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Sex-related gut microbiota in three geographically separated Norway lobster (*Nephrops norvegicus*) populations

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ABSTRACT

Despite the ecological and economic value of the Norway lobster (*Nephrops norvegicus*), its gut microbiota remains largely understudied. The aim of the present study was to investigate the gut bacterial microbiota in three geographically separated *N. norvegicus* populations from the Mediterranean and the North Seas and detect any potential sex-related microbiota differences, by high-throughput sequencing of the V3-V4 16S rRNA gene diversity of the gut tissue. Egg-bearing females from the Greek population, were also included in this analysis. A total of 2385 operational taxonomic units (OTUs) were identified and between 417 and 1290 OTUs were present in each population/sex group. The dominant OTUs belonged to the Fusobacteriia and Bacteroidia (Sweden), Bacilli and Gammaproteobacteria (Italy) and Spirochaetia and Bacilli (Greece) bacterial classes. In the eggs, the Actinobacteria, Alphaproteobacteria and Gammproteobacteria prevailed. Four OTUs related to the *Oceanispirochaeta*, *Kordiimonas, Desulfovibrio, Carboxylicivirga* genera and one unaffiliated OTU were positively correlated (p values between 0.001 and 0.04) with body size, indicating their potential role in the nutrition and growth of *N. norvegicus*. No statistically significant differences were found between males and females in any of the three populations. However, statistically significant differences between populations for each sex, were found for all females (p values between 0.008 and 0.032) and for the males between the most distant populations, i.e. Italy-Sweden ($p = 0.021$) and Greece-Sweden ($p = 0.015$). The egg microbiota was statistically significant different from both the adult female ($p = 0.027$) and male ($p = 0.046$) gut microbiota. Overall, this study revealed that the *N. norvegicus* gut microbiota is differentiated between geographically distant populations and that sex-related differences are not significant.

1. Introduction

The hologenome and holobiont concepts (Zilber-Rosenberg and Rosenberg, 2008) have opened a new way to view animal life (Theis et al., 2016; Webster, 2017) in terms of their ontogeny and function (Stencel and Wloch-Salamon, 2022; Troussellier et al., 2017). Thus, during the last few years, the scientific interest in holobiont research has increased for animals. However, there are still animal species from various habitats, whose microbiomes remain largely unknown, despite their ecological and/or economic importance and the ongoing rapid technological advancements in DNA/RNA technologies.

One such species is the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) a decapod crustacean, langoustine or scampi, which is a typical clawed lobster with a slender body, long claws and large dark eyes. *Nephrops norvegicus* is considered one of the most important species for fisheries and is considered a particularly valuable commercial crustacean species in Europe. It is a benthic crustacean that is dependent on muddy-type sediments suitable for the construction of burrows. It is widely distributed across the continental shelves of the northeast Atlantic Ocean and the Mediterranean Sea, from Iceland and Norway in

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the north, to Morocco and the Adriatic seas in the south (Bell et al., 2013). Populations dwelling in colder waters around Iceland and the Faroe Islands have a biennial breeding cycle whereas those in the Mediterranean region follow an annual breeding cycle (Bell et al., 2013). *N. norvegicus* are opportunistic predators that consume a wide variety of prey species including crustaceans, polychaetes, molluscs and echinoderms (Bell et al., 2013). *N. norvegicus* population genetics within the Mediterranean Sea was analysed using multiple methods, which revealed low or moderate genetic differentiation between geographical regions (Atlantic vs. Mediterranean) but no geographical pattern of genetic differentiation, thus genetic variability seems to be randomly distributed among populations (Maltagliati et al., 1998; Passamonti et al., 1997). Nevertheless, various aspects of the different populations were strongly related to the spatial and environmental features of the Mediterranean (Commission et al., 2022).

The animal gut microbiome, i.e., the collective genetic material of all microbes found in the gut, is considered a central biological feature for the reproduction, development, nutrition, growth, and health/immunity of the host (Diwan et al., 2023; Singh et al., 2025) and is even considered in wildlife conservation (Kanika et al., 2025; West et al., 2019). The microbiome concept is so established that the microbiome has been proposed as a means and a target of manipulation for improving farmed animal production (Luna et al., 2022). The gut microbiome is also useful for assessing the impact of environmentally induced stress in animals (Evariste et al., 2019) and for identifying responses to such disturbances (See et al., 2025). Although the microbiome of decapods have attracted some scientific interest (Foysal, 2023), even to date *N. norvegicus*, a holobiont, has been sporadically studied. The bacterial microbiota of *N. norvegicus* has been investigated both in natural and experimentally reared populations (Meziti and Kormas, 2013; Meziti et al., 2012, 2010) and pathogens such as the dinoflagellate *Hematodinium* (Small et al., 2006) have also been studied. Thus, the microbiome of this species is largely unknown, especially in the context of the most recently developed and high-throughput sequencing technologies. For these reasons, the aim of the present study was to (a) investigate the gut bacterial microbiota in three geographically separated *N. norvegicus* populations from the Mediterranean and the North Seas, (b) detect any potential sex-related microbiota differences and (c) suggest potential core bacterial taxa for the species populations. It is hypothesized that the animal's gut bacterial communities are highly similar because the animals live in habitats with rather low seasonal variations and very similar environmental conditions and because there is little, if any, genetic differentiation between the populations.

