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R/V *Shinsei Maru*/ROV *Hyper-Dolphin* Cruise Report

KS-20-2

Thermal tolerance of the hydrothermal vent nebaliid *Nebalia tagiri* inhabiting Kagoshima Bay

Kagoshima Bay

Jan. 15, 2020–Jan. 22, 2020

Joint Usage/Research Center for Atmosphere and Ocean Science
(JURCAOS)
Japan Agency for Marine-Earth Science and Technology
(JAMSTEC)

Contents

Abstract

To understand the thermal tolerance of the nebaliid crustacean *Nebalia tagiri* inhabiting hydrothermal vents in Kagoshima Bay, ROV dives were conducted in January 2020 using ROV *Hyper-Dolphin*. Four dives (dive #2101–2104) were conducted in the hydrothermal area at a depth of 200 m, but no metazoan (including nebaliid crustacean) was seen probably because of their extremely low dissolved oxygen concentrations (DO) (<0.01 ml/L). *Nebalia tagiri* distributes not only at the hydrothermal vent sites but also at tubeworm sites at a depth of 100 m in the bay. Therefore, three dives (dive #2105–2107) were conducted at the tubeworm site where the DO was much higher (DO: about 2.7–3.9 ml/L) than that of the vent sites, and we finally collected more than several thousands of the nebaliid using a suction sampler installed on ROV and bait traps deployed beside a tubeworm colony. Onboard experiments were conducted, and the maximum survival temperature of *N. tagiri* was elucidated.

1. Participants aboard (Morioka)

1-1. Research group

Chief Scientists	FUJIWARA, YOSHIHIRO
	Japan Agency for Marine-Earth Science and Technology
Scientist	TUCHIDA, SHINJI
	Japan Agency for Marine-Earth Science and Technology
Scientist	KIKUCHI, TOSHIHIKO
	Yokohama National University
Scientist	YAMANAKA, TOSHIROU
	Tokyo University of Marine Science and Technology
Scientist	YAMAKI, AYUTA
	Enoshima Aquarium
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	Tokyo University of Marine Science and Technology
Marine Tec.	MORIOKA, MIKI
	Nippon Marine Enterprises, Ltd.

1-2. Operation team of the ROV *Hyper-Dolphin*

Operation Manager	KIDO, TEPPEI
2nd ROV Operator	SAKAKIBARA, YUDAI
2nd ROV Operator	KIKUYA, SHIGERU
2nd ROV Operator	TAYAMA, YUDAI
3rd ROV Operator	YAMGUCHI, TAIKEN
3rd ROV Operator	ASANO, WATARU

1-3. Captain and crew of the R/V *Shinsei Maru*

Captain	SHISHIKURA, TAKAAKI
Chief Officer	EGASHIRA, TAKESHI
2nd Officer	YUKAWA, TOMOHIRO
3rd Officer	OKADA, MASAKI
Chief Engineer	KUROSE, WATARU
1st Engineer	TADOOKA, NAOHITO
2nd Engineer	YAMAGUCHI, KATSUTO
3rd Engineer	SUGIMOTO, SHINGO
Chief Electronics Op.	NASU, TOKINORI
2nd Electronics Op.	MATSUI, RYOSUKE
Boat Swain	HIROSAKI, KANAME
Able Seaman	NAKAE, KENJI
Able Seaman	MATSUO, YOSHIAKI
Able Seaman	UENO, SHINYA
Able Seaman	NASU, KENTA
Able Seaman	HORI, FUMIHIRO
Sailor	YOSHIMI, YUDAI

Sailor	NAGAYAMA, TOSHIKI
No.1 Oiler	YAMAGUCHI, YUKIHIRO
Oiler	WATANABE, TAKUYA
Oiler	HIDAKA, TORU
Assistant Oiler	KAWANO, MOTOHIRO
Assistant Oiler	MANNA, SHOUTA
Chief Steward	CHIKUBA, YUKIHIDE
Steward	TAKAMA, TOSHIYUKI
Steward	NOJIRI, TAKEHIRO

2. Proposal (Fujiwara)

様式1

2019 年度 研究船共同利用研究申込書

平成 30 年 7 月 27 日

研究船共同利用運営委員会 委員長 殿

研究代表者（申込者）

所 属 機 関　海洋研究開発機構
職　　名　　分野長代理
氏　　名　　藤原　義弘　　印
年　　齢　　49

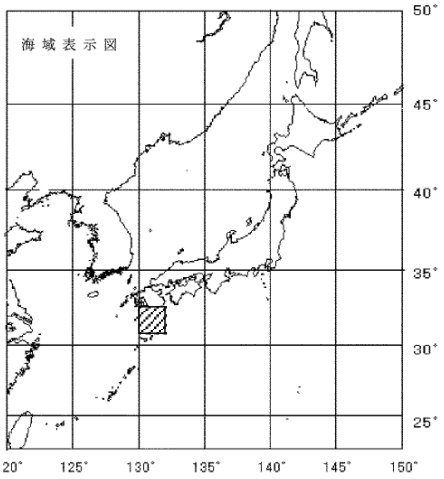
研究船を利用した研究を下記のとおり申し込みます。

研究課題	コノハエビはポンペイワームを超える？～鹿児島湾熱水噴出域産コノハエビ類の高温耐性に関する研究～

	氏　　名	所 属 機 関 ・ 職 名	研究分担内容	旅費負担	乗船・非乗船	期 間 及 び 海 域 等
研究代表者・分担者	藤原　義弘	海洋研究開発機構・分野長代理	とりまとめ	無	○	1．必要観測日数　5 日間
	土田　真二	海洋研究開発機構・技術主幹	コノハエビ生態	無	○	
	河戸　勝	海洋研究開発機構・技術主任	遺伝子解析	無	○	2．観測希望時期　9-2 月
	菊池　知彦	横浜国立大学・教授	コノハエビ分類	無	○	
	平田　琢真	横浜国立大学・大学院生 D2	コノハエビ分類	無	○	3．観測海域　鹿児島湾
	増田　殊大	東京大学大気海洋研究所・特任研究員	チムニー壁内部観察用水中顕微鏡開発	無	○	4．希望船舶（○で囲む） 新青丸 ~~よこすか~~ ~~かいれい~~
	八巻　鮎太	かごしま水族館・技術職員	コノハエビ飼育実験	無	○	
	山中　寿朗	東京海洋大学・教授	同位体解析	無	○	5．乗合航海の可否 否（ROV を使用するため）
						6．震災関連研究航海 （該当する場合は○で囲む）

＊受付年月日		＊採　　否		＊整理番号	

＊印欄は記入しないでください。

<table>
<tr><td>震災との関連</td><td colspan="2">特になし</td></tr>
<tr><td>観測希望時期等</td><td colspan="2">開発中のチムニー壁内部観察用水中顕微鏡の水中試験を次年度初旬に予定していることから 9 月以降の観測を希望するが，希望時期以外でも調査できるよう調整する．</td></tr>
<tr><td rowspan="3">使用観測設備・機器</td><td>申込者が持込む観測機器（名称・数量・重量）
チムニー壁内部観察用水中顕微鏡（ROV 搭載型）・1 式・5kg
スラープガン（ROV 搭載型）・1 式・7.8kg
多連キャニスタ（ROV 搭載型）・1 式・50kg
RINKO 式溶存酸素濃度計（ROV 搭載型）・1 式・1kg
タイムラプスカメラ（海底設置型）・1 式・100kg
現場採水器（ROV 搭載型）・1 式・30kg
カメラ付き小型グラバ（鹿児島市所有）・1 式・500kg</td><td rowspan="3">観測海域（枠で囲んでハッチをつけてください。）
（この地図からはみ出す場合は，適宜別の地図と入れ替えてもかまいません。）
 
</td></tr>
<tr><td>搭載を希望する可搬型機器（添付「利用の手引き」参照）
（大型の可搬型機器の搬入搬出には，多額の経費を必要とするため，採択後の運航計画作成に当りご相談させていただく場合があります。）

「ハイパードルフィン」</td></tr>
<tr><td>搭載を希望する共同利用観測機器（別紙 6 共同利用観測機器一覧参照）

マルチプルコアラー</td></tr>
<tr><td colspan="2"></td><td>研究代表者の連絡先
所属機関：海洋研究開発機構
職　名：分野長代理
氏　名：藤原義弘</td></tr>
</table>

●研究目的・内容

研究の目的：本研究の目的は，鹿児島湾で発見された 200℃の熱水を噴出するチムニー壁内部に多数生息するコノハエビ類の高温耐性およびその生態学的特徴を明らかにすることである．

科学的・技術的内容：本研究では以下の 5 項目を中心に実施する．①温度計測プローブを備えたチムニー壁内部観察用顕微鏡を用いて，*in situ* でコノハエビ類の生息する微小環境を明らかにするとともに生息温度を示す，②温度勾配装置を用いた船上実験により，コノハエビ類の至適生息温度を明らかにする，③船上加温実験を実施して，コノハエビ類の生息限界温度を示す，④タイムラプスカメラを用いてコノハエビ類の生息する熱水噴出チムニーを 24 時間以上撮影し，コノハエビ類の生態を記録する，⑤鹿児島湾周辺の還元環境に出現するコノハエビ類を用いて②③実験を実施し，熱水噴出チムニーに生息するコノハエビ類の特徴を明らかにする．

作業仮説：「鹿児島湾産コノハエビ類はこれまでに発見されているなかで最も高温に耐性を示す後生動物である．」

着想に至った経緯：熱水噴出域には数多くの固有種が生息する．その多くは化学合成細菌との共生関係を示し，共生細菌は熱水中に含まれる硫化水素などの還元的化学物質を利用して化学合成により有機物を生合成する．そのため，これらの生物はある程度，噴出する熱水に近づく必要があるが温度耐性の問題があり，多くの種では 3～20℃程度の水温帯に暮らしている．

真に高温耐性を持つ動物として知られるのはエラゴカイ科多毛類であり，同科の一種であるポンペイワーム *Alvinella pompejana* はチムニーの壁面に棲管を作ってその中に生息しており，80℃の高温でも生存が可能であることが報告されている．しかしながら，ポンペイワームは体長 10cm を越える比較的大型の動物であり，体の後端部が高温に暴露されている場合でも，前端部は 20℃程度の環境に鰓を大きく広げており，体全体が常時高温に曝されていることはない．

我々は 2008 年に実施された「ハイパードルフィン」潜航調査において，鹿児島湾の水深 200m 海域から採集された熱水チムニーの壁内部に小型甲殻類の 1 種であるコノハエビ類（全長約 10mm）が多数生息していることを発見した．チムニー壁は薄い場所では厚さ数 cm 以下であり，チムニー壁を挟んで内外の温度差は 190℃を超えているため，コノハエビ類はこの極度の温度勾配の中で生息していることになる．そこで本研究では，このコノハエビ類の生息温度と温度耐性を明らかにするために本調査航海を提案した．

