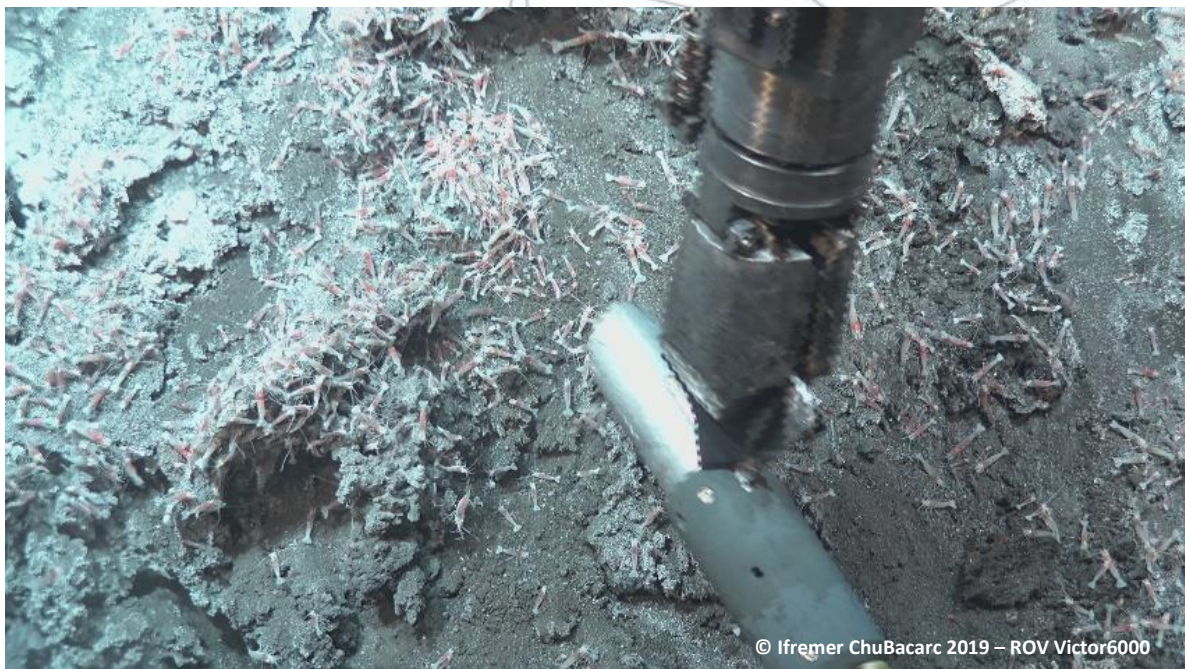




Cruise report CHUBACARC Leg2 – 2nd May – 7th June 2019

Symbiosis part – V. CUEFF-GAUCHARD



Fiche documentaire

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Résumé/ Abstract : Activities scheduled on board Symbioses of <i>Alviniconcha</i> and shrimp <i>Alvinocaridae</i> . Laurence Meslet-Cladière sampling of water and sediments to enrich fungi. Anne Lorrain for an ANR dealing with mercury, asking for gastropods, mussels and sediments. Number of samples: CHU for Chubacarc; PLXX number of dive, ASPI ou GBT or PBT is the tool and CR for shrimp, AX for Alviniconcha, CH for chimney samples	
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Valérie CUEFF-GAUCHARD / vcueff@ifremer.fr	REM/EEP/LM2E
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1 Symbioses of *Alviniconcha* and Alvinocaridae spp

1. *Alviniconcha* spp symbioses

Previous work conducted at Ifremer/EEP (post-doc of Sven Laming 2017-2018) on symbiosis in the three *Alviniconcha* species (*A. kojimai*, *A. boucheti*, and *A. strumneri*) found at vent sites in the Futuna area showed that symbiotic diversity within the host is higher than previously described. Several types of intracellular symbionts were observed in the gills, with different distribution depending on host species, localisation within the gill, and possibly vent site. In addition, we found bacterial communities living in the gut of the snails that could also contribute to their nutrition. We also investigated juveniles early after their recruitment as well as post-larval stages in order to characterize symbiont acquisition, and evaluate the possible influence of the bacterial proliferation in post-recruitment stages on subsequent species distribution.

