

Figure S1. Rarefaction curve of homogenate and plated clam samples reads obtained by 16S sequencing. OM: Homogenate clam samples; MA 22 °C: clam samples plated on marine agar and incubated at 22 °C; TCBS 22 °C: clam samples plated on Thiosulfate-citrate-bile salts-sucrose agar and incubated at 22 °C; CV 22 °C: clam samples plated on CHROMagar Vibrio media and incubated at 22 °C.

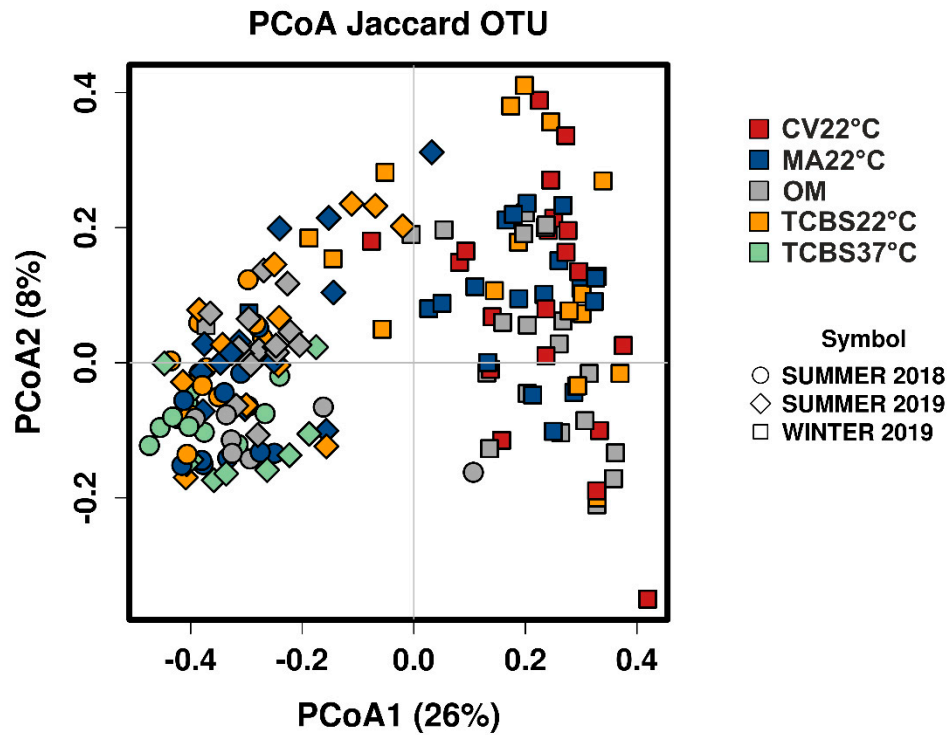


Figure S2. *recA-pyrH* amplicon-based microbial principal coordinate analysis (PCoA) representation of homogenate and plated clam samples according to Jaccard index. In PCoA representation, the season factor corresponding to the collection time of clam samples is also reported. OM: Homogenate clam samples; MA 22 °C: clam samples plated on marine agar and incubated at 22 °C; TCBS 22 °C: clam samples plated on thiosulfate-citrate-bile salts-sucrose agar and incubated at 22 °C; CV 22 °C: clam samples plated on CHROMagar Vibrio media and incubated at 22 °C.

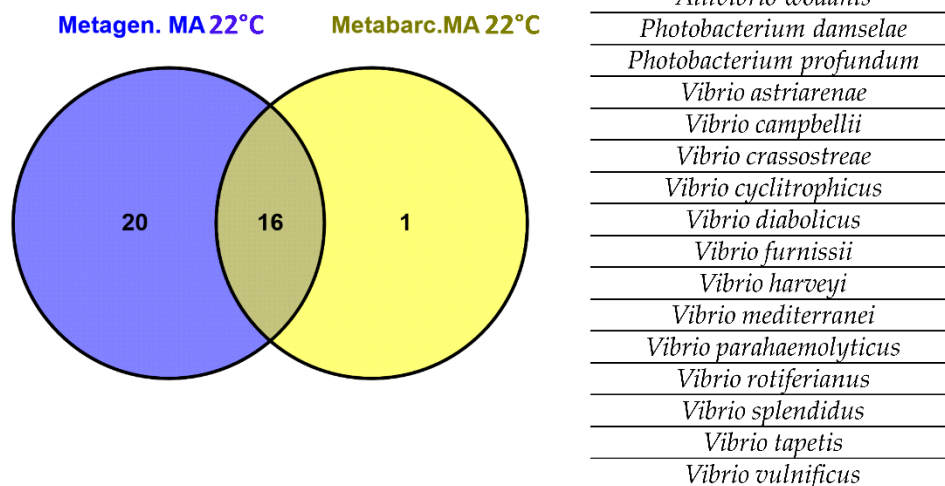


Figure S3. Venn diagram showing the number of species identified in clam samples plated in MA medium and incubated at 22 °C, according to shotgun metagenomics and *recA-pyrH* metabarcoding. The table reports the details of the 16 species shared between the two methods. MA 22 °C: clam samples plated on marine agar media and incubated at 22 °C.

