) and Working Group on Ballast and Other Ship Vectors (WGBOSV).
Linkages to other organizations	The work of this group is potentially aligned with similar work by the Intergovernmental Oceanographic Commission of UNESCO (IOC) and the International Maritime Organization (IMO).