2. Materials and methods

2.1. Sample collection

In the European Union, decapods are not classified as experimental animals under legislation (Directive 2010/63/EU and SFS 2019:66), therefore no animal ethical permits for their collection are required. In Sweden the samples for this study were collected during the ordinary International Bottom Trawl Survey, performed under the EU Data Collection Framework. In Italy the samples for this study were part of the daily landings of standard commercial fishing vessel operating in the Strait of Sicily therefore these samples are subject to IACUC regulations. In Greece the samples were acquired during onboard fishing operations with commercial bottom trawler in the frame of the National Data Collection Framework Program; by the time of capture all samples were already dead. All sampling efforts was limited to minimize large-scale impacts on the populations and is conducted using methodologies to ensure animal welfare and all speciments didn't go through any experimental procedure.

Samples from the North Aegean Sea were collected southwest off Thasos Island (Greece, 40.46°N 24.6°E) between 190 and 435 m depth on 31 October 2023 by a bottom otter trawl boat. The Italian samples were collected in the Strait of Sicily (37° 16' N, 13° 02'W, at 350–400 m depth) on 13 September 2023 by a commercial bottom trawler. Samples from the Kattegat, eastern North Sea (56.22°N 12.16°E, at 33 m depth), were collected by a research vessel equipped with a bottom trawling fishing net on 28 August 2023. In all cases, fishing took place around dawn and early hours of morning hours. From each sampling site, a total of seven male and seven females were collected for further analysis (Table S1, Figure S1).

2.2. Sample processing and microbiota analysis

For each individual and while on board the fishing vessel, the animals were placed on ice immediately and the abdomen was severed from the cepaholothorax immediately after collection of the animals. The estimated time between fishing and sampling did not exceed four hours. The entire gut tissue was then carefully extracted by holding the abdomen with one hand and gently pulling the telson with the other on a pre-sterilised plastic board for each sample and wearing gloves which were replaced with new ones for each sampling. The collected gut tissue samples were stored immediately in DNA/RNA shield (Zymo Research, USA) and stored at -80^0C after a few days. All gut samples contained no digesta. Gut bacterial communities composition of the gut tissue was determined after bulk DNA was extracted from approximately 0.25 mg of whole gut tissue (or eggs in the case for specimens from Greece) with the DNeasy PowerSoil Pro Kit (Qiagen, Germany) with no modifications from the suggested protocol. The eggs were carefully removed from the female individuals with presterilsed spatula and placed immediately in particle-free sterile sea water and rinsed three times; after the final rinse the eggs were placed in DNA/RNA shield and stored at -80^0C. The V3–V4 regions of the bacterial 16S rRNA genes were amplified from the extracted DNA with the primer pair S-D-Bact-0341-b-S-17 and S-D-Bact-115 0785-a-A-21 (Klindworth et al., 2012). Sequencing of the amplicons was performed on a MiSeq Illumina instrument (2 ×300 bp) at the MRDNA Ltd. (Shallowater, TX, USA) sequencing facilities. The raw DNA sequences from this study have been submitted to the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) in the BioProject PRJNA1070646 (BioSample SAMN39675726). The standard operating procedure of MOTHUR software (v.1.48.0) (Schloss et al., 2011; Schloss et al., 2009) was used for processing all the raw 16S rRNA sequence reads. Sequences assigned to mitochondria and chloroplasts, and single singletons were excluded from further analyses. The operational taxonomic units (OTUs) were determined at 97 % cutoff similarity level and were classified with the SILVA database release 138.1 (Quast et al., 2013; Yilmaz et al., 2014). The final OTUs table was normalized to 33, 164 sequence reads. Rarefaction curves (Figure S2) reached the plateau phase for the number of sequence reads we used for our analysis. The Nucleotide BLAST (http://blast.ncbi.nlm.nih.gov) tool was used for identifying the closest relatives of the resulting OTUs.

Testing of the differences across all samples were implemented in the PAleontological STudies (PAST) software (Hammer et al., 2001) by applying non-metric multidimensional scaling (nMDS) based on the unweighted pair group method with the arithmetic mean Bray–Curtis similarity. In addition, Bary-Curtis similarity permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations, between the three geographical locations and sex of the *N. norvegicus* gut microbiota and eggs, was applied.