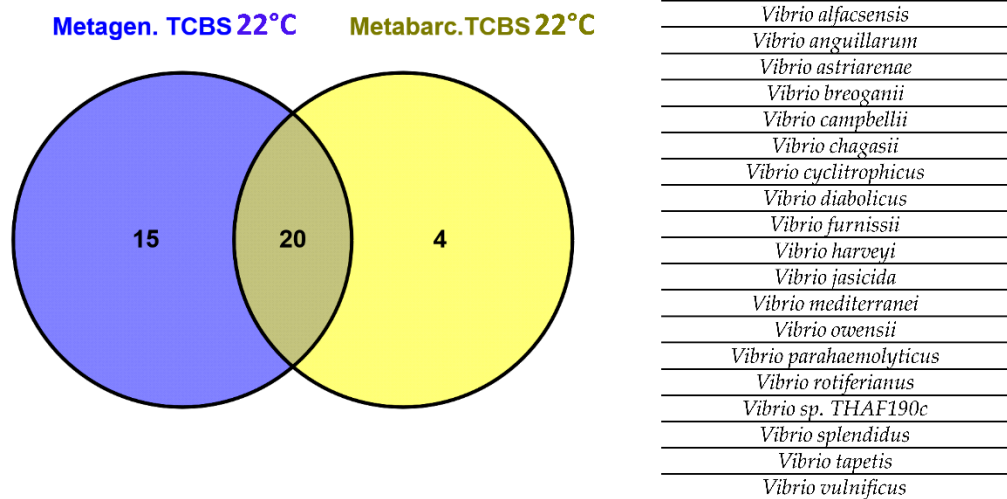


Figure S4. Venn diagram showing the number of species identified in clam samples plated in TCBS medium and incubated at 22° C, according to shotgun metagenomics and *recA-pyrH* metabarcoding. TCBS 22 °C: clam samples plated on thiosulfate-citrate-bile salts-sucrose agar and incubated at 22 °C.