なお，これまでの研究により本コノハエビ類については以下の結果を得ている．①形態観察および分子系統解析の結果，本種は *Nebalia* 属の新種である可能性が極めて高い（新種記載中），②バルクの炭素窒素安定同位体比およびアミノ酸窒素同位体比解析から本種は化学合成細菌に栄養のほとんどを依存している，③SEM，TEM 観察の結果，体表および体内に化学合成共生細菌の存在は確認できない，④本種の消化管はよく発達し，消化管内は様々な構造物で満たされている（バクテリアマットのグレイジングの可能性），⑤フィールド調査の結果，本種はチムニー壁内で繁殖している（抱卵個体や育房で幼生を保育中の個体を確認済み），⑥本種の総排泄孔付近には未知の寄生(?)生物が多数付着している．

●研究計画

試料採集：コノハエビ類の採集は鹿児島湾内熱水噴出域（水深 200m），同湾内サツマハオリムシサイト（水深 100m）および野間岬沖（水深 250m）で実施する．分子系統解析により，鹿児島湾内 2 サイトのコノハエビ類は同一種であり，野間岬沖のものは湾内のものとは別種であることを明らかにしている．そこで異なる水深・環境に生息する同一種と，同様の水深に生息する別種を用いて比較研究を実施する．

鹿児島湾深部においては，コノハエビ類はチムニー壁の内部に生息しているため，「ハイパードルフィン」のマニピュレータを用いてチムニーの一部を採集する（1 日：4 時間潜航×2 回）．サツマハオリムシサイトのコノハエビ類はハオリムシの棲管の間に生息しているため，ROV 搭載のスラープガンを用いて採集する（1 日：4 時間潜航×2 回）．野間岬沖の個体は海底に沈んだ鯨骨の隙間やその周辺に分布しているため，同様にスラープガンで吸引採集する（1 日：4 時間潜航×2 回）．加えてマルチプルコアラーを用いて，熱水噴出孔や湧水域といった特異点以外の堆積物中に対象とするコノハエビ類が分布するかを確認する（0.5 日：2 時間×3 地点）．なおサツマハオリムシサイトについては漁船の影響で ROV 調査が困難な場合がある．その場合，同サイトでの使用実績があるカメラ付き小型グラバによる生物採集を行う．

環境計測：各地点において，小型計測装置を用いて生息環境の水温，溶存酸素濃度などの物理化学因子を計測する（試料採集と同時に実施）．測定装置には小型メモリ DO 計 RINKO I（JFE アドバンテック製）等を用いる．また，現在開発中の温度プローブを備えたチムニー壁内部観察用水中顕微鏡を用いてチムニー壁の内部を直接観察するとともに温度計測を実施する（1 日：4 時間潜航×2 回）．加えて，現場採水器を用いてコノハエビ類が生息する環境の海水を採水し（試料採集と同時に実施），船上実験に備える．

タイムラプス観測：コノハエビ類が生息するチムニー脇に「ハイパードルフィン」を用いてタイムラプスカメラを設置し（0.5 日：4 時間潜航×1 回），24 時間以上のタイムラプス撮影および環境計測（計測装置類はカメラシステムに搭載）を実施する．タイムラプスカメラは音響切り離し信号により自己浮上するため，回収のための潜航は必要ない．

温度勾配実験(船上)：温度勾配装置（0～100℃）に各ロット 20 個体以上のコノハエビ類を加え，コノハエビ類の行動を動画にて記録する．また同時にサーモグラフィーカメラを用いて，温度勾配装置の表面温度を記録する．これらの情報を統合し，コノハエビ類の至適生息温度を明らかにする．実験には可能な限り現場採水した海水を用いる．

高温耐性実験(船上)：任意に温度コントロール可能な小型恒温槽を用いて，15 分暴露時のコノハエビ類（$n > 20$）の

半数致死温度を明らかにする．実験には可能な限り現場採水した海水を用いる．

●他航海への応募　最近の航海採択・不採択状況

他航海への応募：

- 「深海底開発において既存技術の適用に死角は無いか？　セメントコンクリート構造物の大深度における材料学的および構造学的安定性の検証」（山中，代表）（本公募との関連なし）
- 「ホウキガニ類およびタギリカクレエビ類の幼生分散」（土田，分担）（本公募との関連なし）

最近の航海採択・不採択状況：

- 平成 26 年度 東北海洋生態系調査研究船（学術研究船）「新青丸」 共同利用研究申込「東北津波域における海洋生態系の変動メカニズムの研究」（採択）

●研究業績（2016 年以降，査読付き）

Aguzzi J, Fanelli E, ard TC, Schirone i, Leo FCD, Doya C, Kawato M, Miyazaki M, Furushima Y, Costa C, Fujiwara Y (2018) Faunal activity rhythms influencing early community succession of an implanted whale carcass offshore Sagami Bay, Japan. Scientific Reports 8. doi:10.1038/s41598-018-29431-5

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3. Dive survey results

3-1. Dive list

Dive # Date	Main purposes	Site	Landing Leaving bottom	Latitude (N)	Longitude (E)	Depth (m)
#2101	Bio, water and sediment sampling	SES Site	8:44	31-39.7499N	130-46.2981E	199
2020/1/18			13:08	31-39.7444N	130-46.2944E	201
#2102	Deployment of camera system	SES Site	15:30	31-39.7412N	130-46.2867E	200
2020/1/18			16:08	31-39.7463N	130-46.2929E	200
#2103	Recovery of camera system	SES Site	8:35	31-39.7434N	130-46.2813E	200
2020/1/19			8:50	31-39.7448N	130-46.2949E	200
#2104	Bio, water sampling and collecting sediments from chimney	WHV Site	11:34	31-40.0700N	130-45.7030E	199
2020/1/19			16:06	31-40.0720N	130-45.6839E	198
#2105	Bio and water sampling	Tubeworm Site	8:37	31-39.7619N	130-48.0719E	108
2020/1/20			10:51	31-39.7562N	130-48.0648E	102
#2106	Release of bait trap and collecting bio, water samples	Tubeworm Site	13:36	31-39.7603N	130-48.0716E	108
2020/1/20			16:03	31-39.7560N	130-48.0633E	103
#2107	Recovery of bait trap and collecting bio, water samples	Tubeworm Site	8:44	31-39.7624N	130-48.0666E	105
2020/1/21			13:00	31-39.7580N	130-48.0646E	103

3-2. Dive report

Dive Report HD#2101

Date: January 18, 2020

Site: Wakamiko Caldera, Kagoshima Bay, ***Depth:*** 201 m

Landing (Lat., Lon., Time, Depth): 31-39.7499N 130-46.2981E, 8:44, 199 m

Leaving (Lat., Lon., Time, Depth): 31-39.7444N 130-46.2944E, 13:08, 201 m

Pilot: TAYAMA, Yudai ***Co-Pilot****:* KIKUYA, Shigeru

Observer: FUJIWARA, Yoshihiro

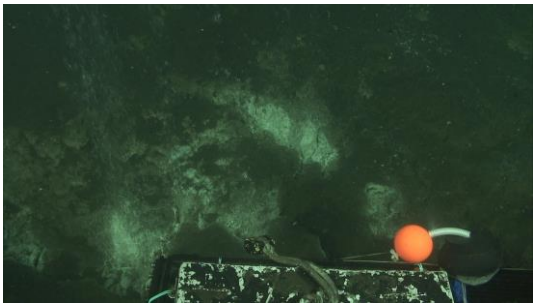
Theme: Study on thermotolerance of *Nebalia tagiri* living inside hydrothermal-chimney wall.

Purpose of dive:

1. *In situ* observation of *Nebalia tagiri* living inside chimney wall
2. Sampling of *Nebalia tagiri*
3. Sampling of hydrothermal vent fluid and ambient seawater

Dive Summary

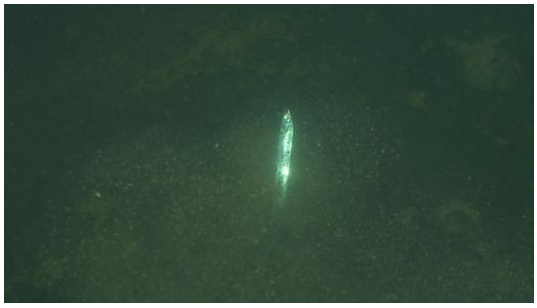
HD#2101 dive was conducted to observe and collect the hydrothermal vent nebaliid *Nebalia tagiri*. We landed beside a "Tagiri (bubbling)" site and observed inside sediments using "Vent Scope". We clearly observed bacterial mats on and in sediments using the scope, but any benthic organisms were not found. Sediments and rocks were collected using manipulators and a suction sampler installed on ROV. Emitting water from the bottom of Tagiri site and the ambient water were collected using a vacuum water sampler and two Niskin bottles, respectively. Two individuals of fresh but dead hairtails (Tachi-uo) were observed on the bottom. Upon recovery, sediments and rocks were sorted, but there was no visible metazoans.