3. Results

The three investigated populations had specimens of different sizes, based on their carapace length and body weight (Table S1). The smallest animals were caught in Italy and the largest in Greece. Statistically significant differences between females-males, based on PERMANOVA, occurred only for the animals from Sweden ($p = 0.027$, F=19.810). Among animals of the same sex, the male specimens from Italy differed significantly from those from Sweden ($p = 0.011$, F=125.100) while the

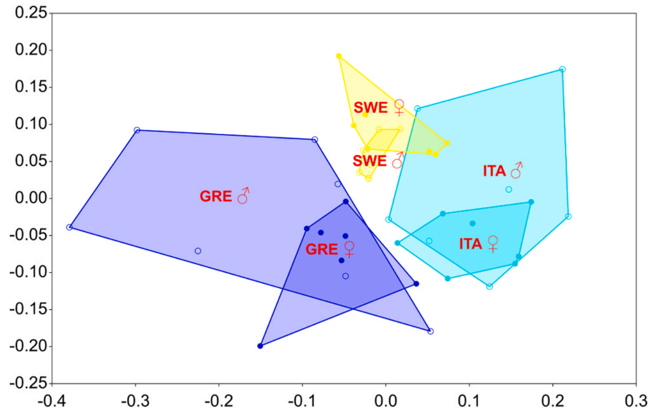

Fig. 1. Non-metric multidimensional scaling (nMDS) of male (♂) and female (♀) individuals of *Nephrops norvegicus* gut from Greece (GRE), Italy (ITA) and Sweden (SWE).

females from Italy were different from those from Greece (p = 0.015, F=32.600) and Sweden (p = 0.014, F=277.100). The three populations were clearly separated with their male and female specimens overlapping in terms of their gut microbiota structure (Fig. 1). The relative contributions of bacteria at the class-level were also similar between females and males in each of the three populations, with Fusobacteriia and Bacteroidia prevailing in the Swedish specimens, Bacilli and Gammaproteobacteria prevailing in the Italian specimens, and Spirochaetia along with Bacilli prevailing in the female Greece gut samples while the male samples were very diverse. In the eggs samples from the specimens from Greece, the Actinobacteria, Alphaproteobacteria and Gammproteobacteria prevailed (Fig. 2).

A total of 2385 bacterial OTUs were identified across all samples. Females had between 417 (Greece) and 931 (Sweden) OTUs while the respective range for males was between 489 (Italy) and 1290 (Greece), while 180 OTUs were found in eggs from Greece (Table 1). The structure of the eggs bacterial communities was statistically different from both the gut microbiota of both females and males (Table 2). Moreover, there was no overlap between the dominant (relative abundance ≥80 %) OTUs in these samples (Table S2). Females had higher number of shared OTUs with the eggs bacterial microbiota (24.9 %) compared to males (10.6 %) (Figure S3). For each population, no significant differences were observed between females and males, but statistically significant differences occurred for each sex between all populations except between males from Greece and Italy (Table 2).

The populations from Italy and Sweden were highly dominated by the same OTU for both females and males, a Photobacterium-related (OTU-0002) and a *Psychrilyobacter*-related (OTU-0001), respectively (Table 1). The females of the Greek population were dominated (17.8 %) by the Spirochaetia-related OTU-0004 while the dominant Cyanobiaceae-related OTU-0018 for males prevailed with 8.4 % relative abundance (Table S2).

In each population, a different set of OTUs were found to occur in abundances ≥ 1 % concomitantly in both the females and males, i.e. denoted as "most important OTUs" in the study, in each population (Fig. 3, Table 3). Three of these, 17 in total, most important OTUs, namely OTU-0001, −0003 and −0013, were found in all three

