



Diversity and microhabitat features of crustacean copepods collected in colonization experiments at deep-sea hydrothermal vent field at the Mid-Atlantic Ridge (37 N, Lucky Strike)

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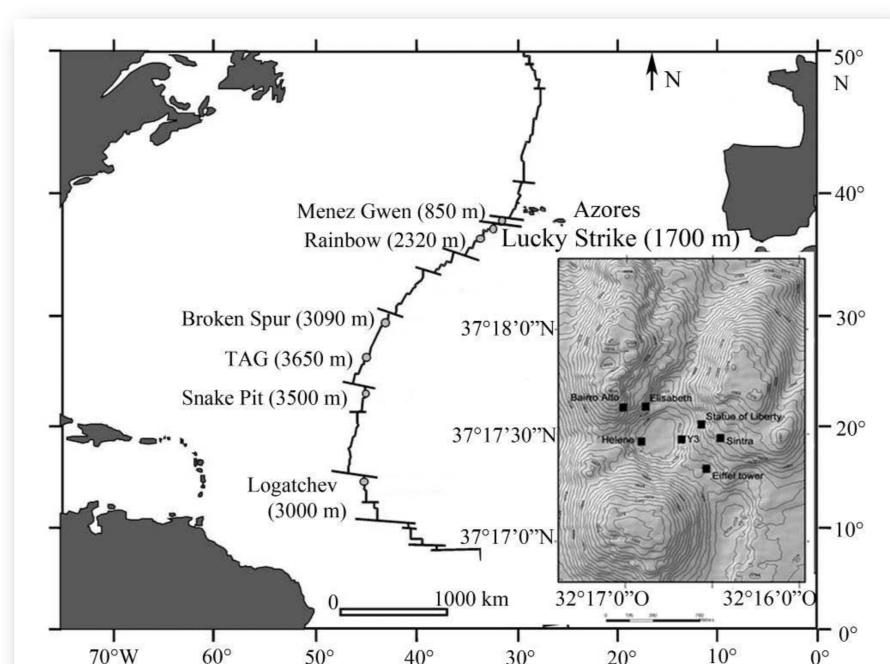


Fig 1. Location of the Lucky Strike vent field on the Mid-Atlantic Ridge (MAR) at 37°17.59N, 32°169W. The inset shows the hydrothermal vent field with the localisation of several active sulphide edifices around a central lava lake, including the Eiffel Tower edifice in the south-east. Cuvelier et al 2014

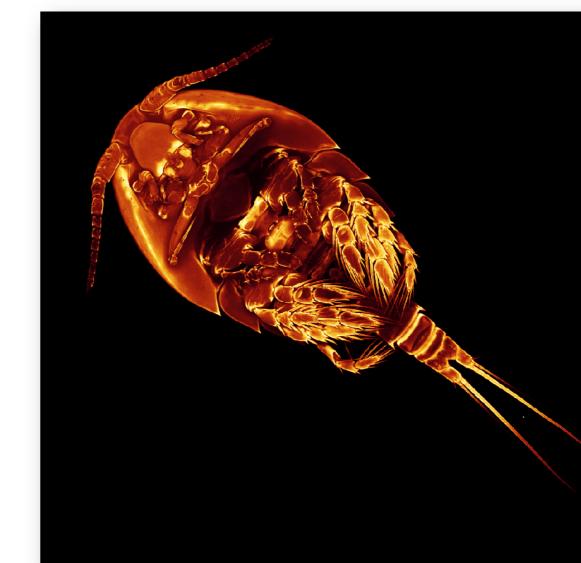


Fig 6. A confocal image of a new species of a deep-sea hydrothermal vent copepod Aphotopontius sp. nov. (Copepoda: Siphonostomatoida: Dirivultidae) from Lucky Strike, Mid-Atlantic Ridge, collected during MoMAReto Cruise (France: Ifremer) at a depth of 1698 m. http://www.eurekalert.org/multimedia/pub/52124.php