Tagiri (bubbling) site

Vent scope observation

Bacterial mat on sediment



Dead hairtail

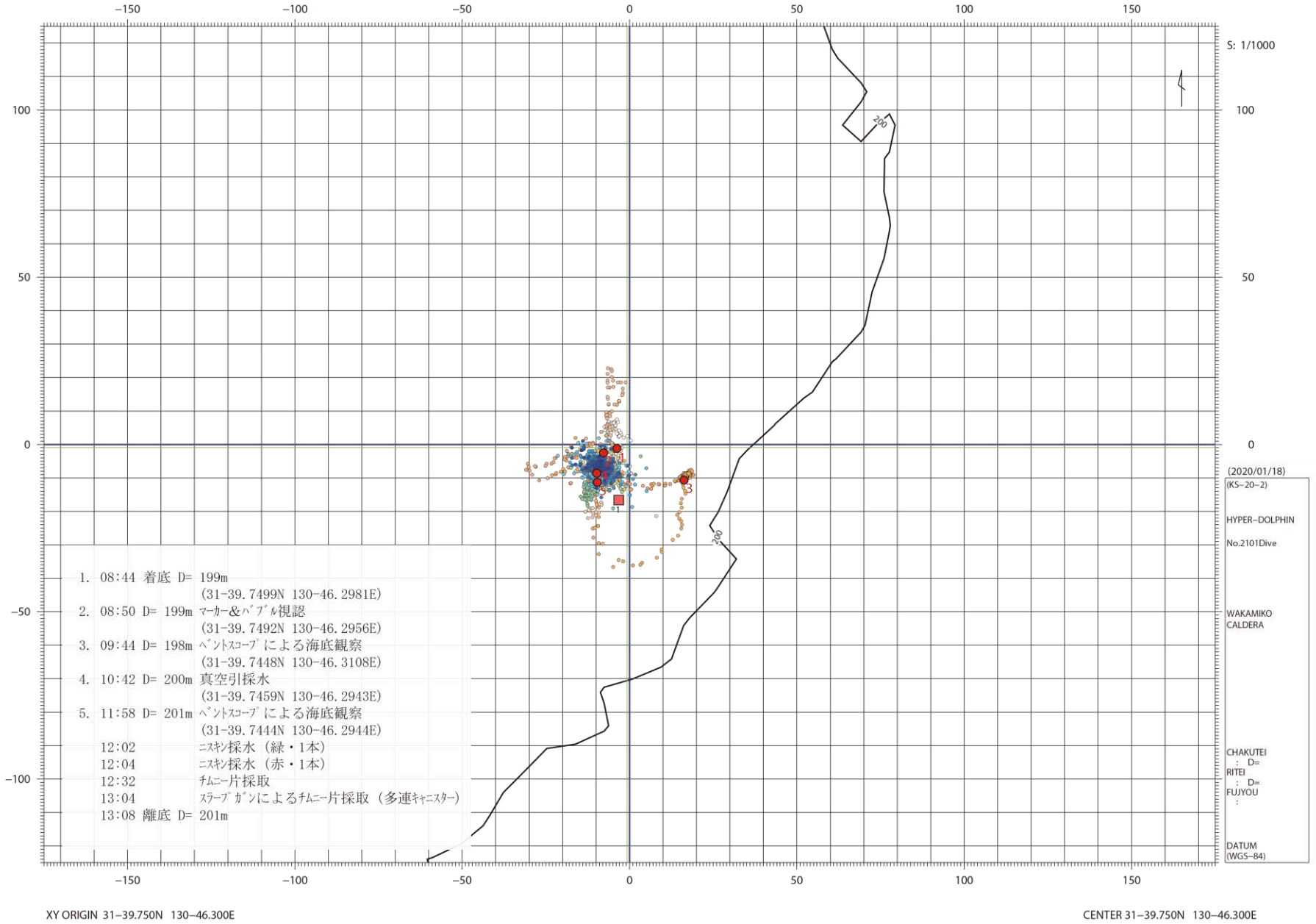
Payload Equipment:

1. Sample box x2
2. Suction sampler, multi-bottled canister, and single-boxed canister
3. Vent Scope
4. Niskin bottle (2.2 L) x2
5. Vacuum water sampler

Sampling Points and Markers:

Time	Position	Depth (m)	Events
8:50	31-39.7492N 130-46.2956E	199	Tagiri site and old marker
9:33	31-39.7448N 130-46.3108E	198	Vent Scope observation
10:40	31-39.7459N 130-46.2943E	200	Vacuumed water sampling
11:58	31-39.7444N 130-46.2944E	201	Vent Scope observation
12:02	31-39.7444N 130-46.2944E	201	Water sampling using Niskin (green)
12:04	31-39.7444N 130-46.2944E	201	Water sampling using Niskin (red)
12:34	31-39.7444N 130-46.2944E	201	Rock sampling
13:04	31-39.7444N 130-46.2944E	201	Sediment sampling using suction sampler

Dive track HD#2101



Dive Report HD#2102

Date: January 18, 2020

Site: Wakamiko Caldera, Kagoshima Bay, ***Depth:*** 201 m

Landing (Lat., Lon., Time, Depth): 31-39.7499N 130-46.2981E, 8:44, 199 m

Leaving (Lat., Lon., Time, Depth): 31-39.7444N 130-46.2944E, 13:08, 201 m

Pilot: TAYAMA, Yudai ***Co-Pilot****:* KIKUYA, Shigeru

Observer: TSUCHIDA, Shinji

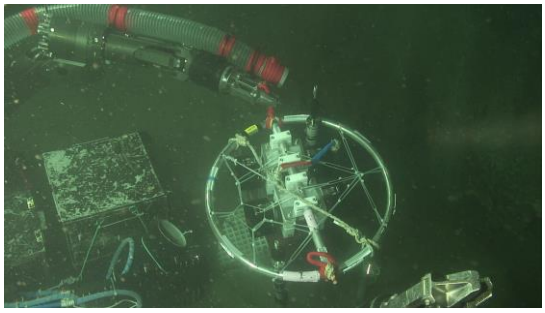
Theme: Study on thermotolerance of *Nebalia tagiri* living inside hydrothermal-chimney wall.

Purpose of dive:

1. Deploying a camera system to monitor the activity of bubbling and venting at SES site

Dive Summary

HD#2102 dive was conducted to deploy a camera system for monitoring activity of Tagiri (bubbling and venting) and benthic animals. We landed at the bottom about 15m south-west from the Tagiri site and soon moved to it. After half an hour, we arrived at the site and deployed the camera system front of the Tagiri. This is the short dive for setting the camera.



Deploying the camera system

Lighting of the camera to Tagiri

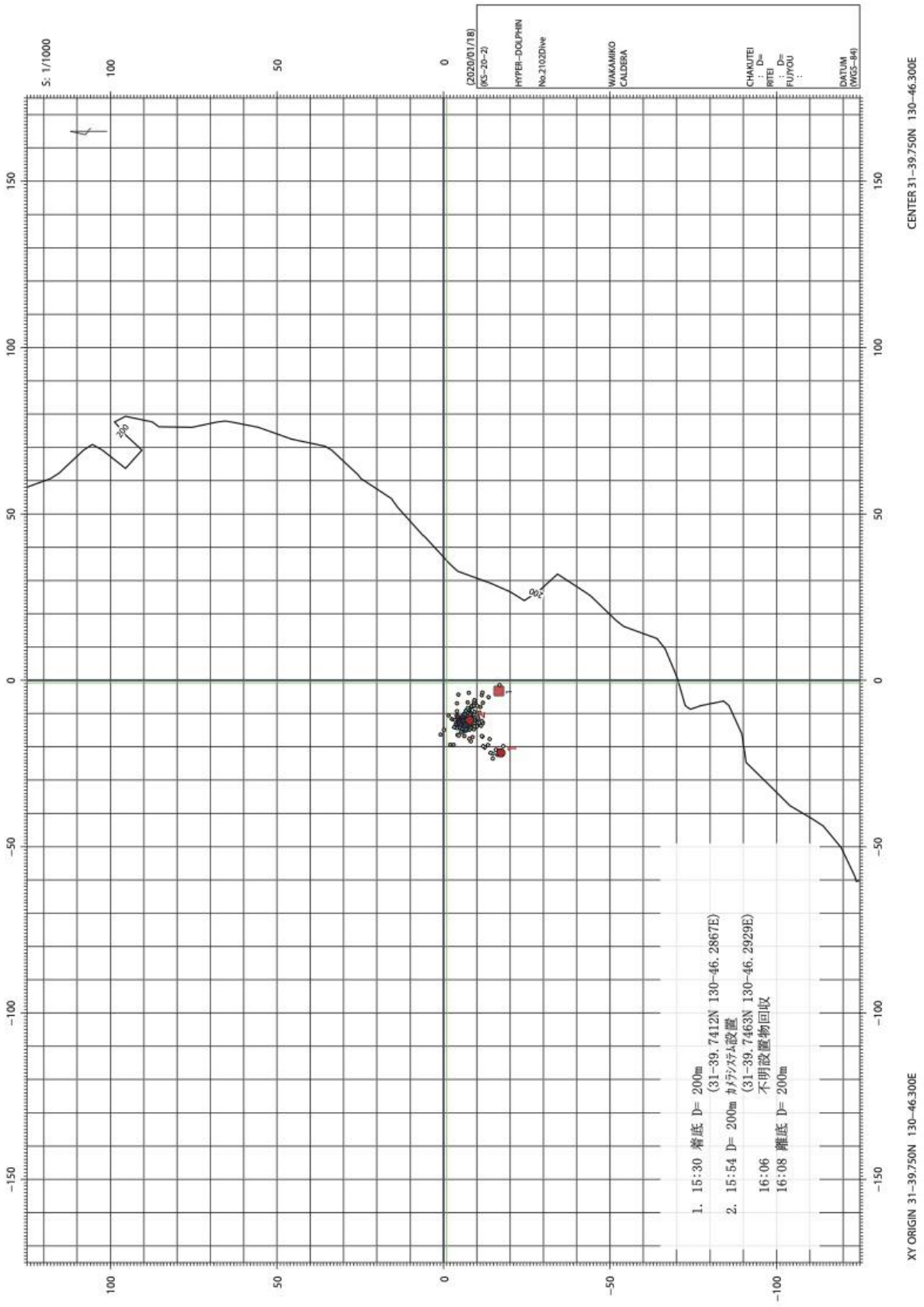
Payload Equipment:

1. Sample box x2 with Kumade scoop
2. Suction sampler, multi-bottled canister, and single-boxed canister
3. Camera system with baited trap
4. Niskin bottle (2.2 L) x2

Sampling Points and Markers:

Time	Position	Depth (m)	Events
15:54	31-39.7463N 130-46.2867E	200	Deploying the camera system

Dive track HD#2102



Dive Report HD#2103

Date: January 19, 2020

Site: WHV site, Wakamiko Caldera, Kagoshima Bay, ***Depth:*** 200 m

Landing (Lat., Lon., Time, Depth): 31-39.7434N 130-46.2813E, 8:35, 200 m

Leaving (Lat., Lon., Time, Depth): 31-39.7448N 130-46.2949E, 8:50, 200 m

Pilot: SAKAKIBARA, Yudai ***Co-Pilot****:* TAYAMA, Yudai

Observer: YAMAKI, Ayuta

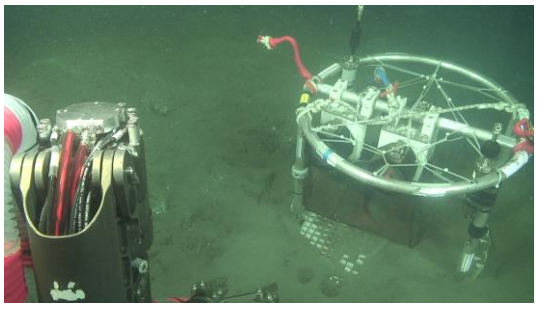
Theme: Study on thermotolerance of *Nebalia tagiri* living inside hydrothermal-chimney wall.

Purpose of dive:

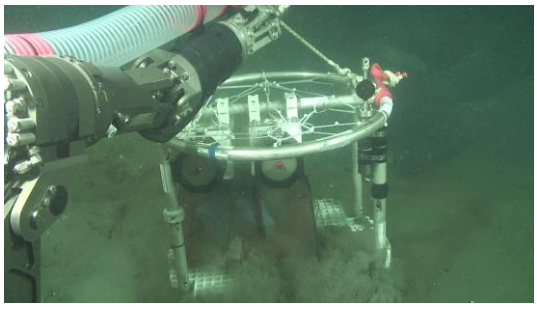
1. Collection of the Camera system and Bait trap.