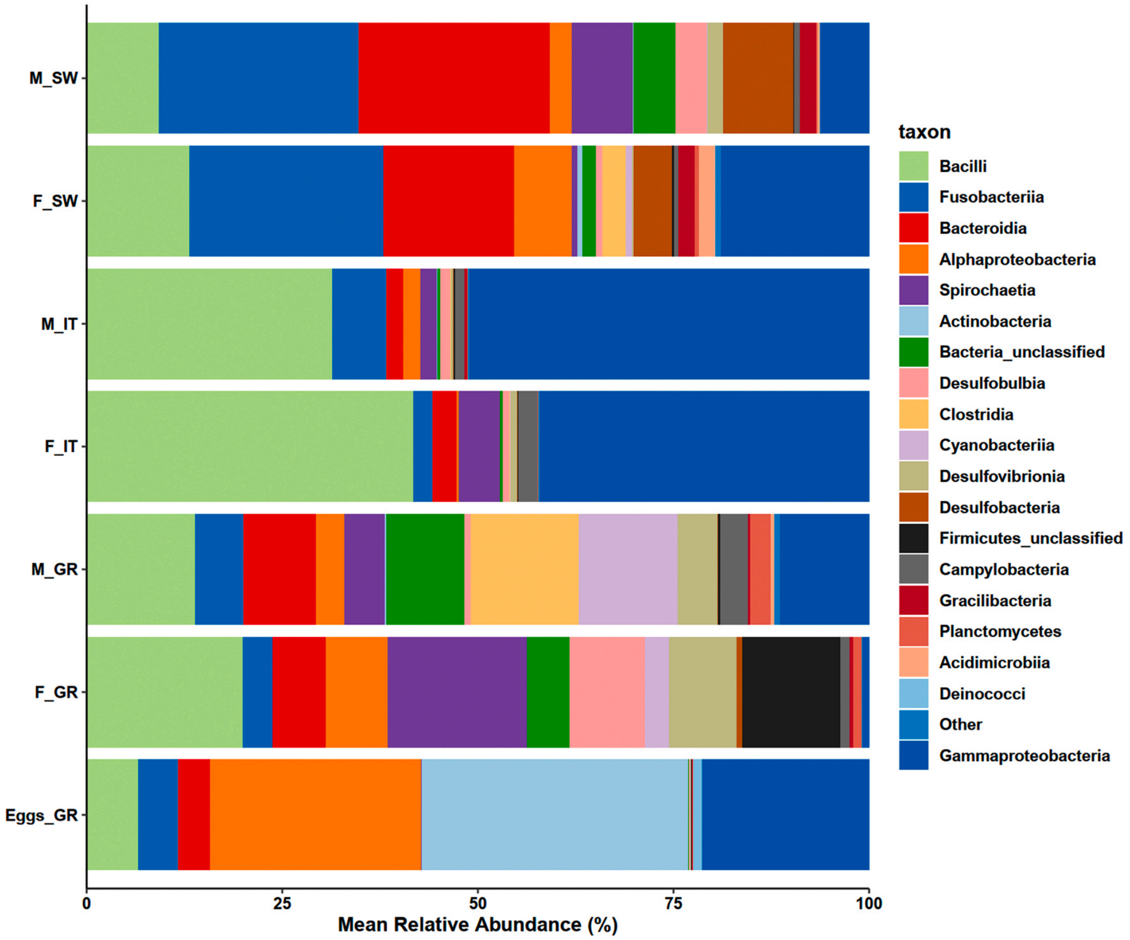

Fig. 2. Relative abundance of class-level operational taxonomic units in the female (F) and male (M) *Nephrops norvegicus* gut from Greece (GR), Italy (IT) and Sweden (SW).

Table 1

Midgut bacterial operational taxonomic units (OTUs) from three *Nephrops norvegicus* populations. Sequence reads= 33,164; N = 7 for male and female individuals, N = 5 for eggs.

		Average carapace length (mm) (coefficient of variation)	Average total body weight (g) (coefficient of variation)	No. of OTUs (average, coefficient of variation)	No. of OTUs with ≥ 80 % relative abundance	Most dominant OTU (% relative abundance)
Greece	Eggs	-	-	180 (86, 4.2 %)	17	OTU−0020 (18.5 %) *Cutibacterium*
	Females	46.0 (11.6 %)	74.0 (30.9 %)	417 (128, 24.1 %)	11	OTU−0004 (17.8 %) *Oceanispirochaeta*
	Males	46.7 (14.6 %)	77.0 (42.0 %)	1290 (335, 64.2 %)	20	OTU−0018 (8.4 %) *Cyanobium*
Italy	Females	34.9 (4.5 %)	32.4 (10.3 %)	434 (136, 24.0 %)	7	OTU−0002 (20.2 %) *Photobacterium*
	Males	36.9 (4.0 %)	36.3 (13.9 %)	489 (153, 30,4 %)	10	OTU−0002 (20.3 %) *Photobacterium*
Sweden	Females	45.0 (0.0 %)	64.6 (2.5 %)	931 (252, 63.0 %)	14	OTU−0001 (24.5 %) *Psychrilyobacter*
	Male	45.0 (0.0 %)	67.9 (1.6 %)	527 (161, 36,7 %)	11	OTU−0001 (25.5 %) *Psychrilyobacter*

Table 2

Permutational analysis of variance (PERMANOVA) of eggs, male and female individuals of *Nephrops norvegicus* gut bacterial communities from Greece (GRE), Italy (ITA) and Sweden (SWE). * : $p < 0.05$, * *: $p < 0.002$, * ** : $p < 0.01$.

	Eggs-Females	Eggs-Males	Female-Males		Females	Males
GRE	p = 0.027 * F= 3.162	p = 0.046 * F= 2.190	p = 0.897 F= 1.565	GRE-ITA	p = 0.032 * F= 2.916	p = 0.580 F= 1.579
ITA	X	X	p = 1.000 F= 0.616	ITA-SWE	p = 0.027 * F= 2.981	p = 0.021 * F= 2.668
SWE	X	X	p = 0.960 F= 1.668	GRE-SWE	p = 0.008 * ** F= 3.611	p = 0.015 * F= 3.438

populations. The population from Sweden had 5/10 most important OTUs occurring exclusively in these samples, while the populations from Italy and Greece had only 2/8 and 1/9, respectively. Regression analysis of the most important OTUs abundances vs. their total body weight, showed two cases of statistically significant negative regressions (OTU-0002, −0005) and five cases of positive regressions (OTU-0004, −0010, −0011, −0013, −0015) (Table 3). There were no “most important” OTUs between the eggs and females or males in the Greek population (Figure S4). In each population, the females:males ratios of abundant OTUs, i.e. relative abundance of ≥ 1 % in each population, which exhibited high (>10) or low (<0.1) values, revealed that various and no overlapping between females and males OTUs contributed differentially to these bacterial communities (Fig. 4). Each population had a different set of OTUs with 0.1 <ratio> 10 for all OTUs (Figure S5). The population from Italy had the lowest number of OTUs (8) with females:males ratio > 10 and the highest (74) in the population from Sweden. The population from Sweden showed the lowest numbers of OTUs (14) with ratios < 0.1 while the highest number of this ratio occurred in the population from Greece (Figure S5).