In order to expand that work, systematic sampling of adult specimens of the three species was conducted during CHUBACARC in all assemblages collected for biodiversity studies that were also assessed for habitat chemical conditions (see reports by O. Rouxel). Upon collection boxes recovery on board, all specimens were sorted in the cold room using gloves. Species were identified based on external characteristics such as the arrangement of periostracal hairs on the shell, as well as body coloration.

Sets of three specimens per species were selected for dissection of the tissues and conditioning for symbiosis studies. For each, gills were separated in three parts: anterior, middle and posterior, and a subset of gill filaments were FISH imagery (formaldehyde fixation), electron microscopy studies (glutaraldehyde fixation) and DNA diversity for each part. The digestive whorl was conditioned either for FISH imagery, electron microscopy studies or DNA diversity. A piece of the gonad was also preserved similarly to the digestive whorl, in order to check for possible bacterial transmission to the next generation. Pieces of foot muscle were frozen for later barcoding in order to validate morphological specimen identification and for isotopic analyses. Specimens treated for symbiotic analyses were also sampled for genetic approaches conducted by Didier Jollivet, Jade Castel et al. All rests of dissections were frozen at -80°C, as well as a piece of shell for later confirmation of morphological species diagnostic.

Moreover, samples of gonads of the two *Alviniconcha* species were collected on animals also symbiosis and genetic studies. Gonads were fixed whole in a borax buffered 4% formalin solution. They will be used for histological studies aiming at assessing gametogenesis, fertilization processes, reproductive synchrony, and potential developmental mode of larvae.

Before dissection, each specimen was photographed and 6 biometric measurements were recorded: shell length (SL), shell width (SW), aperture height (distance between the tip of the columella and the suture point of the outer lip of the aperture) (ei), maximal aperture width (fh), the body whorl thickness at the level of the suture point of the outer lip (ce), and the body whorl thickness at the level of the maximal aperture width (bf) (fig 1)

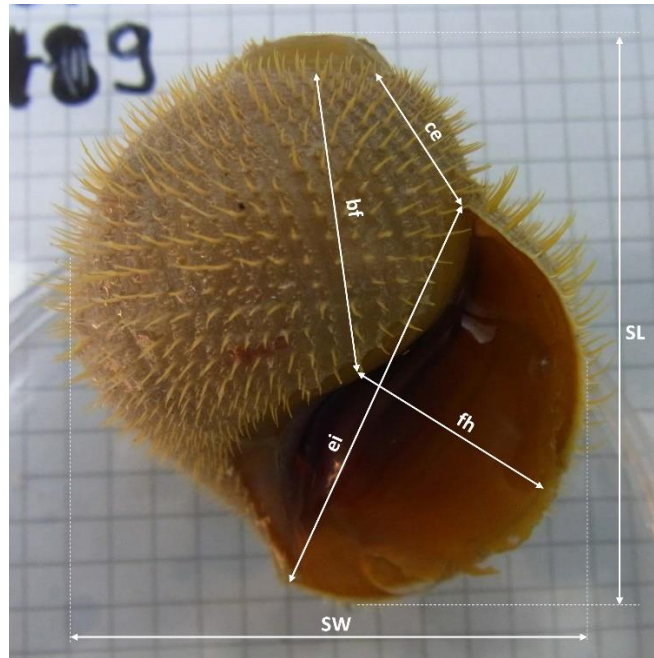


Figure 1 : biometric measurements recorded for each *Alviniconcha* specimen

A total of 113 adult specimens were treated: 27 were dissected, 14 were frozen without dissection as back-up and 72 were only used for gonads preservation in formalin. We found only two species, *A. strummeri* wasn't present neither in Manus Basin nor in Woodlark site. We have collected 65 *A. boucheti* including 12 individuals for symbiosis studies and 48 *A. kojimai* including 29 individuals for symbiosis studies. In total, gonads of 113 specimens were collected. Details of specimen dissections are presented in the table on the following pages (Table 1).