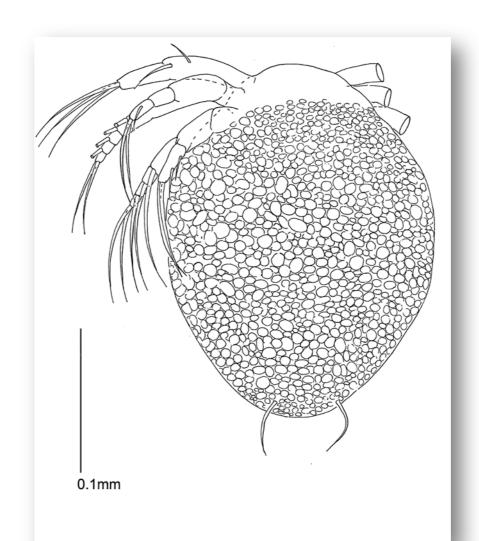


Fig 7. Stygiopontius pectinatus Humes, 1987, nauplius of stage I: A, habitus, ventral; B, habitus, lateral, left side; C, antennule, ventral; D, antenna, ventral; E, mandible, dorsal. Ivanenko V.N., P. Martínez Arbizu & J. Stecher. 2007.

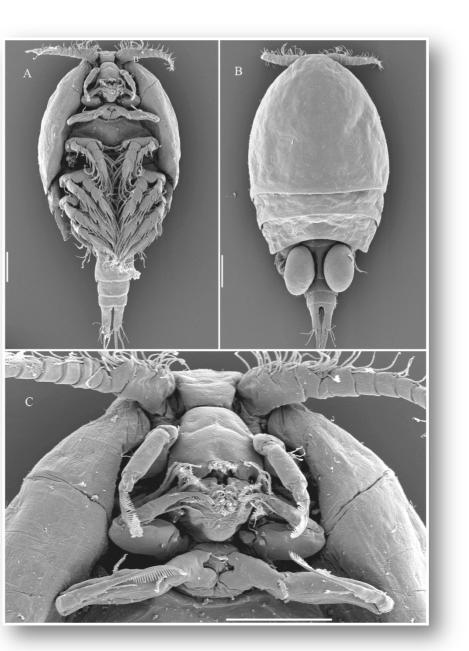


Fig 8. Stygiopontius pectinatus (female) SEM micrographs. A: habitus, ventral view. B: habitus, dorsal view. C: oral cone and anterior appendages. Scale bars 100 um. Gollner S, Ivanenko VN, Arbizu

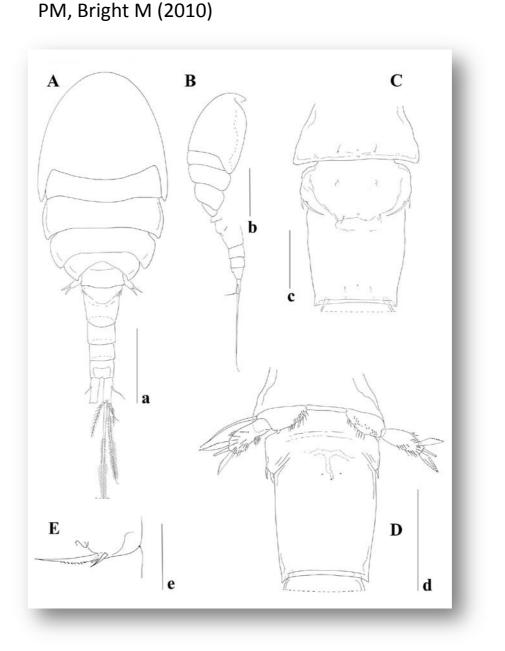
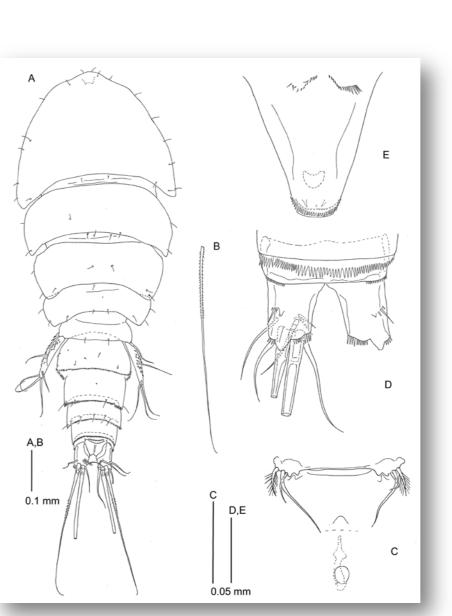


Fig 9. Ivanenko V.N. & D. Defaye. 2004., ♀ holotype: A, habitus, dorsal; B, terminal seta of caudal ramus (shown partially in Fig 1 A); C, leg 6 and genital field, ventral; D, anal somite and caudal rami, ventral; E, labrum, anterior.