4. Discussion

In our study, the gut bacterial communities in three different *N. norvegicus* populations including both male and female samples, were analysed to detect potential differences between geographic regions and sexes. As the exact age/developmental stage of the collected animals is difficult to determine safely, we used the total body weight and carapace length as indicators of growth, which can be related to some of the occurred OTUs.

Minor differences were observed between male and female individuals in the Swedish and Italian populations, while differences observed between male and female gut samples from Greece at both the class and OTU levels might be associated with females that were carrying eggs and that thus were probably consuming different food sources. In particular, the influence of available feed on the gut microbiota of *N. norvegicus* has been investigated and confirmed in previous studies (Meziti et al., 2012, 2010).

The overall dominant OTUs detected in this study suggest that more algae-related feeding resources exist for all populations apart from the Italian polulation. More specifically the genus *Psychrilyobacter*, which dominates the Swedish population, is a Fusobacteriota bacterium that is very commonly detected in association with marine animals such as oysters (Fernandez-Piquer et al., 2012), snails (Aronson et al., 2016), mussels (Santibáñez et al., 2022) and crabs (Zhang et al., 2017), while it also exhibits free-living lifestyles and is a part of the rare biosphere (Liu et al., 2023; Yadav et al., 2021). A recent study combining cultivation-dependent and cultivation-independent methods, revealed several functions of a *Psychrilyobacter* isolate abalone where this specific genus is thrived. Among others, the isolate could utilize monosaccharides and disaccharides but not polysaccharides, implying that it is possibly involved in later fermentation steps but not in initial food degradation (Liu et al., 2023). The authors reported that *Psychrilyobacter* is a versatile fermenter that, in collaboration with other bacteria, is very important in the digestion of the algae consumed by the host. However, *N. norvegicus* does not feed on algae although remains of plants have been found in its stomach (Cristo and Cartes, 1998). Previous studies on seasonal changes in the *N. norvegicus* gut microbiome have suggested that temporal changes in the water column leading to increased algal material concentrations in sediment might increase the presence of alginolytic communities in the *N. norvegicus* gut microbiome (Meziti et al., 2010).

Populations from Greece are characterized by sexual variability, leading to the prevalence of a conspicuous Spirochaetaceae representative in females. The genus *Oceanispirochaeta,* observed in this study, contains three fully described species that are obligately anaerobic sediment chemoorganotrophs and rely mainly on mono- and disaccharide fermentation (Dubinina et al., 2020; Subhash and Lee, 2017). Although the importance of *Spirochaeta* in the termite gut has been well studied (Breznak and Leadbetter, 2006), there is not much information regarding their contribution to marine animals. Recently, spirochaetes