Table 1 : List of *Alviniconcha* spp adult specimens conditioned for symbiosis and life cycle studies

Date	Dive	Sampling tool	Community	site	Species	nb of individuals	Ifremer number	Roscoff number	Treatment
17/05/2019	733 (14)	GBT2	Alviniconcha 1	PacManus - Big Papi	<i>Alviniconcha boucheti</i>	1	Ax472	Alv527	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax473	Alv528	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax474	Alv529	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	19	Ax481 to Ax499	Alv531 to Alv550	gonads for histology
18/05/2019	733(14)	GBT8	Alviniconcha 2	PacManus - Fenway	<i>Alviniconcha boucheti</i>	12	Ax500 to Ax511	Alv551 to Alv562	gonads for histology
23/05/2019	736 (17)	GBT3	Alviniconcha 1	Manus - Suzette	<i>Alviniconcha kojimai</i>	1	Ax475	Alv621	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax476	Alv622	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax477	Alv623	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	7	Ax512 to Ax518		Whole individuals frozen as back-up
24/05/2019	736 (17)	GBT10	Alviniconcha 2	Manus - North Su	<i>Alviniconcha kojimai</i>	1	Ax478	Alv645	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax479	Alv646	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax480	Alv647	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	19	Ax519 to Ax537	Alv648 to Alv666	gonads for histology
25/05/2019	737 (18)	GBT10	Alviniconcha 1	Manus - South Su	<i>Alviniconcha boucheti</i>	1	Ax538	Alv667	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax539	Alv668	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax540	Alv669	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
25/05/2019	737 (18)	PBT5	chimney	Manus - South Su	<i>Alviniconcha kojimai</i>	1	Ax541	Alv691	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax542	Alv692	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax543	Alv693	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax544	Alv694	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax545	Alv695	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax546	Alv698	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
25/05/2019	737 (18)	GBT7	Alviniconcha 2	Manus - South Su	<i>Alviniconcha boucheti</i>	2	Ax547 to Ax548	Alv721 to Alv722	gonads for histology
28/05/2019	738 (19)	GBT10	Alviniconcha 1	Woodlark - La Scala	<i>Alviniconcha kojimai</i>	1	Ax549	Alv745	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax550	Alv746	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax551	Alv747	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	7	Ax555 to Ax561		Whole individuals frozen as back-up
31/05/2019	739 (20)	PBT5	chimney	Woodlark - La Scala	<i>Alviniconcha boucheti</i>	1	Ax552	Alv769	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax553	Alv770	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	1	Ax554	Alv771	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha boucheti</i>	20	Ax565 to Ax584	Alv775 to Alv795	gonads for histology
31/05/2019	739 (20)	GBT10	Alviniconcha 2	Woodlark - La Scala	<i>Alviniconcha kojimai</i>	1	Ax562	Alv772	Digestive tract, piece of gonad: DNA + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax563	Alv773	Digestive tract, piece of gonad: FISH + pieces of gills (ant/mid/post) for DNA/FISH/SEM
					<i>Alviniconcha kojimai</i>	1	Ax564	Alv774	Digestive tract, piece of gonad: SEM + pieces of gills (ant/mid/post) for DNA/FISH/SEM

2. Alvinocaridae sp symbiosis

Sampling of shrimps in suction device jars: most of the time, the shrimps were sampled long before the ROV recovery. So they were not very fit so dead. We only sampled 1 *Alvinocaris komai*, all the other sampled shrimps were *Rimicaris variabilis*. The sampled shrimps have been dissected for FISH preservation with fixation with 3% formaldehyde, for scanning electronic microscopy with fixation with glutaraldehyde or for DNA analyses with dry freezing at -80°C. As they were little, a part of them were dissected under magnifying glass when possible (more than 2,5 cm long). Otherwise, the specimens have been packed in their entirety. In total, 112 shrimps (111 *Rimicaris variabilis* and 1 *Alvinocaris komai*) were treated, 38 were dissected and we have prepared 74 whole specimens. In Manus Basin, lots of female were gravid. Details of specimen dissections are presented in the table 2.