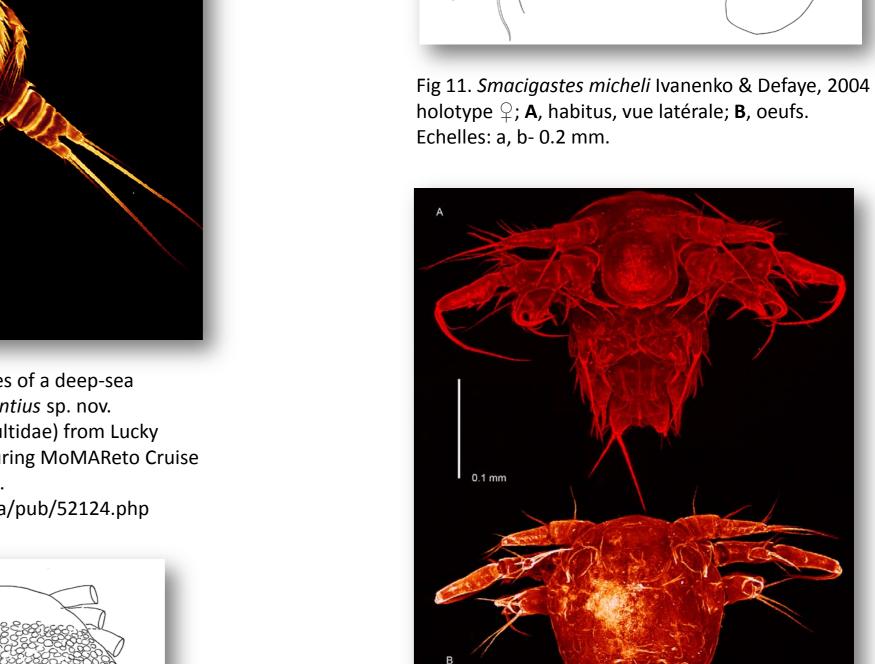


Fig 12. Smacigastes micheli Ivanenko & Defaye, 2004. Confocal photo of premolting stage VI nauplius. (A) Habitus, ventral. (B) Habitus, dorsal.

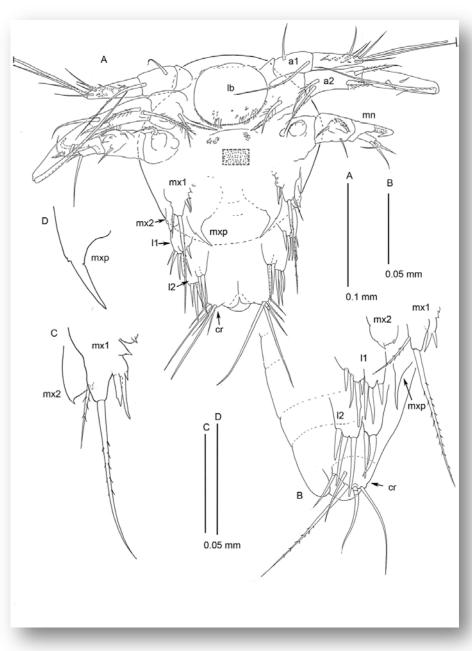


Fig 13. Smacigastes micheli Ivanenko & Defaye, 2004. Stage VI nauplius. (A) Habitus, ventral, showing buds of maxillule, maxilla, maxilliped, Leg 1 and Leg 2, papulose surface illustrated within square. (B) Posterior part of body showing buds of maxillule, maxilla, maxilliped, Leg 1 and Leg 2, lateral. (C) Buds of maxillule and maxilla, ventral. (D) Bud of (rift) maxilliped. a1, antennule; a2, antenna; mn, mandible; m·1, bud of maxillule; m·2, bud of maxilla; mxp, bud of maxilliped; l1, bud of Leg 1; l2, bud of Leg 2; cr, bud of