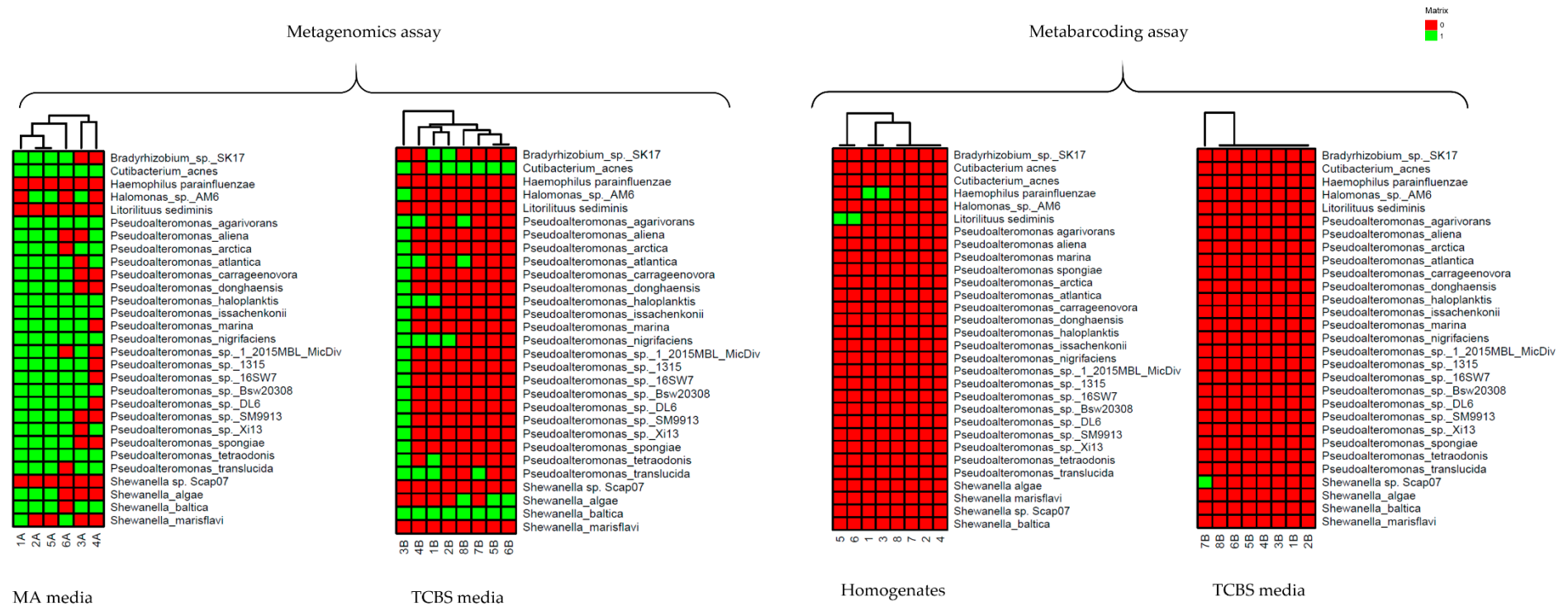


Figure S5. Heat map showing the presence (green) and absence (red) of marine bacteria, *Vibrio* species ex-cluded, found in clam samples according to metagenomics and *recA-pyrH* metabarcoding.

Table S1. Summary of the total 54 clam samples collected during summer and winter seasons. Samples are subdivided according to the culture-independent and -dependent methods. Clam samples were plated on the following: A: MA22 °C, Marine agar incubated at 22 °C; B: TCBS 22 °C, Thiosulfate-citrate-bile salts-sucrose agar incubated at 22 °C; C: CV 22 °C, CHROMagar Vibrio media incubated at 22 °C; D: TCBS 37 °C, Thiosulfate-citrate-bile salts-sucrose agar incubated at 37 °C. Sampling season: Summer; Winter. Sampling sites: CH: Chioggia; CO: Colmata; GO: Goro; MA: Marano; PM: Porto Marghera; SC: Scardovari. 1: Pre-depuration samples; 2: Post-depuration samples.

Sites	Season	Depuration	CULTURE- INDEPENDENT		CULTURE- DEPENDENT				
			Homogenates	MA22 °C		TCBS 22 °C		TCBS 37°C	CV22 °C
				Metagen.	Metabarc.	Metagen.	Metabarc.	Metabarc.	Metabarc.
CH	Summer	1	1	1A	1A	1B	1B	1D	
CH	Summer	2	2	2A	2A	2B	2B	2D	
CH	Winter	1	3	*	3A	3B	3B		3C
CH	Winter	2	4	4A	4A	4B	4B		4C
PM	Summer	1	5	5A	5A	5B	5B	5D	
PM	Summer	2	6	6A	6A	6B	6B	6D	
PM	Winter	1	7	*	7A	7B	7B		7C
PM	Winter	2	8	*	8A	8B	8B		8C
SC	Summer	1			9A		9B	9D	
SC	Summer	2			10A		10B	10D	
CO	Summer	1	11		11A		11B	11D	
CO	Summer	2	12		12A		12B	12D	
GO	Summer	1			13A		13B	13D	
GO	Summer	2			14A		14B	14D	
MA	Summer	1	15		15A		15B	15D	
MA	Summer	2	16		16A		16B	16D	
CH	Winter	1	17		17A		17B		17C
CH	Winter	2	18		18A		18B		18C
CH	Winter	1	19		19A		19B		19C
CH	Winter	2	20		20A		20B		20C
CH	Winter	1	21		21A		21B		21C
CH	Winter	2	22		22A		22B		22C

PM	Winter	1	23	23A	23B	23C
PM	Winter	2	24	24A	24B	24C
SC	Winter	1	25	25A	25B	25C
SC	Winter	2	26	26A	26B	26C
SC	Winter	1	27	27A	27B	27C
SC	Winter	2	28	28A	28B	28C
CO	Winter	1	29	29A	29B	29C
CO	Winter	2	30	30A	30B	30C
CO	Winter	1	31	31A	31B	31C
CO	Winter	2	32	32A	32B	32C
GO	Winter	1	33	33A	33B	33C
GO	Winter	2	34	34A	34B	34C
GO	Winter	1	35	35A	35B	35C
GO	Winter	2	36	36A	36B	36C
MA	Winter	1	37	37A	37B	37C
MA	Winter	2	38	38A	38B	38C
MA	Winter	1	39	39A	39B	39C
MA	Winter	2	40	40A	40B	40C
CH	Summer	1	41	41A	41B	41D
CH	Summer	2	42	42A	42B	/
CH	Summer	1	43	43A	43B	43D
CH	Summer	2	44	44A	44B	44D
PM	Summer	1	45	45A	45B	45D
PM	Summer	2	46	46A	46B	46D
SC	Summer	1	47	47A	/	/
SC	Summer	2	48	48A	48B	48D
CO	Summer	1	49	49A	49B	49D
CO	Summer	2	50	50A	50B	50D
GO	Summer	1	51	51A	/	/
GO	Summer	2	52	52A	52B	52D
MA	Summer	1	53	53A	53B	53D