Sampling using FISH BOX: (starting of dive 734 / 15): To be sure to have good sampling for metatranscriptomic studies, I prepared the 2 box with homemade RNA later in case there is a problem during the dive.

As the complete FISH system takes a large part of the ROV basket (Figure 2), we used it only one time during Leg 2. The hose of the slurp gun in on the right and seems quite difficult to manage using Maestro. There is a way to further improve the pipe part for suction.

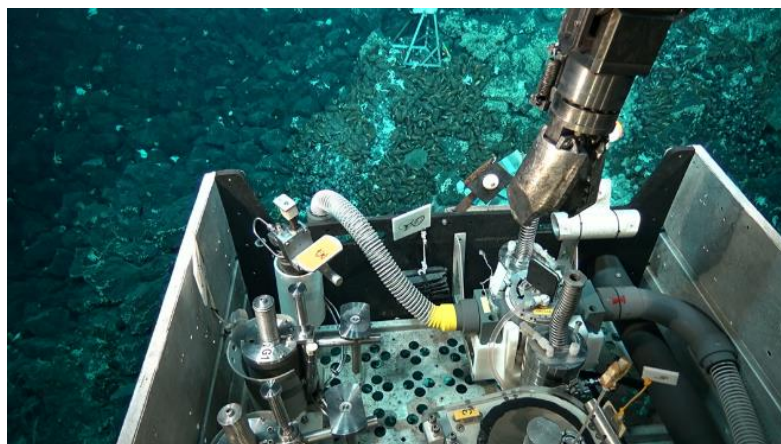


Figure 2 : FISH system inside the ROV basket

In situ, the 2 FISH boxes were correctly managed. So we sampled on 2 different sites: the FISH 1 on Big Papi site (PacManus) and the FISH2 on Roman ruins site (PacManus) (Figure 3). The pin was removed correctly but the ROV had to use the clam to close properly the box at 1700 m depth. The vacuum system worked properly without loose of charge. It only took between 15 and 30 min for each FISH box.

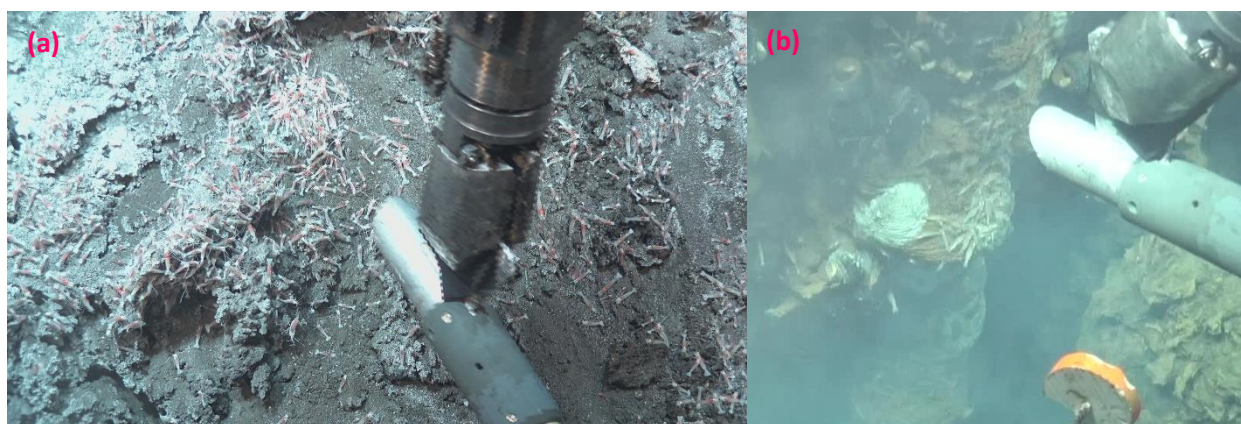


Figure 3 : suction of shrimps for FISH1 (a) and FISH2 (b)

After recovery in the lab, FISH1 contained very small shrimps. A large part were sucked inside the fixative bottles with the seawater because pipes were bigger than shrimps. The shrimps left in the bowl (14 on 45) were preserved as whole specimens either in commercial RNA later or in Trizol®.