caudal ramus

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Abstract. Diverse copepods previously unknown were found and described from the Eiffel Tower edifice located in the Lucky Strike vent field (37 degrees N) (Mid-Atlantic Ridge MAR, 1698 m). The copepoda were collected in colonization experiments (SMAC arrays) that were deployed in 1997 during the MARVEL cruise and recovered in 1998 during the PICO cruise. Other specimens were collected during the MoMARETO cruise during which a physicochemical characterization of copepod microhabitats was done. The cyclopoid copepod Hepterina confusa Ivanenko & Defaye, 2004 (Cyclopoida: Cyclopinidae), the harpacticoid copepods Smacigastes micheli Ivanenko & Defaye, 2004 (Harpacticoida: Tegastidae) and Tisbe dahmsi Ivanenko, Ferari, Defaye, 2011 (Crustacea, Copepoda: Harpacticoida: Tisbidae), and the dirivultid copepod Aphotopontius atlanteus Humes, 1996 (Siphonostomatoida: Dirivultidae) were found abundantly in the in situ colonization experiments (SMAC arrays) deployed on Bathymodiolus azoricus mussel assemblages at the Eiffel Tower edifice. Another colonization experiment, deployed near a black smoker, exhibits a different pattern with dominance of the harpacticoid families Ameiridae and Argestidae of the Ectinosomatidae family. These colonization experiments revealed that copepods of the genus Tisbe were substantially more abundant in the trays deployed on mussel assemblages than to those in the vicinity of black smokers. Nevertheless, no clear response regarding the effect of organic enrichment on copepod abundance was observed. The type of environment where the arrays were deployed appears to have a stronger influence on copepod abundances and composition than the treatment applied within each tray. Directly on the Eiffel Tower edifice, Tisbe copepods were found within different Bathymodiolus azoricus assemblages. These assemblages were alternatively dominated by the Dirivultidae or the Tegastidae,

Keywords: copepods, diversity, microhabitat, deep-sea, hydrothermal vent, Mid-Atlantic Ridge, colonization experiments

the former being dominant in 67% of the samples.

Introduction Before our study only three species of dirivultid copepods (Aphotopontius atlanteus Humes, 1996, A. temperatus Humes, 1997, and S. rimivagus Humes, 1997) were known at the Lucky Strike (LS) vent field (37°N), one of the largest and active known hydrothermal fields of the MAR (Comtet & Desbruyères, 1998; Desbruyères et al., 2000). However, recent study synonymized Aphotopontius temperatus Humes, 1997 with A. atlanteus (Ivanenko & Defaye, 2006; Ivanenko et al., in preparation). A poecilostomatoid copepod *Ambilimbus arcuscelestis* Ivanenko et al., 2005 has been described from the Rainbow vent field (36°N) and is the only record for the family Erebonasteridae Humes, 1987 from the MAR (Ivanenko et al., 2005). A harpacticoid copepod Bathylaophonte azorica Lee & Huys, 1999 (Laophontidae), was described from the Menez Gwen vent field (Lee & Huys, 1999). Heptnerina confusa Ivanenko & Defaye, 2004 (Cyclopoida: Cyclopinidae) and Smacigastes micheli Ivanenko & Defaye, 2004 (Harpacticoida: Tegastidae), have been described from LS (Ivanenko & Defaye, 2004a,b). The monotypic genus *Heptnerina* Ivanenko & Defaye, 2004 is phylogenetically most related to the specious genus Cyclopina Claus, 1863 recorded worldwide from shallow waters. The description of S. micheli represented the first record of tegastids from the deep-sea environment, since these copepods are primarily known from shallow waters, either free-living or associated with bryozoans, cnidarians and algae (Humes, 1981a, b, 1984; Ivanenko et al., 2008a, b). Recent investigations of chemosynthetic communities, such as deep-sea hydrothermal vents of the East Pacific Rise and deep-sea cold seeps of the Gulf of Mexico, as well as from a shallow whale-fall near the Swedish coast, lead to the discovery of new species of tegastid copepods (Gollner et al., 2008; Plum & Martínez Arbizu, 2009; Willems et al., 2009). During the last decades, a series of in situ colonization experiments and