Dive Summary

HD#2103 dive was conducted to collect the Camera system and Bait trap which were settled on this site at the last dive, yesterday. We landed beside the settled Camera system and Bait trap. Soon after landing, we collected the system and trap and leave the sea floor.



Camera system and Bait trap on the sea floor



Collection of the Camera system and Bait trap

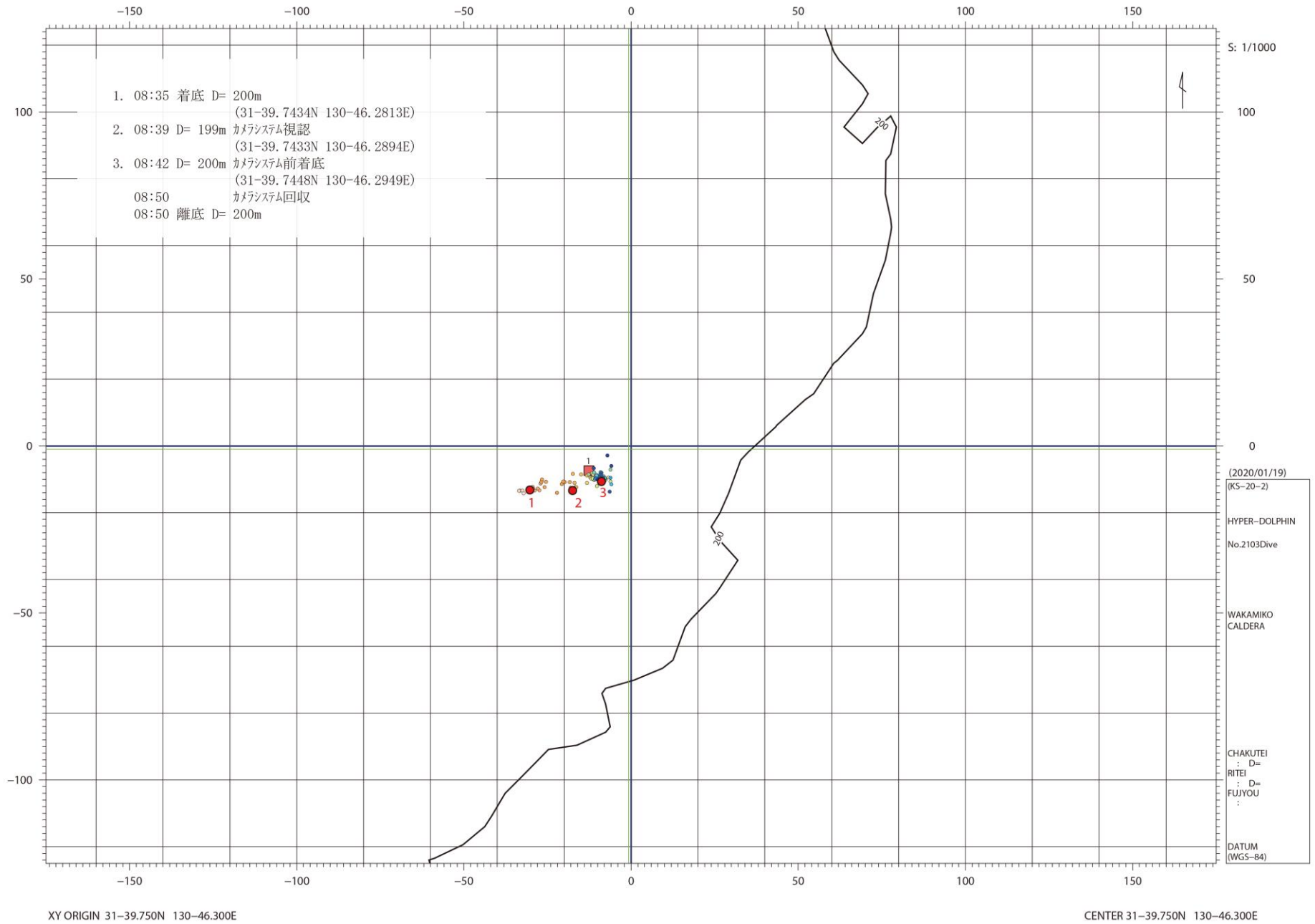
Payload Equipment:

1. Sample box x2
2. Suction sampler, multi-bottled canister, and single-boxed canister
3. Niskin bottle (2.2 L) x2

Sampling Points and Markers:

Time	Position	Depth (m)	Events
8:50	31-39.7448N 130-46.2949E	200	Camera System

Dive track HD#2103



Dive Report HD#2104

Date: January 19, 2020

Site: Wakamiko Caldera, Kagoshima Bay, ***Depth:*** 201 m

Landing (Lat., Lon., Time, Depth): 31-40.0700N 130-46.2981E, 8:44, 199 m

Leaving (Lat., Lon., Time, Depth): 31-40.0720N 130-45.6839E, 13:08, 198 m

Pilot: SAKAKIBARA, Yuta ***Co-Pilot****:* TAYAMA, Yudai

Observer: YAMANAKA, Toshiro

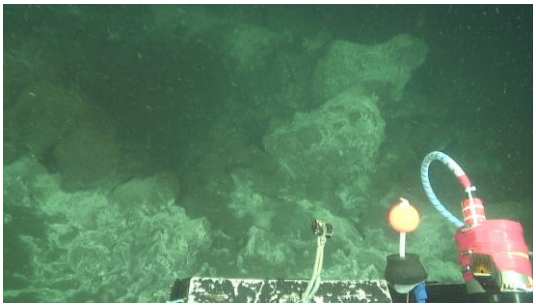
Theme: Study on thermotolerance of *Nebalia tagiri* living inside hydrothermal-chimney wall.

Purpose of dive:

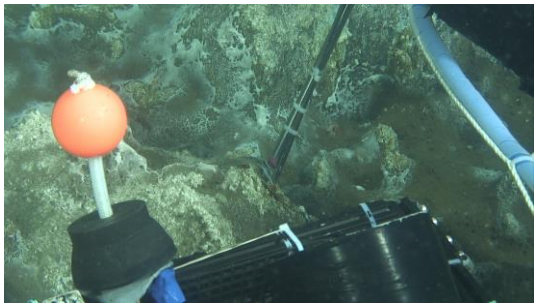
1. *In situ* observation of *Nebalia tagiri* living inside chimney wall
2. Sampling of *Nebalia tagiri* with chimney fragments
3. Sampling of hydrothermal vent fluid and ambient seawater

Dive Summary

HD#2104 dive was conducted to observe and collect the hydrothermal vent nebaliid *Nebalia tagiri*. We landed beside WHV site and searched active chimneys. After reaching to the bottom, according to sonar reflection image the ROV was moved toward to North. We saw two remarkable hydrothermal shimmering and fumarole sites covered with white materials on the way to first sampling site (31˚40.1077'N, 130˚45.6782'E; Kyokucho Site), where active hydrothermal venting from several fissures (without chimney) covered with many cobble – boulder size rocks on the area were observed. At the site venting fluid was collected using a vacuum water sampler. Then, we observed inside sediments using "Vent Scope". We clearly observed bacterial mats on and in sediments using the scope, but any benthic organisms were not found. Sediments and rocks were collected using manipulators into the front box and a suction sampler installed on ROV. After finish the sampling, we moved to South and found remarkable sonar reflection. When we reached to the reflection, White Cone chimney at WHV site has been rediscovered after eight years. Sediments and rocks were collected using manipulators from the foot of the chimney into the back box. Then, the ROV left the bottom. Upon recovery, sediments and rocks were sorted, but there was no visible metazoans.

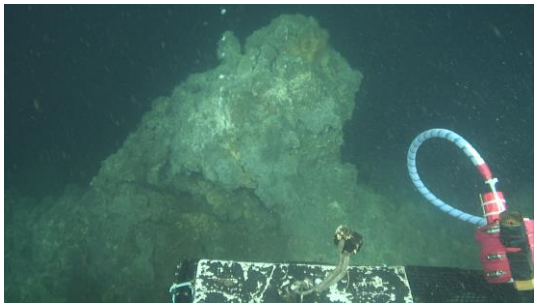


Kyokucho site



Vent scope observation

Sediment sampling with suction sampler



White Cone Chimney at WHV site

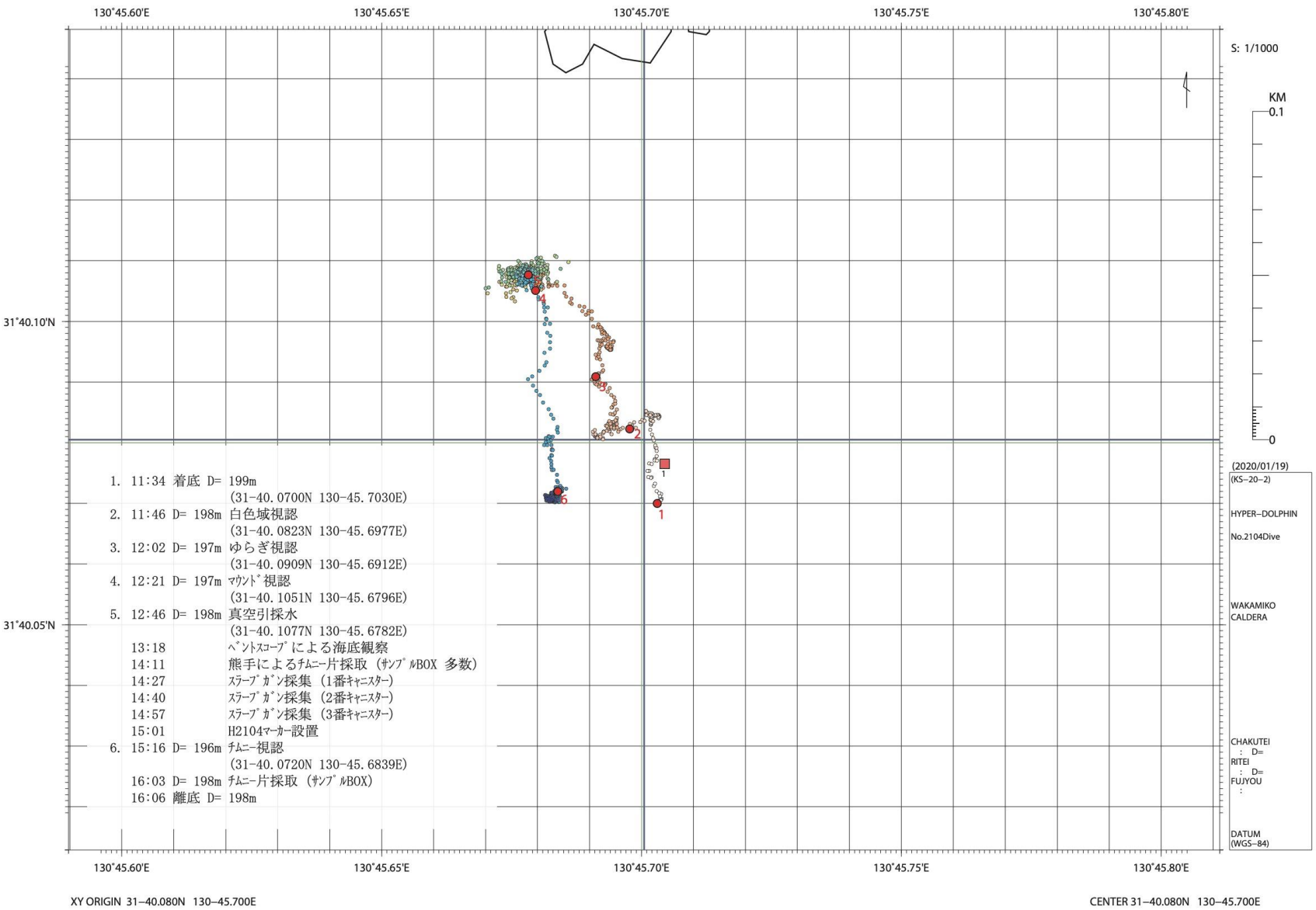
Payload Equipment:

1. Sample box x2
2. Suction sampler, multi-bottled canister, and single-boxed canister
3. Vent Scope
4. Vacuum water sampler

Sampling Points and Markers:

Time	Position	Depth (m)	Events
12:21	31-40.1051N 130-45.6796E	198	Hydrothermal venting site (no chimney)
12:46	31-40.1077N 130-45.6782E	198	Vacuumed water sampling
13:18	*ditto*	198	Vent Scope observation
14:11	*ditto*	198	Rock sampling using a rake (front box)
14:57	*ditto*	198	Sediment sampling using suction sampler (canister #1-3)
15:01	*ditto*	198	Deployed Marker (H2104)
15:16	31-40.0720N 130-45.6839E	196	Reached to White Cone Chimney
16:03	*ditto*	196	Rock sampling using a rake (back box)

Dive track HD#2104



Dive Report HD#2105

Date: January 20, 2020

Site: Wakamiko Caldera, Haorimushi Site, Kagoshima Bay, ***Depth:*** 108 m

Landing (Lat., Lon., Time, Depth): 31-39.7619N 130-48.0719E, 8:37, 108 m

Leaving (Lat., Lon., Time, Depth): 31-39.7562N 130-48.0648E, 10:51, 106 m

Pilot: TAYAMA, Yudai ***Co-Pilot****:* KIKUYA, Shigeru

Observer: Tomohiko KIKUCHI

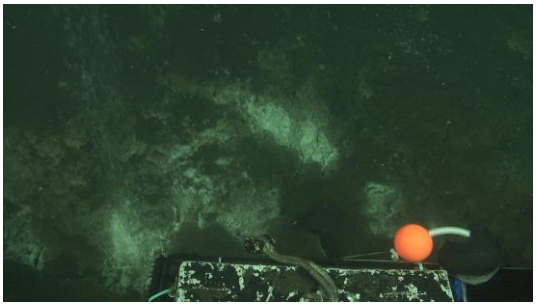
Theme: Collection of *Nebalia tagiri* and other crustaceans from tubeworm colonies at Haorimushi Site.

Purpose of dive:

1. *In situ* observation of tubeworm colonies
2. Water sampling with Niskin bottles at Haorimushi Site
3. *In situ* observation of *Nebalia tagiri* in the colonies of tubeworm by means of Vent Scope
4. Sampling of small benthic organisms with suction sampler in the tubeworm colonies
5. Sampling of tubeworm with manipulators

Dive Summary

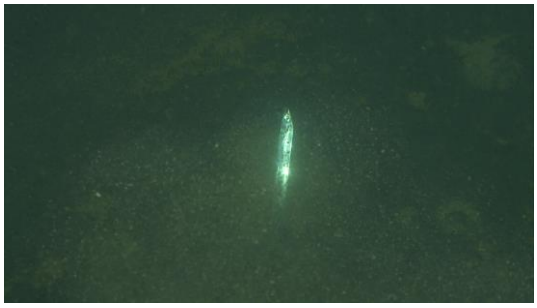
HD#2105 dive was conducted to observe and collect *Nebalia tagiri* and other small benthic crustaceans in and outside of tubeworm colony at “Haorimushi Site” of Kagoshima Bay. We landed beside a “Haorimushi” site and sampled ambient water at close range from tubeworm colonies. Next, observation of benthic animals in the tubeworm colony with “Vent Scope” was carried out. Though we tried to take much time for observation, no animals observed clearly in the colonies with the scope. Many benthic animals with sediments and rocks were collected using a suction sampler installed on ROV, however, only 5 specimens of Leptostraca (*Nebalia tagiri*) were collected. At last, we moved to small colonies of tubeworm and take a handful of tubeworms with manipulators.



Tagiri (bubbling) site

Vent scope observation

Bacterial mat on sediment



Dead hairtail

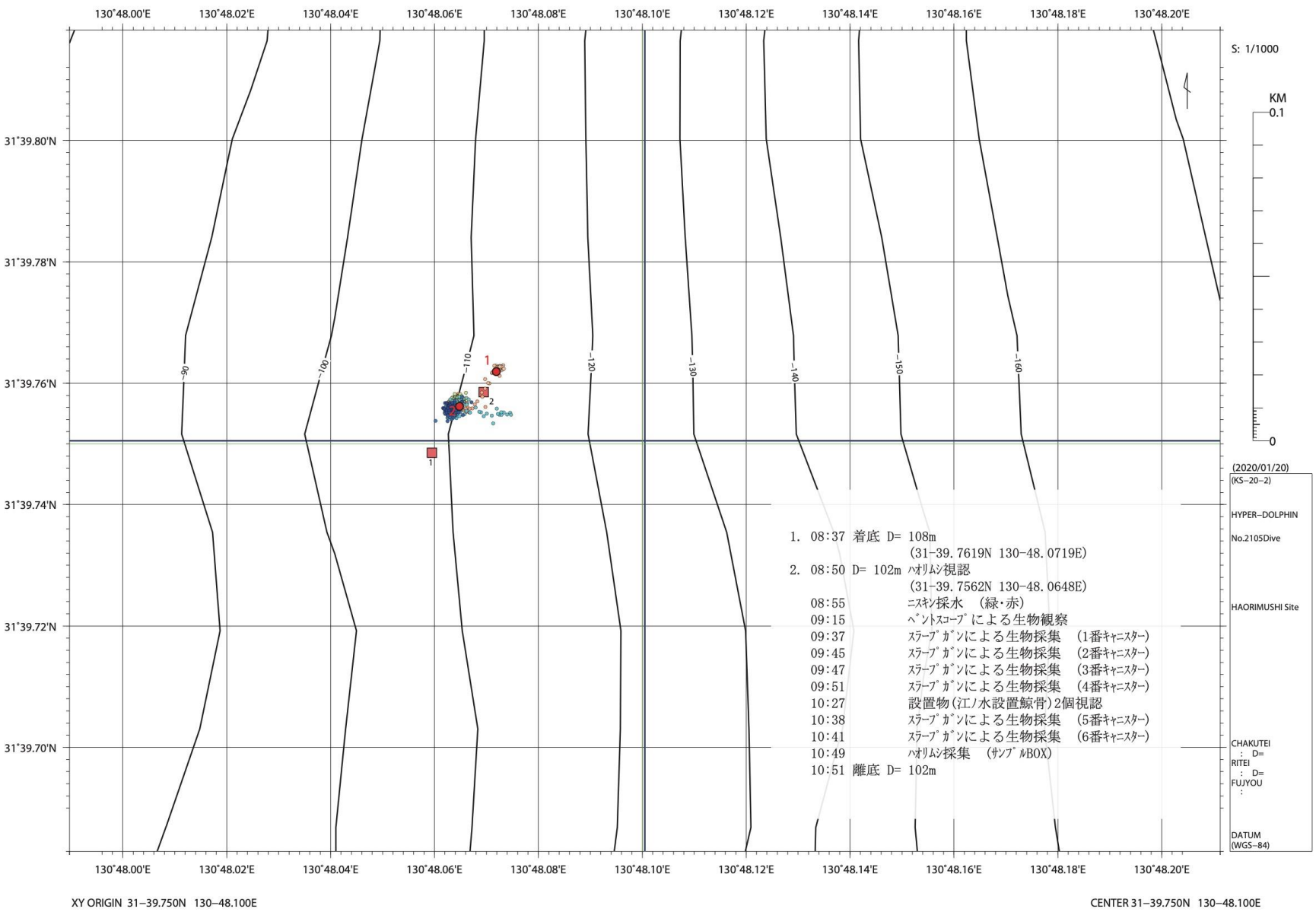
Payload Equipment:

1. Sample box x2
2. Suction sampler, multi-bottled canister, and single-boxed canister
3. Vent Scope
4. Niskin bottle (2.2 L) x2

Sampling Points and Markers:

Time	Position	Depth (m)	Events
8:50	31-39.7562N 130-48.0648E	102	Tube worm colonies
8:55	31-39.7562N 130-48.0648E	102	Water sampling using Niskin bottel (2.2L)
9:00:	31-39.7562N 130-48.0648E	102	Water sampling in progress
09:15	31-39.7562N 130-48.0648E	102	Vent Scope observation
12:02	31-39.7562N 130-48.0648E	102	Biological sampling using suction sampler
12:04	31-39.7562N 130-48.0648E	102	Biological sampling in progress
12:34	31-39.7562N 130-48.0648E	102	Biological sampling in progress
10:49	31-39.7562N 130-48.0648E	102	Tubeworm sampling using suction sampler

Dive track HD#2105



Dive Report HD#2106

Date: January 20, 2020

Site: HAORIMUSHI Site, Kagoshima Bay, ***Depth:*** 108 m

Landing (Lat., Lon., Time, Depth): 31-39.7603N 130-48.0716E, 13:36, 108 m

Leaving (Lat., Lon., Time, Depth): 31-39.7444N 130-46.2944E, 16:03, 103 m

Pilot: KIKUYA, Shigeru ***Co-Pilot****:* SAKAKIBARA, Yuta

Observer: MITA, Kouhei

Theme: Study on thermotolerance of *Nebalia tagiri* living inside hydrothermal-chimney wall.