Shrimps inside FISH2 were bigger so I dissected 12 of them and preserved 20 whole specimens either in commercial RNA later or in Trizol®. After 1 night inside RNA later at 4°C, I transferred a part of whole specimens and dissected tissues in Trizol®.

Details of sampled specimens are presented in the table 2.

2 Other sampling

1. Sampling for Anne Lorrain

Samples of *Ifremeria*, *Alviniconcha* and *Bathymodiolus* (rest of the body after genetician sampling) and sediments have been collected. Samples have been kept in plastic bags and frozen at -80°C.

When possible, I sampled on both sites Manus Basin and Woodlark (no mussel at Woodlark).

Samples:

Manus basin

CHU-PL15-GBT1-BB341 : *Bathymodiolus manusiensis*

CHU-PL15-GBT5-In523 : *Ifremeria nautilei*

CHU-PL16-CT2 : sediment

CHU-PL17-GBT3-Alv634 : *Alviniconcha kojimai*

Woodlark – La Scala

CHU-PL19-GBT10-Alv748 : *Alviniconcha kojimai*

CHU-PL20-CT8 section 0-5 cm (site 2) : sediment

2. Sampling for Laurence Meslet Cladière

During Leg2, several samples of chimney and one sample of sediment have been conditioned in penicillin vial as asked, sealed, flushed with nitrogen and kept at 4°C.

Manus Basin

CHU-PL18-PBT6-CH15 : fraction of homogenized chimney (South Su site)