faunal sampling have been conducted at the LS vent field, but only recently researchers have started to look specifically at the meiofauna. Furthermore, very few studies have focussed on the distribution of meiofauna in relation to environmental factors. As a result of these colonization experiments, about 22.000 specimens belonging to more than 20 copepod species have been collected, representing the orders Calanoida (Spinocalanidae), Cyclopoida (Cyclopinidae), Harpacticoida (Ameiridae, Ancorabolidae, Argestidae, Canthocamptidae, Ectinosomatidae, Miraciidae, Pseudotachidiidae, Donsiellinae, Tegastidae, Thalestridae, Tisbidae), Poecilostomatoida (Erebonasteridae), and Siphonostomatoida (Dirivultidae). Among these specimens, three species were found to be abundant in these hydrothermal vent field samples: the cyclopoid *H. confusa*, the harpacticoid *S. micheli* and a new species of the specious, harpacticoid genus *Tisbe* Lilljeborg, 1853 (Tisbidae). The family Tisbidae has been actively investigated from shallow-waters, as free-living or associated with invertebrates (Humes, 1957; Dahms & Schminke, 1993, 1995; Dahms et al., 1991; Chullasorn et al., 2009).

al. (2009).

Material and methods Site locality: 37°17.29'N, 32°16.45'W; Atlantic Ocean, Mid-Atlantic Ridge, Azores Triple Junction, Lucky Strike, Eiffel Tower edifice; depth 1698m (Fig 1). Specimens were sampled at different time periods: between 1997 and 1998 with *in situ* colonization experiments (SMAC arrays: "Small Module Autonome de Colonisation") and in 2006, through direct sampling with the ROV VICTOR6000 (Figs 2-3). For in situ experiments, the designed arrays (SMAC A, B and C) were deployed in 1997 by the submersible Nautile (during the MARVEL cruise with RV L'ATALANTE) (Fig 4). Each array contained 4 trays filled with small glass beads and protected from large carnivores by mesh. In addition to the beads, some of the trays were filled with an organic supplement (NordSeaMink quality fish meal) or enriched with either elemental sulfur (S⁰) or hydrogen sulfide to mimic the conditions found at hydrothermal vents. Other specimens (nauplii, copepodids and adults) were collected during the MoMARETO cruise in 2006 on the R/V Pourquoi pas? by the ROV VICTOR6000. During this cruise, the microhabitats of twelve different faunal assemblages (C1 to C12) were characterized in terms of temperature and chemical conditions (Sarradin et al. 2009, De Busserolles et al. 2009) (Fig 5). Abiotic conditions in the vicinity of the fauna during the MoMARETO were assessed on each sampling unit by in situ measurements and water sampling. Total dissolved iron (TdFe) and total dissolved sulfide (TdS: H2S+HS-+S--) concentrations were measured in situ using the chemical analyser CHEMINI (Vuillemin et al. 2009). Temperature (T°C) was measured with an autonomous temperature probe (NKE) attached to the sampling inlets. Water samples were collected with the PEPITO sampling device (Sarradin et al. 2009). The pH was measured on board at 25°C using a combined pH electrode (Ingold®) for sulfide-rich medium after calibration with NBS buffers (pH 4 and 7). Finally, total dissolved copper (TdCu), was measured by stripping chronopotentiometry (SCP) with a gold electrode (Riso et al. 1997). All abiotic sampling and analytical procedures are described in Sarradin et

After the physico-chemical characterization of each microhabitat, the fauna was sampled semi-quantitatively using Victor's suction sampler and arm grab. Once brought on board, faunal samples from each location were washed through 250 μm and 63 μm mesh sieves and fixed with 4 % buffered formalin. After two days, they were transferred to 70 % ethanol. Once in the laboratory, the meiofauna from the 63 µm fraction was separated from heavier particles by centrifugation in Ludox (Burgess 2001) and meiofaunal specimens then were stained with Rose Bengal. Specimens from both fractions (250 μm and 63 μm) were identified to the lowest possible taxonomic level. In this paper only tegastid copepods are considered; the other faunal groups will be addressed in ongoing studies. The regressions and multivariate statistical analysis (RDA) were conducted using a forward selection procedure with unrestricted permutations using the pack for library (S. Dray, University of Lyon) implemented in the free software R 2.8.1 (R Development Core Team 2008) as well as the Vegan package (Oksanen et al. 2011). Canonical redundancy analyses (RDA) were performed to evaluate the links between environmental conditions and faunal observations. They allow for a simultaneous representation of the observations in two or three dimensions, selecting the linear combination of environmental variables that maximizes the dispersion of species scores (Ter Braak 1986, Sarrazin et al. 1999). In the forward selection procedure, the environmental variables are tested one by one and are included if they are statistically significant. Copepoda were prepared for confocal microscopy as follows. Soft tissues were removed without damaging the external anatomy by transferring a copepod to staining vials and immersing for circa 10