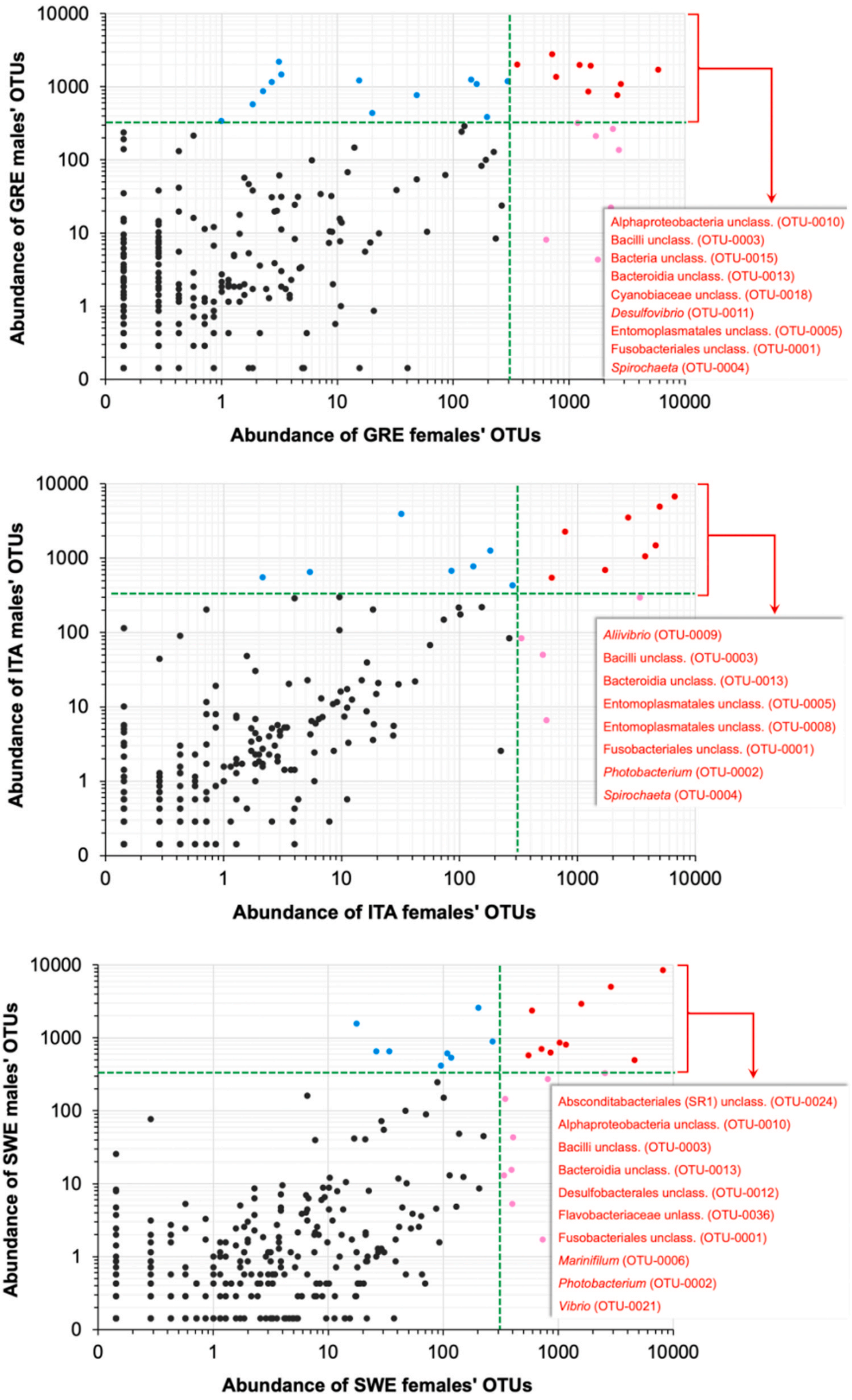

Fig. 3. Most important (relative abundance ≥1 % in both male and female individuals) bacterial operational taxonomic units (OTUs) of the *Nephrops norvegicus* gut from Greece (GRE), Italy (ITA) and Sweden (SWE). Red dots: bacterial operational taxonomic units (OTUs); blue and pink dots: important OTUs, i.e. ≥ 1 % relative abundance in males and females only, respectively.

associated with a marine sponge were reported to mediate terpene production for possible protection of their host against oxidative stress (Waterworth et al., 2024) and were also speculated to be involved in nitrite metabolism and the degradation of complex sugars in the gut of the mudshrimp *Gilvossius tyrrhenus* (formerly *Pestarella tyrrhena*) (Demiri et al., 2009).

The genus *Photobacterium* is commonly detected in the gut microbiomes of *N. norvegicus* and other crustaceans (Foysal, 2023; Jiang et al.,

Table 3

Regression of the total body weight vs. the abundance of each of the most important operational taxonomic units (OTU) from three *Nephrops norvegicus* populations. N = 42; negative and positive regressions are in red and blue letters, respectively; * : $p < 0.05$; G: Greece, I: Italy, S: Sweden.

OTU	Bacterial taxon	Closest (≥98 %) relative in NCBI (GenBank accession no.)	p	r
0001 (G/I/S)	*Psychrilyobacter*	Uncultured bacterium clone SS1_B_01_74 (EU050918)	0.165	0.218
0002 (I/S)	*Photobacterium*	*Photobacterium phosphoreum* (AB179540)	0.031 *	−0.333
0003 (G/I/S)	Mycoplasmataceae (Ca. Hepatoplasma)	Uncult. bacterium from *N. norvegicus* gut (JN092163)	0.219	−0.194
0004 (G/I)	*Oceanispirochaeta*		0.001 *	0.487
0005 (G/I)	Mycoplasmataceae (Ca. Hepatoplasma)	Uncult. bacterium from *N. norvegicus* gut (JN092262)	0.016 *	−0.368
0006 (S)	*Ancyomarina*	Uncultured Bacteroidetes bacterium clone FII-OX025 (JQ579674)	0.131	0.237
0008 (I)	Mycoplasmataceae (Ca. Hepatoplasma)	Uncult. bacterium from *N. norvegicus* gut (JN092255)	0.089	−0.266
0009 (I)	*Aliivibrio*	*Aliivibrio wodanis* (LR721750)	0.121	−0.243
0010 (G/S)	*Kordiimonas*	Uncult. Alphaproteobacteria from *N. norvegicus* gut (JN092256)	0.040 *	0.319
0011 (G)	*Desulfovibrio*		0.001 *	0.503
0012 (S)	Desulfobacteraceae unclass.	Uncult. Desulfobacterales from *N. norvegicus* gut (GQ866071)	0.273	0.173
0013 (G/I/S)	*Carboxylicivirga*	Uncult. Bacteroidia from mud crab *(Scylla paramamosain)* gut (HE610318)	0.033 *	0.330
0015 (G)	Bacteria		0.015 *	0.373
0018 (G)	Cyanobium	Uncultured bacterium clone 2CE2-5m−91 (GU062169)	0.927	0.015
0021 (S)	*Vibrio*	Uncult. bacterium from *N. norvegicus* gut (JN092213)	0.125	0.240
0024 (S)	*Candidatus* Absconditabacteriales		0.470	0.115
0036 (S)	Flavobacteriaceae unclass.	Uncultured bacterium clone EzyYyy31 (KX172210)	0.464	0.116

2023; Meziti et al., 2012; Moi et al., 2017). This genus can produce several beneficial compounds such as polyunsaturated fatty acids, lipases, esterases and antimicrobial compounds and is also a good candidate for probiotic use (Jiang et al., 2023; Le Doujet et al., 2019). In our study, this genus was dominant in the Italian population and important for both the Swedish and Italian populations. Its negative correlation with size may be related to its prevalence in younger populations. Notably, in an older study examining the *N. norvegicus* gut microbiota in reared populations receiving different feeds, *Photobacterium* was the most abundant genus in mussel-fed individuals and appeared to be a potential probiotic (Meziti et al., 2012).