Woodlark – La Scala

CHU-PL19-PBT2-CH17: fraction of homogenized chimney

CHU-PL20-CT8 section 0-5 cm: sediment

CHU-PL20-PBT2-CH19: fraction of homogenized chimney

Tableau 2: List of shrimps sampled for symbiosis

Date	Dive	Sampling tool	site	Species	nb of individuals	Ifremer number	Treatment
18/05/2019	733 (14)	ASPI6	PacManus - Solwara8	<i>Rimicaris variabilis</i>	9 whole shrimps	CR101 to CR109	4 DNA, 1 FISH, 2 SEM, 2 isotop
		ASPI7	PacManus - Solwara8	<i>Rimicaris variabilis</i>	4 dissected shrimps	CR79 to CR82	1 DNA (head part 1, digestive tract) + FISH (head part 2), 1 FISH (head part 1, digestive tract) + DNA (head), 1 DNA (head part 1, digestive tract) + SEM (head part 2), 1 SEM (head part 1, digestive tract) + DNA (head)
				<i>Rimicaris variabilis</i>	11 whole shrimps	CR83 to CR93	6 DNA, 3 FISH, 2 SEM
		ASPI8	PacManus - Solwara8	<i>Rimicaris variabilis</i>	4 dissected shrimps	CR75 to CR78	1 DNA (head part 1, digestive tract) + FISH (head part 2), 1 FISH (head part 1, digestive tract) + DNA (head), 1 DNA (head part 1, digestive tract) + SEM (head part 2), 1 SEM (head part 1, digestive tract) + DNA (head)
				<i>Rimicaris variabilis</i>	7 whole shrimps	CR94 to CR100	3 DNA, 2 FISH, 2 SEM
19/05/2019	734 (15)	FISH2	PacManus - Roman ruins	<i>Rimicaris variabilis</i>	12 dissected shrimps	CR110 to CR121	12 RNA (RNA later or Trizol)
				<i>Rimicaris variabilis</i>	20 whole shrimps	CR122 to CR141	20 RNA : 10 Trizol and 10 RNA later
19/05/2019		FISH1	PacManus - Big Papi	<i>Rimicaris variabilis</i>	14 whole shrimps	CR142 to CR155	14 RNA : 7 Trizol and 7 RNA later
		ASPI1	PacManus - Roman ruins	<i>Rimicaris variabilis</i>	7 dissected shrimps	CR156 to CR162	2 DNA (head part 1, digestive tract) + FISH (head part 2), 2 FISH (head part 1, digestive tract) + DNA (head), 2 DNA (head part 1, digestive tract) + SEM (head part 2), 1 SEM (head part 1, digestive tract) + DNA (head)
		ASPI6	PacManus - Solwara6	<i>Rimicaris variabilis</i>	12 whole shrimps	CR168 to CR179	6 DNA, 3 FISH, 3 SEM
		ASPI7	PacManus - Solwara6	<i>Rimicaris variabilis</i>	3 dissected shrimps	CR163 to CR165	1 DNA (head part 1, digestive tract) + FISH (head part 2), 1 FISH (head part 1, digestive tract) + DNA (head), 1 SEM (head part 1, digestive tract) + DNA (head)
		ASPI8	PacManus - Solwara6	<i>Rimicaris variabilis</i>	2 whole shrimps	CR166 to CR167	1 DNA, 1 SEM
24/05/2019	736 (17)	ASPI2	Manus - Suzette	<i>Rimicaris variabilis</i>	4 dissected shrimps	CR182 to CR183 and CR188 to CR189	1 DNA (head part 1, digestive tract) + FISH (head part 2), 1 FISH (head part 1, digestive tract) + DNA (head), 1 DNA (head part 1, digestive tract) + SEM (head part 2), 1 SEM (head part 1, digestive tract) + DNA (head)
				<i>Rimicaris variabilis</i>	5 whole shrimps	CR192-CR193-CR195-CR196-CR197	2 DNA, 3 FISH
		ASPI3	Manus - North Su	<i>Rimicaris variabilis</i>	2 whole shrimps	CR190 and CR194	1 DNA, 1 FISH
		ASPI4	Manus - Suzette	<i>Rimicaris variabilis</i>	6 dissected shrimps	CR180 to CR181 and CR184 to CR187	2 DNA (head part 1, digestive tract) + FISH (head part 2), 2 FISH (head part 1, digestive tract) + DNA (head), 1 DNA (head part 1, digestive tract) + SEM (head part 2), 1 SEM (head part 1, digestive tract) + DNA (head)
				<i>Rimicaris variabilis</i>	1 whole shrimp	CR191	1 DNA
25/05/2019	737 (18)	ASPI8	Manus - South Su	<i>Alvinocaris komai</i>	1 dissected shrimp	CR198	1 DNA (head part 1, digestive tract) + FISH (head part 2)
29/05/2019	738 (19)	ASPI2	Woodlark - La Scala	<i>Rimicaris variabilis</i>	9 whole shrimps	CR204 to CR212	9 DNA
		ASPI3	Woodlark - La Scala	<i>Rimicaris variabilis</i>	3 dissected shrimps	CR199 to CR201	1 DNA (head part 1, digestive tract) + FISH (head part 2), 1 FISH (head part 1, digestive tract) + DNA (head), 1 SEM (head part 1, digestive tract) + DNA (head)
				<i>Rimicaris variabilis</i>	2 whole shrimps	CR202 to CR203	2 DNA
31/05/2019	739 (20)	ASPI1	Woodlark - La Scala	<i>Rimicaris variabilis</i>	6 dissected shrimps	CR213 to CR218	2 DNA (head part 1, digestive tract) + FISH (head part 2), 2 FISH (head part 1, digestive tract) + DNA (head), 1 DNA (head part 1, digestive tract) + SEM (head part 2), 1 SEM (head part 1, digestive tract) + DNA (head)
				<i>Rimicaris variabilis</i>	14 whole shrimps	CR219 to CR232	6 DNA, 4 FISH, 4 SEM