minutes in a K-protease digestive medium from QIAGEN following the

recommended protocol. The specimen was then transferred to distilled

stained with Yellow Fuchsin. The staining procedure was adapted from

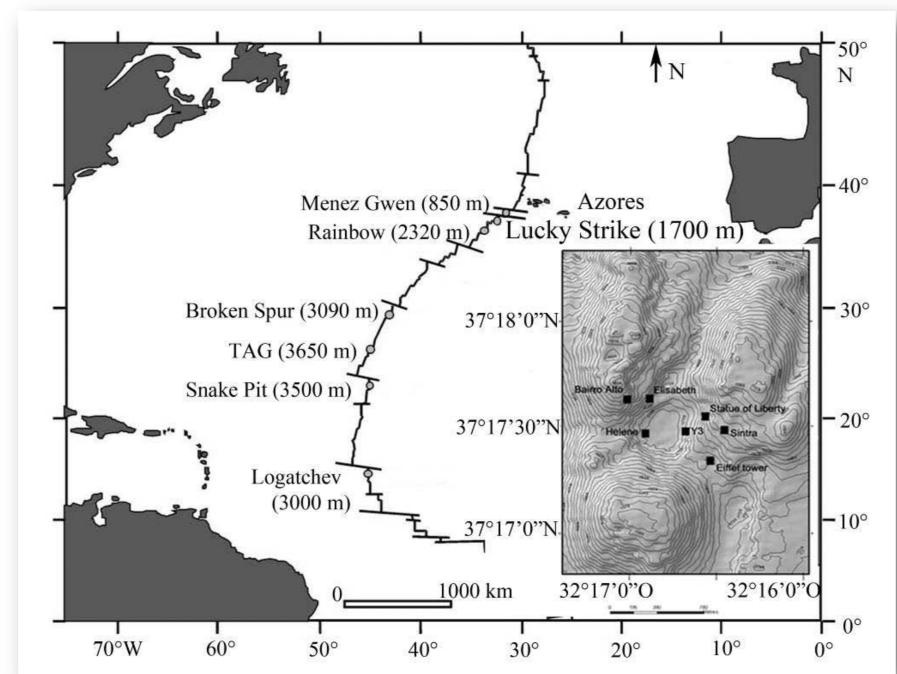
Michels & Büntzow (2010) by substituting the Congo Red solution with

water to wash out any remain of K-protease, and the cuticle was

a solution of Yellow Fuchsin.

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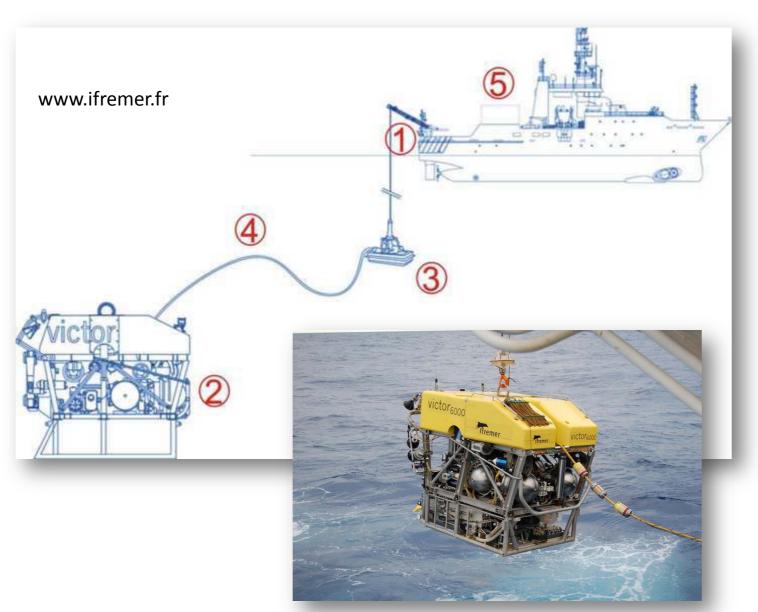


Fig 5. Localisation and depth of the sites at the Eiffel Tower edifice (MAR)

at 37°17.59N- 32°169W where different substrata were deployed in 2006

and recovered in 2008. On each locality a panel of slate (A) and wood (B),

each equipped with a NKE temperature probe, were deployed. Remark

the change in localisation of panel A3 in 2008. Cuvelier et al 2014

Copepods from diverse families (Aegisthidae, Ancorabolidae,

Pseudotachidiidae, Spinocalanoida, Tegastidae, and Tisbidae),

mostly unrecorded for the LS vent field, were found in the 250

μm and 63 μm fractions, while morphologically diverse nauplii

were represented mainly in the 63 µm fractions (Figs 6-13).