Purpose of dive:

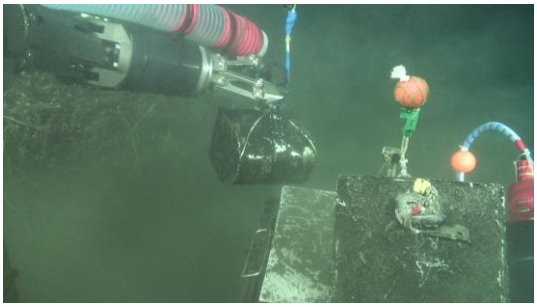
1. *In situ* observation of *Nebalia tagiri* living inside chimney wall
2. Sampling of *Nebalia tagiri*
3. Sampling of hydrothermal vent fluid and ambient seawater

Dive Summary

HD#2106 dive was conducted to observe and collect the nebaliid *Nebalia tagiri* living around the bush of tube worms. We landed beside HAORIMUSHI site and released bait traps to catch *Nebalia tagiri.* After deploying bait traps, we searched around HAORIMUSHI Site to find other bushes of tube worms, but we couldn't find out. Going back to landed point, we collected water samples using two Niskin bottles. Sediments and bio samples were collected from the under part of tube worms using vacuum water samplers. After having a look at how bait traps work, sediments and tube worms were collected using "Kumade" sampler and stored in the two sample boxes. Upon recovery, sediments were sorted and lugworms, amphipods, ostracods, Tanaidecea, brittle stars, and many other kinds of organisms were found. Not so much, but some *Nebalia tagiri* were found as well.

Bush of tube worms

Collecting bio samples from the bush of tube worms using Vacuum water sampler



Sampling sedimets using “Kumade” samplar

Released bait traps (red, blue)

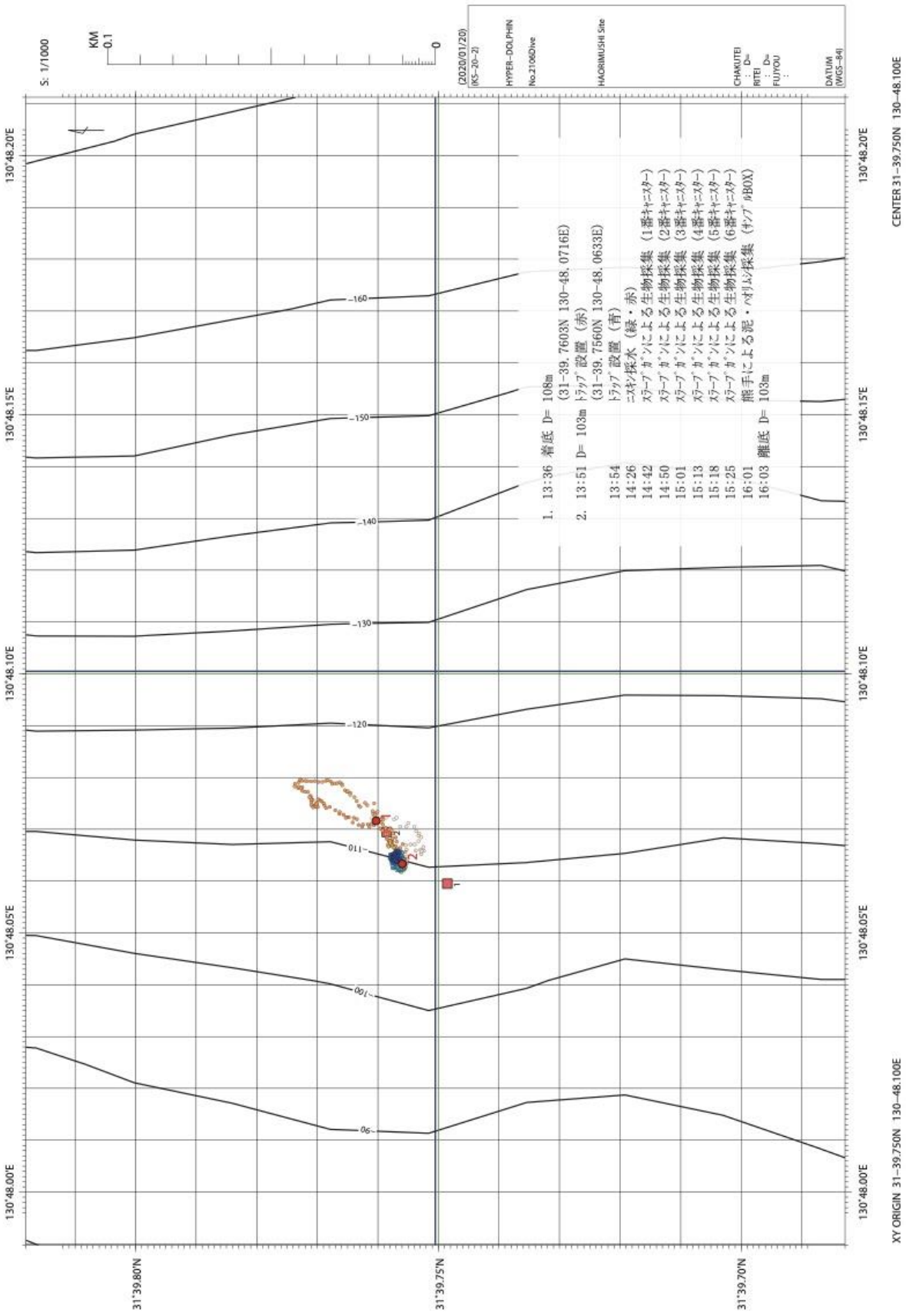
Payload Equipment:

1. Sample box x2
2. Suction sampler, multi-bottled canister, and single-boxed canister
3. Vent Scope
4. Niskin bottle (2.2 L) x2
5. Vacuum water sampler
6. “Kumade” sampler
7. Bait trap (red, blue)

Sampling Points and Markers:

Time	Position	Depth (m)	Events
13:51	31-39.7560N 130-48.0633E	103	Releasing bait trap (red)
13:54	31-39.7560N 130-48.0633E	103	Releasing bait trap (blue)
14:26	31-39.7560N 130-48.0633E	103	Water sampling using Niskin (red, green)
14:42	31-39.7560N 130-48.0633E	103	Bio sampling using Vacuum water sampler (canister 1)
14:50	31-39.7560N 130-48.0633E	103	Bio sampling using Vacuum water sampler (canister 2)
15:01	31-39.7560N 130-48.0633E	103	Bio sampling using Vacuum water sampler (canister 3)
15:13	31-39.7560N 130-48.0633E	103	Bio sampling using Vacuum water sampler (canister 4)
15:18	31-39.7560N 130-48.0633E	103	Bio sampling using Vacuum water sampler (canister 5)
15:25	31-39.7560N 130-48.0633E	103	Bio sampling using Vacuum water sampler (canister 6)
16:01	31-39.7560N 130-48.0633E	103	Collecting sediments and tube worms using “Kumade” sampler

Dive track HD#2106



Dive Report HD#2107

Date: January 21, 2020

Site: Tubeworm site, Kagoshima Bay, ***Depth:*** 105 m

Landing (Lat., Lon., Time, Depth): 31°39.7624'N 130°48.0666'E, 8:44, 105 m

Leaving (Lat., Lon., Time, Depth): 31°39.7580'N 130°48.0646'E, 13:00, 103 m

Pilot: TAYAMA, Yudai ***Co-Pilot****:* KIKUYA, Shigeru

Observer: FUJIWARA, Yoshihiro

Theme: Study on thermotolerance of *Nebalia tagiri* living inside hydrothermal-chimney wall.

Purpose of dive:

1. *In situ* observation of *Nebalia tagiri* living around tubeworm colonies using vent scope
2. Sampling of *N. tagiri*
3. Retrieval of bait trap for *N. tagiri*

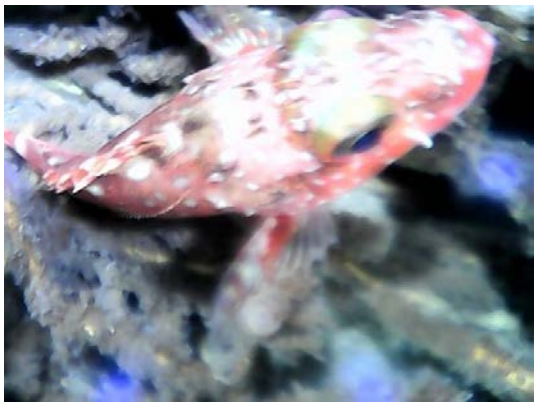
Dive Summary

HD#2107 dive was conducted to observe and collect *Nebalia tagiri* individuals. We landed beside the tubeworm site where we have visited during HD#2105 and HD#2106 and observed inside the tubeworm colony using the "Vent Scope". Suction sampling was conducted at surfaces of sediments around the colony. Two bait traps, deployed during HD#2106, were successfully retrieved. Upon recovery, more than several thousands of *N. tagiri* individuals were collected in the canisters of the suction sampler and in the bait traps. Onboard experiments were conducted, and the maximum survival temperature of *N. tagiri* was elucidated.