Finally, the conspicuous Mycoplasmataceae (*ca.* Hepatoplasma) representatives were also prevalent in this study, similar to previous studies on *N. norvegicus* as well as other crustacean gut microbiomes (Meziti et al., 2012). These bacteria were initially, detected in the midgut glands (hepatopancreas) of the terrestrial isopod *Porcellio scaber* (Wang et al., 2004) and have been shown to benefit their hosts under low-nutrient conditions (Fraune and Zimmer, 2008). Today it seems that this bacterial taxon is frequently abundant in various decapods (Foysal, 2023), such as in the hepatopancreas of the velvet crab *Necora puber* (Martin et al., 2024) and in the gut of deep-sea amphipods (Cheng et al., 2019); it has also been identified in juvenile (Sun et al., 2020) and adult individuals (An et al., 2024) of the mitten crab *Eriocher sinensis*, juvenile Caribbean spiny lobsters *Panulirus argus* (Zamora-Briseño et al., 2020), the mud crab *Scylla paramamosain* (Jiang et al., 2023) and the vent shrimp *Rimicaris exoculata* (Aubé et al., 2022). Genome sequencing of ca. Hepatoplasma (Collingro et al., 2015) as well as Metagenome Assembled Genomes (MAGs) sequencing (Aubé et al., 2022), revealed that typical Mycoplasmataceae had a reduced genome size, with the majority of energy-producing pathways missing, with the exception of glycolysis. Similarly, the majority of nucleotide and amino acid biosynthesis pathways are not present in the genomes, suggesting that ca. Hepatoplasma mainly relies mainly on its host or syntrophic bacteria for its growth (Aubé et al., 2022).

Overall, the majority of the dominant and ‘important’ OTUs in our dataset belonged to groups and genera that had been previously detected in *N. norvegicus* or other crustaceans gut microbiome studies (Meziti et al., 2012; Meziti et al., 2010; Aubé et al., 2022; Jiang et al., 2023). Most importantly, the closest relatives detected, for the majority of the ‘important OTUs’, were phylotypes from previous *N. norvegicus* gut microbiome studies mainly performed in Greece more than one decade ago implying the presence of a core gut *N. norvegicus* microbiome regardless of geographical boundaries. Similar findings were reported for the mud crab *Scylla paramosain,* where ca. Hepatoplasma, *Vibrio, Photobacterium, Carboxylicivirga* were identified as core gut microbiota genera from different coastal regions in southern China (Jiang et al., 2023).

To date, no known interaction between the *N. norvegicus* sex and its gut microbiota exists. However, it is known that some vertebrate steroids (e.g. oestradiol, progesterone and testosterone) influence and are dependent on the developmental stage and reproduction of the *H. americanus* and *N. norvegicus* lobsters; these steroids are most likely species-specific (Burns et al., 1984; Chang, 1997; Fairs et al., 1989). Bacteria seem to play a role in regulating the levels of steroid hormone in mammals. For example, the activity of the β-glucuronidase produced by gut bacteria, such as *Bacteroides* and *Clostridium,* regulates the levels of active oestrogen by breaking glycosidic bonds between glucuronic acid and oestrogen (Cotton et al., 2023). β-glucuronidases are widespread among several bacterial taxa (Lombard et al., 2013; Wardman et al., 2022), some of which are among the most abundant found in this study (e.g. Clostridia, Sphingomonadaceae, Spirochaetia). Bacteria also seem to be also involved in the degradation or the production/reactivation of testosterone since changes in the gut microbiota changes can be accompanied by changes in testosterone levels (Cotton et al., 2023). Other invertebrates, such as cephalopods, have an accessory nidamental gland that is known to harbour specific microbiota for assisting the defence of their females against pathogens and fouling organisms (Vijayan et al., 2024). In addition, our current dataset cannot provide any potential metabolic features of the investigated microbial communities as several of the most important OTUs cannot be securely affiliated with any of the known bacterial taxa (see Table S2). These yet-to-be cultivated Bacteria, which hinder the full characterization of their ecological demands, may prevent us from having a more complete picture of the life cycle of this animal as well as its commercial cultivation.