Physico-chemical characterization of S. micheli microhabitats

relatively narrow temperature range for this ecosystem (De

temperature values (C2, C8 and C10) also had the highest

were near ambient (4.4°C) in C1, C9, C11 and C12. All

higher hydrothermal influence.

chemical conditions.

significant. Fig 14.

Busserolles et al. 2009). The microhabitats with the warmest

concentrations of TdFe and TdS. On the other hand, the coolest

location C9 had one of the lowest TdS and TdFe concentrations

and the highest TdCu concentration. The mean temperatures

environmental variables were significantly correlated with

temperature but while sulfide and iron concentrations were

with temperature (De Busserolles et al. 2009). The pH varied

from 6.00 (C10) to 7.34 (C9), being more acidic in the areas of

characterized by relatively low temperatures (4.8 to 7.5°C) that

microhabitats sampled (C8 and C2). No tegastid copepods were

The faunal and environmental data were transformed prior to a

distances in Principle Component Analysis (Legendre & Gallagher

2001). The Hellinger distance gives low weight to rare taxa. This

of rare taxa in the samples can be attributable to sampling error

standardized because they are measured in different sampling

meaningful covariance. The procedure consists in centring all

explained 31% of the variance in distribution . An analysis of

second, explaining 10 % of the variance, was not (F=1.5423,

descriptors on a zero mean and reducing them to unit standard

deviation (Legendre & Legendre 1998). The redundancy analysis

(RDA) performed on *S. micheli* abundances from all microhabitats

variance testing for significance of the RDA showed that only the

first axis was significant (F=4.6736, p=0.01667, α <0.05) while the

p=0.29000, α >0.1). Only three of the five environmental variables

tested (TdS, TdCu and T°C) significantly explained the copepod

distribution. TdS is the more significant (p=0.015), followed by

TdCu (p=0.146) and then, T°C (p=0.434). TdFe and pH were not

distance is used because in the deep-sea the sporadic appearance

multivariate statistical analysis. Faunal abundance data were

transformed to conserve Hellinger, rather than Euclidian,

(Gauthier et al. 2010). The environmental variables were

units and must be reduced to a common scale to calculate

represent low hydrothermal inputs . All developmental stages

were absent from the most extreme microhabitat (C10) and

found in C7 despite the presence of intermediate physico-

Overall, the microhabitats colonized by S. micheli were

tegastid abundance was low in the two other hotter

positively correlated, TdCu and pH showed negative correlations

(microhabitat) varied from 4.79°C to 8.79°C, which represents a

Mean temperatures conditions on each sampling unit

Lubbockiidae, Miraciidae, Oncaeidae, Oithonidae,

Canthocamptidae, Cyclopinidae, Dirivultidae, Ectinosomatidae,

Figs 2-3. The remote controlled vehicle VICTOR 6000 gear includes: A direct-winding hydraulic winch, the 8,500 m 20 mm umbilical, weighing in all: 30 tonnes(1) The vehicle 3.1 x 1.8 x 2.1 m, weight 4 tonnes and 600 kg for the module (2) The hard ballast 1.5 x 0.8 x 0.5 m, weight 1.2 tonne (3) The tether measuring from 100 to 300 m 35 mm (4) 5 20-ft containers (5): vehicle power •winch's hydraulic unit control room mechanics storage



Fig 4. Colonization experiment on the Eiffel Tower edifice of the Lucky Strike vent field. Ivanenko et al. 2011