Retrieval of a bait trap deployed beside a tubeworm colony

Vent scope observation inside the tubeworm colony

Magnification image of tubeworms' tube using Vent Scope



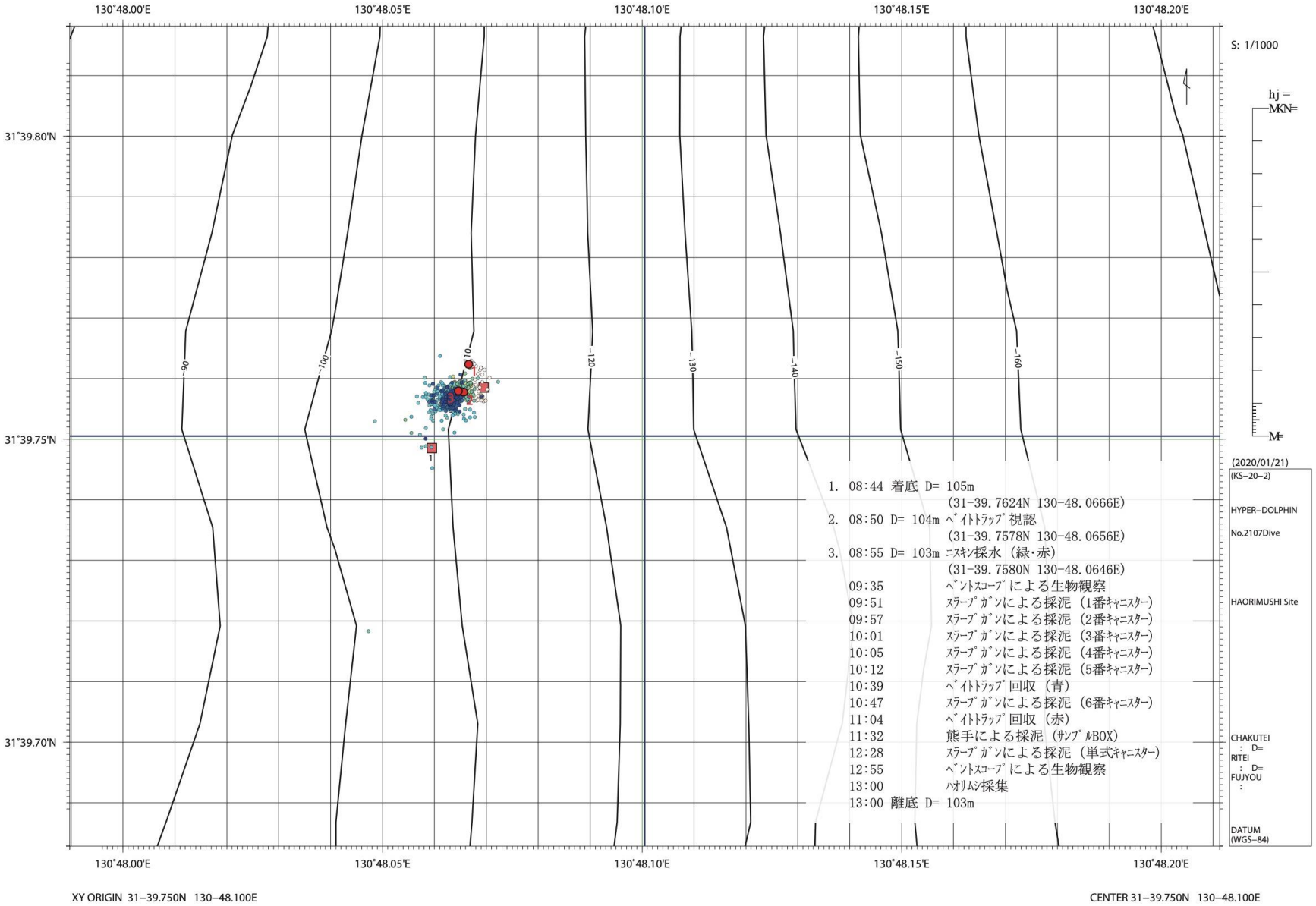
Magnification image of *Sebastiscus tertius* using Vent Scope

Payload Equipment:

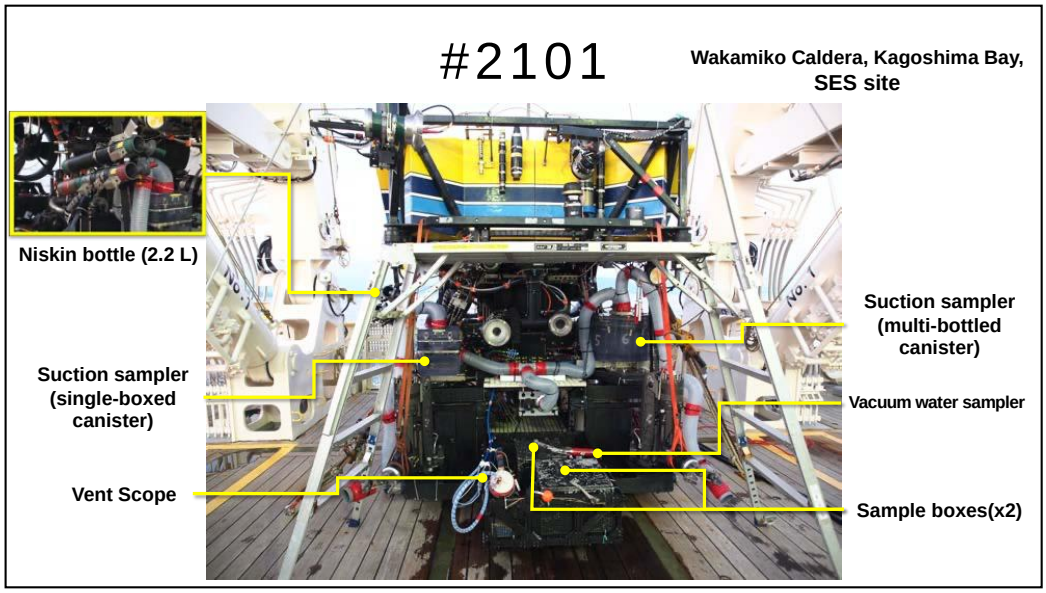
1. Sample box x2
2. Suction sampler, multi-bottled canister, and single-boxed canister
3. Vent Scope
4. Niskin bottle (2.2 L) x2

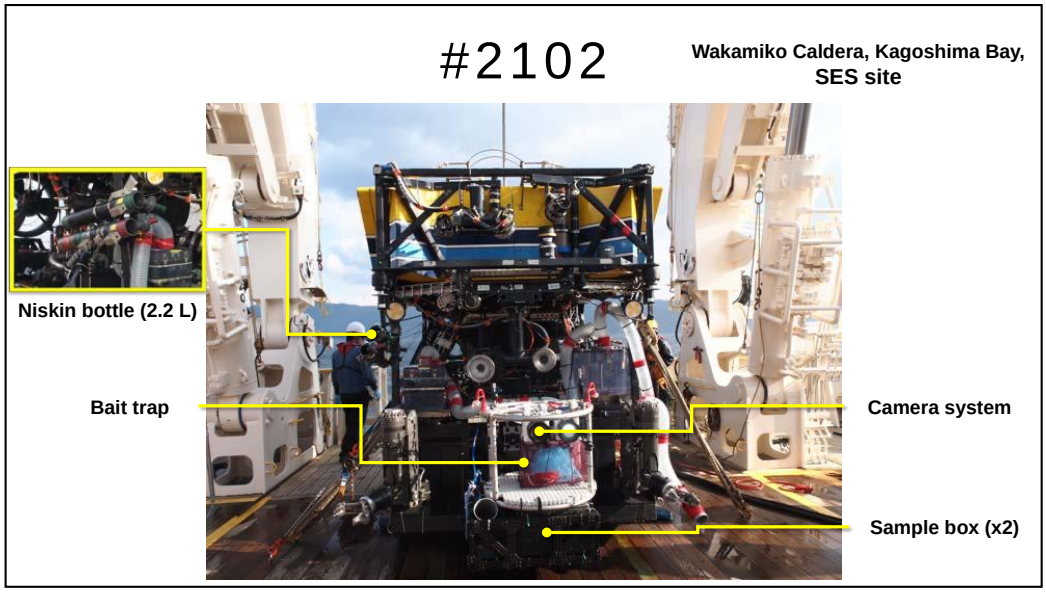
Sampling Points and Markers:

Time	Position	Depth (m)	Events
8:55	31°39.7580'N, 130°48.0646'E	103	Water sampling using two Niskin bottles
9:35	Ditto	Ditto	Vent Scope observation
9:51	Ditto	Ditto	Sediment sampling using suction sampler
10:39	Ditto	Ditto	Retrieval of a bait trap (blue)
11:04	Ditto	Ditto	Retrieval of a bait trap (red)
11:32	Ditto	Ditto	Sediment sampling using "Kumade"
13:00	Ditto	Ditto	Sampling of tubeworm


130°48.00'E
130°48.05'E
130°48.10'E
130°48.15'E
130°48.20'E
31°39.80'N
31°39.75'N
31°39.70'N
S: 1/1000
hj =
MKN=
M=
-90
-100
-110
-120
-130
-140
-150
-160
1
1. 08:44 着底 D= 105m
(31-39.7624N 130-48.0666E)
2. 08:50 D= 104m ﾍﾞｲﾄﾄﾗｯﾌﾟ視認
(31-39.7578N 130-48.0656E)
3. 08:55 D= 103m ﾆｽｷﾝ採水（緑・赤）
(31-39.7580N 130-48.0646E)
09:35 ﾍﾞﾝﾄｽｺｰﾌﾟによる生物観察
09:51 ｽﾚｰﾌﾞｶﾞﾝによる採泥（1番ｷｬﾆｽﾀｰ）
09:57 ｽﾚｰﾌﾞｶﾞﾝによる採泥（2番ｷｬﾆｽﾀｰ）
10:01 ｽﾚｰﾌﾞｶﾞﾝによる採泥（3番ｷｬﾆｽﾀｰ）
10:05 ｽﾚｰﾌﾞｶﾞﾝによる採泥（4番ｷｬﾆｽﾀｰ）
10:12 ｽﾚｰﾌﾞｶﾞﾝによる採泥（5番ｷｬﾆｽﾀｰ）
10:39 ﾍﾞｲﾄﾄﾗｯﾌﾟ回収（青）
10:47 ｽﾚｰﾌﾞｶﾞﾝによる採泥（6番ｷｬﾆｽﾀｰ）
11:04 ﾍﾞｲﾄﾄﾗｯﾌﾟ回収（赤）
11:32 熊手による採泥（ｻﾝﾌﾟﾙBOX）
12:28 ｽﾚｰﾌﾞｶﾞﾝによる採泥（単式ｷｬﾆｽﾀｰ）
12:55 ﾍﾞﾝﾄｽｺｰﾌﾟによる生物観察
13:00 ﾊｵﾘﾑｼ採集
13:00 離底 D= 103m
(2020/01/21)
(KS-20-2)
HYPER-DOLPHIN
No.2107Dive
HAORIMUSHI Site
CHAKUTEI
: D=
RITEI
: D=
FUJYOU
:
DATUM
(WGS-84)
XY ORIGIN 31-39.750N 130-48.100E
CENTER 31-39.750N 130-48.100E

3-3. Payload list with photographs



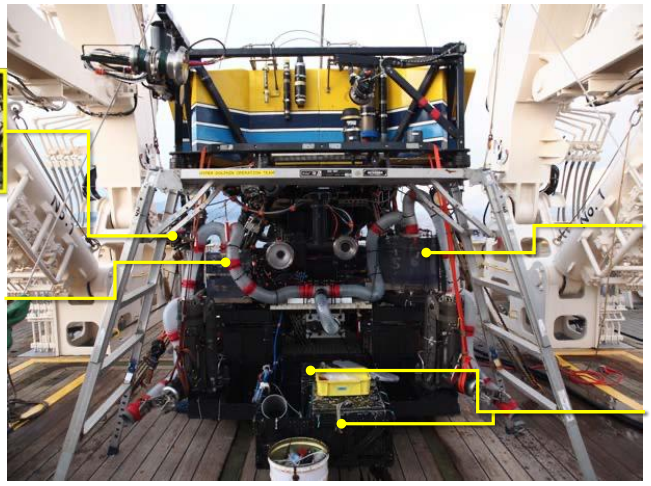


#2103

Wakamiko Caldera, Kagoshima Bay,
SES site

Niskin bottle (2.2 L)