In conclusion, this study revealed that in three geographically distant populations of the Norway lobster *N. norvegicus*, from the Mediterranean and North Seas, the gut microbiota was similar between male and female individuals. Geographic location, however, seemed to distinguish the gut microbiota for each sex. In addition, the eggs had a very distinct

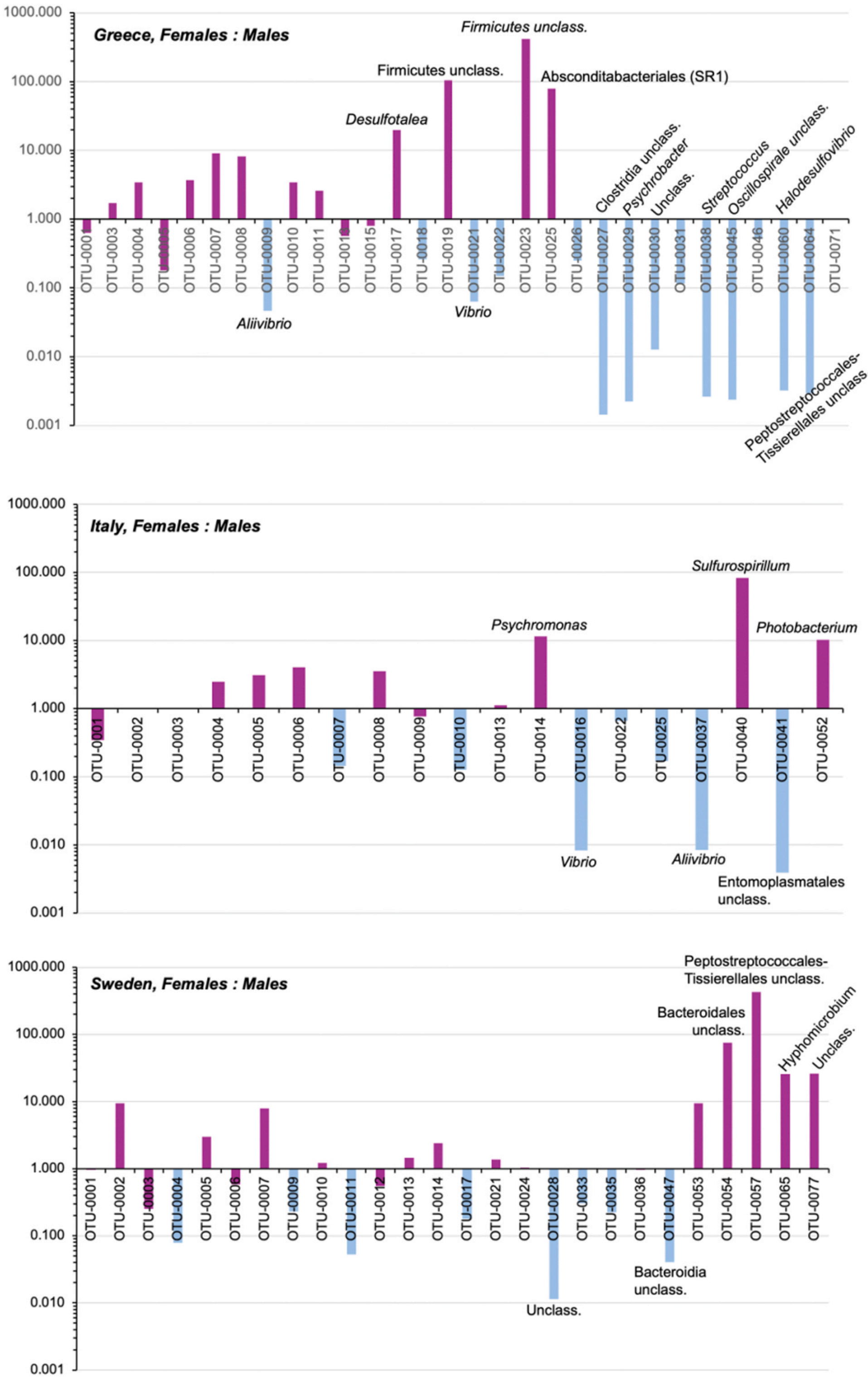

Fig. 4. Ratio of females:males bacterial operational taxonomic units (OTUs) with ≥ 1 % relative abundance in the *Nephrops norvegicus* gut from three populations. Only closest relatives of OTUs with $0.1 \leq \text{ratio} \geq 10$ is shown. Blue bars indicate OTUs occurrence only in males.

microbiota from the adult gut microbiota. Notably, among the dominant bacteria in the gut microbiota was a *ca*. Hepatoplasma representative, which has been reported in a previous *N. norvegicus* gut microbiome study from a different location. The metabolic repertoire of this likely lobster-specific microorganism along with those of other important ones identified in this study, can be a meaningful targets of future isolation efforts and/or meta'omics studies.

CRediT authorship contribution statement

Konstantinos Kormas: Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alexandra Meziti:** Writing – review & editing, Validation, Methodology, Investigation. **Elena Mente:** Writing – review & editing, Investigation. **Gioacchino Bono:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Michele Casini:** Writing – review & editing, Supervision, Methodology. **Eleni Nikouli:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Polina Rusanova:** Writing – review & editing, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2025.100396.

Data availability

Sequencing data are available in NCBI (see manuscript)

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