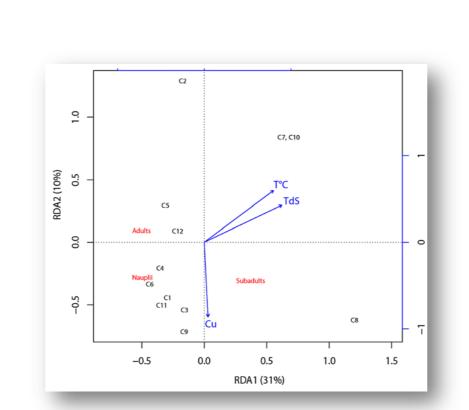


Fig 14. Redundancy analysis (RDA, scaling type 2) performed on the matrix of *Smacigastes micheli* Ivanenko and Defaye 2004 abundances of the different developmental stages (nauplii, subadults, adults) sampled on the Eiffel Tower edifice and the matrix of environmental factors. The first two axes represent 40% of the total variance but only the first axis, explaining 31% of the variance, was significant. Vectors are represented by arrows.

Discussion

This is the first report of abundant and diverse nauplii of copepods in a deep-sea hydrothermal community. Up to now, copepod nauplii were not hypothesized to be living in the extreme conditions (high levels of sulfides, heavy metals and elevated temperatures) present in the vent environment (Tsurumi et al. 2003). Only a few studies report the presence of nauplii (Dinet et al. 1988), and only the lecithotrophic nauplius I of a siphonostomatoid copepod from the family Dirivultidae has been positively identified (Ivanenko et al. 2007). In the Eiffel Tower samples, nauplii belonging to the harpacticoid copepod families Canthocamptidae, Pseudotachidiidae, Miraciidae, and Tegastidae, as well as nauplii which could not be attributed to a family, were identified.

The discovery of several new species and a remarkably high diversity of copepods are a result of the improvement in sampling designs and the increased interest for identification of smaller meiofaunal species. With the exception of a few studies, sampling at vents is often limited to the identification of megaand macrofaunal species and the meiofauna has often been neglected (Gauthier et al., 2010). It is only recently that the meiofauna has really started to be systematically accounted for in vent ecological studies (Tsurumi et al., 2003, Gollner et al., 2006, 2007, Copley et al., 2007). Future ecological studies at vents will surely benefit from more systematic, small-scale spatial studies of faunal assemblage composition and our portrait of species diversity for these peculiar ecosystems will probably evolve significantly during the up-coming decade. In terms of their habitats, the statistical analysis (RDA) showed

that the relative abundances of nauplii and adults of *S. micheli* were higher in the microhabitats characterized by the lowest TdS and lowest T°C conditions while the abundance of the subadult stages appears to be linked to slightly higher hydrothermal inputs. This confirms our initial hypothesis concerning the existence of separated environmental niches for the various developmental stages. Whether this distribution is due to differential physiological tolerances, to resource competition between juvenile copepodids and adults, or to different nutritional needs of the different developmental stages is unknown. Presence of nauplii in the mildest microhabitats with the adults may simply result from the fact that nauplii were recently hatched. On the other hand, it is also possible that both the nauplii and adults share the same microhabitat, with different nutritional niches. The separation of adults and subadults in areas of differing hydrothermal input/ higher microbial resource areas may result from differing nutritional requirements. More generally, it seems that copepod diversity is strongly influenced by environmental conditions at vents and that areas with lower hydrothermal influence (lower temperatures, lower concentrations of TdS) harbour a higher diversity (Tsurumi et al. 2003, Zekely et al. 2006, Gollner et al. 2010).

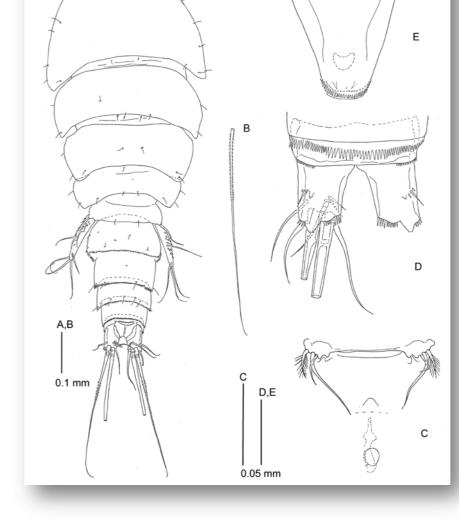


Fig 10. *Tisbe dahmsi* Ivanenko et al. 2011, ♀ holotype: **A**, habitus, dorsal; **B**, terminal seta of caudal ramus (shown partially in Fig 1 A); C, leg 6 and genital field, ventral; D, anal somite and caudal rami, ventral; E, labrum, anterior.