MA	Summer	2	54	54A	54B	/
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* Metagenomics samples not included in the study because of the low quality reads

/ Plated clam samples not included on the analysis because the number of grown colonies was below 10

Metagen.: Metagenomics

Metabarc.: Metabarcoding

Table S2. Fleiss' kappa agreement of *Vibrio* species detection in homogenate and clam samples plated and incubated at 22 °C on MA and TCBS media according to *recA-pyrH* metabarcoding. The *Vibrio* species with a significant statistical Fleiss' kappa are shown in bold. Fleiss' kappa values agreement interpretation: $k < 0$ —poor; $k = 0.01–0.20$ —slight; $k = 0.21–0.40$ —fair; $k = 0.41–0.60$ —moderate; $k = 0.61–0.80$ —substantial; $k = 0.81–1$ —almost perfect

Species	Fleiss 'kappa	Agreement	<i>p</i> -value
<i>Aliivibrio fischeri</i>	0.051	Slight	0.544
<i>Aliivibrio wodanis</i>	0.133	Slight	0.111
<i>Photobacterium damsela</i>	0.226	Fair	0.007
<i>Photobacterium gaetbulicola</i>/ <i>Photobacterium rosenbergii</i>	0.409	Moderate	0.001
<i>Photobacterium profundum</i>	0.493	Moderate	0.001
<i>Vibrio alfacensis</i>	0.4	Fair	0.001
<i>Vibrio alginolyticus</i>	0.052	Slight	0.534
<i>Vibrio anguillarum</i>	0.074	Slight	0.378
<i>Vibrio astriarenae</i>	0.57	Moderate	0.001
<i>Vibrio atlanticus</i>	0.114	Slight	0.171
<i>Vibrio azureus</i>	0.493	Moderate	0.001
<i>Vibrio breoganii</i>	0.181	Slight	0.029
<i>Vibrio campbellii</i>	0.14	Slight	0.09
<i>Vibrio casei</i>	-0.07	Poor	0.933
<i>Vibrio chagasii</i>	0.15	Slight	0.072
<i>Vibrio cholerae</i>	0.031	Slight	0.73
<i>Vibrio cidicii</i>	-0.07	Poor	0.933
<i>Vibrio crassostreae</i>	0.108	Slight	0.195
<i>Vibrio cyclitrophicus</i>	-0.055	Poor	0.519
<i>Vibrio diabolicus</i>	0.367	Fair	0.001
<i>Vibrio europaeus</i>	-0.014	Poor	0.843
<i>Vibrio fluvialis</i>	-0.042	Poor	0.647
<i>Vibrio furnissii</i>	0.107	Slight	0.199
<i>Vibrio harveyi</i>	0.718	Substantial	0.001
<i>Vibrio jasicida</i>	-0.036	Poor	0.666
<i>Vibrio mediterranei</i>	0.676	Substantial	0.001
<i>Vibrio nigripulchritudo</i>	-0.014	Poor	0.866
<i>Vibrio owensii</i>	0.033	Slight	0.696
<i>Vibrio panuliri</i>	0.133	Slight	0.199
<i>Vibrio parahaemolyticus</i>	0.477	Moderate	0.001
<i>Vibrio ponticus</i>	-0.021	Poor	0.966
<i>Vibrio rotiferianus</i>	0.543	Moderate	0.001
<i>Vibrio rumoiensis</i>	-0.021	Poor	0.966
<i>Vibrio scopthalmi</i>	-0.026	Poor	0.866
<i>Vibrio</i> sp. Scap24	-0.021	Poor	0.966
<i>Vibrio</i> sp. THAF100	-0.014	Poor	0.966
<i>Vibrio</i> sp. THAF190c	0.107	Slight	0.299
<i>Vibrio splendidus</i>	0.379	Fair	0.001
<i>Vibrio tapetis</i>	0.358	Fair	0.001
<i>Vibrio tubiashii</i>	0.493	Moderate	0.001

<i>Vibrio vulnificus</i>	0.454	Moderate	0.001
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Table S3. Resulting sequencing depth obtained by the shotgun metagenomics

Sample	Total raw reads
1A	14067580
1B	10467200
2A	7223358
2B	12635596
3B	11110382
4A	11772854
4B	9922952
5A	9991516
5B	4175460
6A	504008
6B	8678986
7B	11407522
8B	9820130