Suction sampler
(single-boxed
canister)



Suction sampler
(multi-bottled
canister)

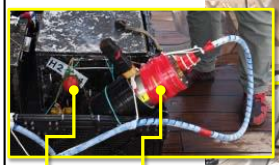
Sample boxes(x2)

#2104

Wakamiko Caldera, Kagoshima Bay,
WHV site

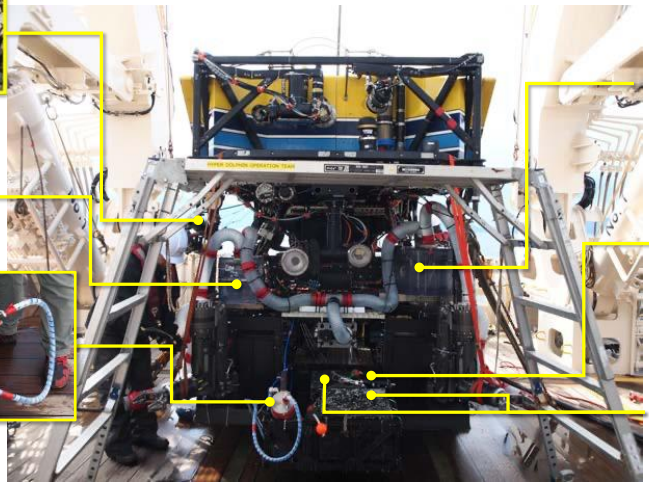
Niskin bottle (2.2 L)

Suction sampler
(single-boxed
canister)



Marker

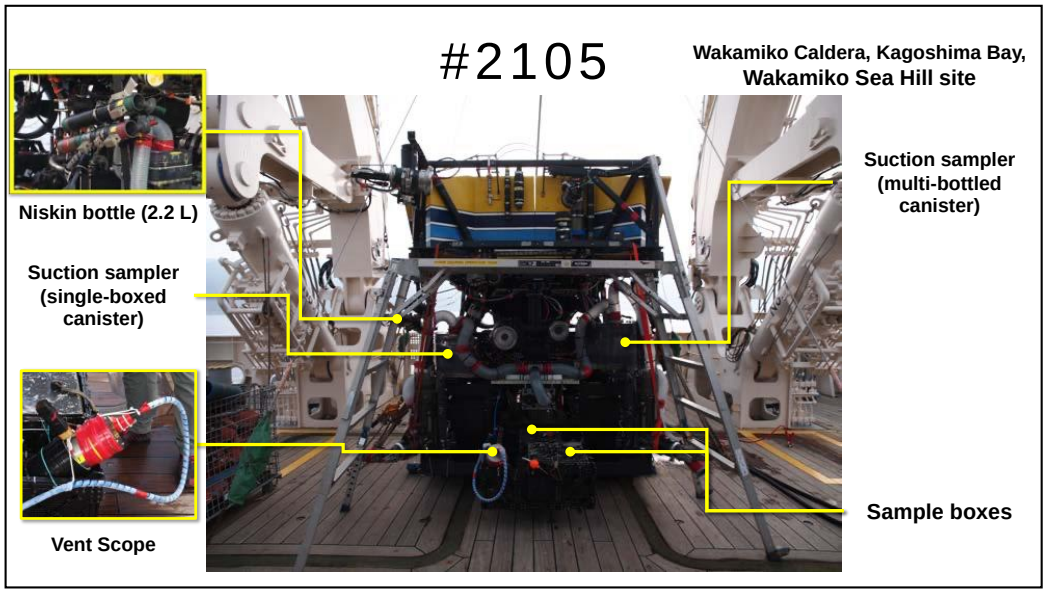
Vent Scope

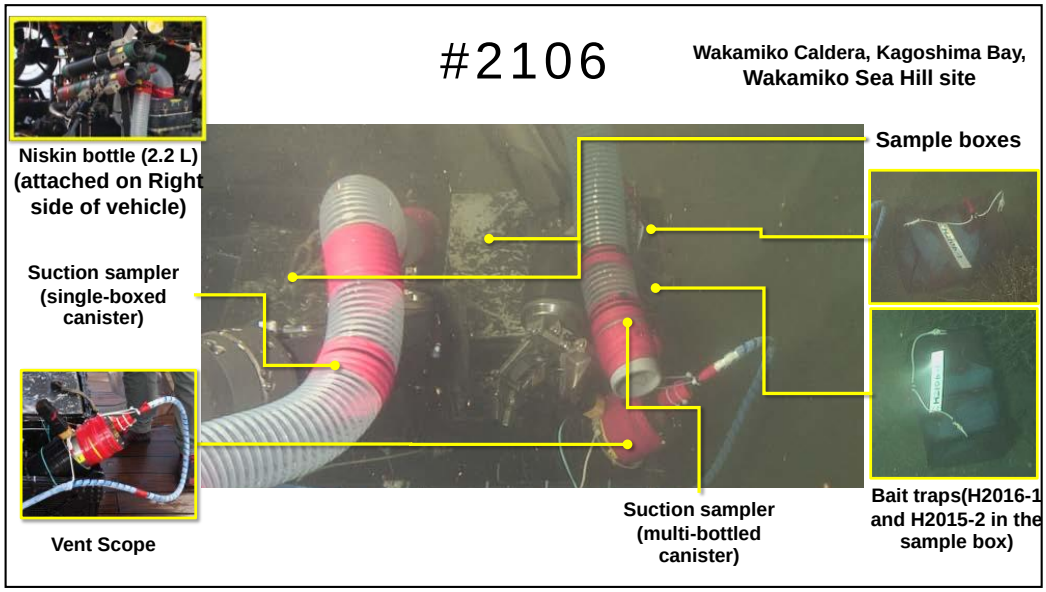


Suction sampler
(multi-bottled
canister)

Vacuum water sampler

Sample boxes


#2105
Wakamiko Caldera, Kagoshima Bay,
Wakamiko Sea Hill site
Niskin bottle (2.2 L)
Suction sampler
(single-boxed
canister)
Vent Scope
Suction sampler
(multi-bottled
canister)
Sample boxes


#2106
Wakamiko Caldera, Kagoshima Bay,
Wakamiko Sea Hill site
Niskin bottle (2.2 L)
(attached on Right
side of vehicle)
Suction sampler
(single-boxed
canister)
Vent Scope
Sample boxes
Suction sampler
(multi-bottled
canister)
Bait traps(H2016-1
and H2015-2 in the
sample box)

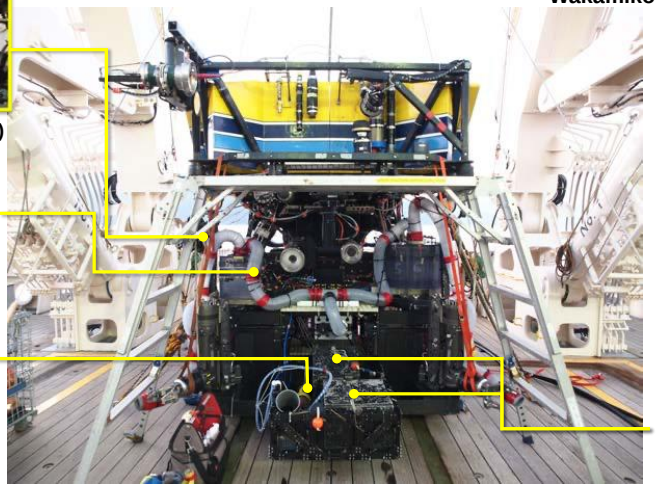
#2107

Wakamiko Caldera, Kagoshima Bay,
Wakamiko Sea Hill site

Niskin bottle (2.2 L)

Suction sampler (single-boxed canister)

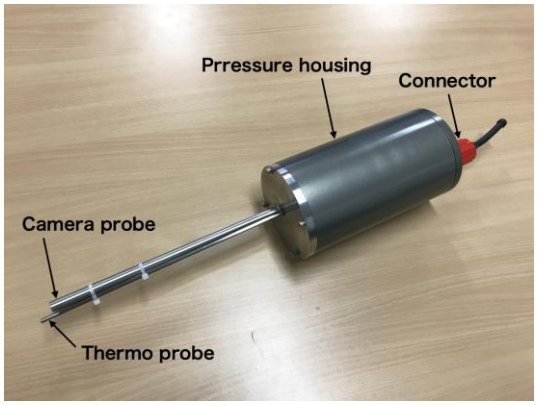
Vent Scope



Sample boxes

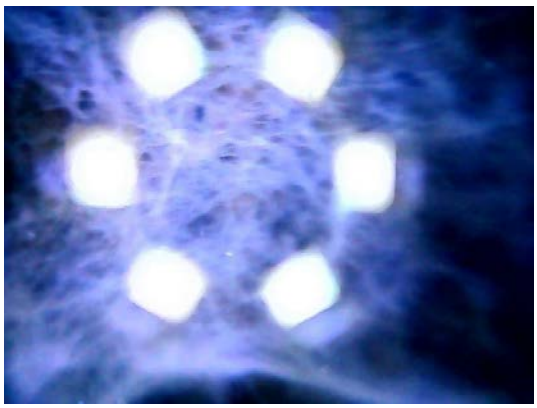
4. Vent scope observation

“Vent Scope” observations were conducted during all the dives in this cruise. The Vent Scope is composed of a camera probe accompanying LED lights, a thermo probe, pressure housing made of aluminum, and a connector. An optional “ball lens” are able to be installed in the camera probe, which magnifies the image (image of bacterial mat shown below), but strong internal reflection of the lens occurs. Video images (VGA, H264 codec) and temperature are monitored onboard, and such data is stored in a micro SD card installed in the pressure housing. Temperature profiles were not obtained correctly due to a mechanical problem. Fine images were taken in situ, but no nebaliid shrimps were observed using the Vent Scope.

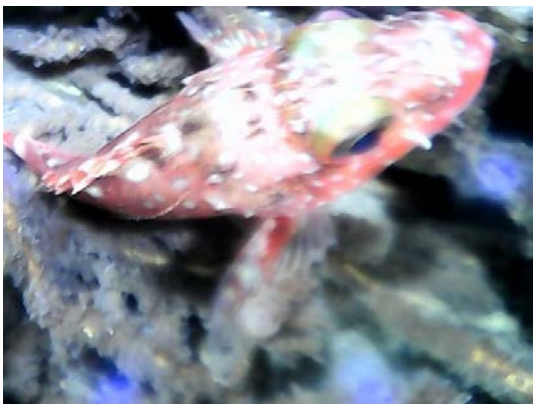


Vent scope

Vent scope operation during HD#2107



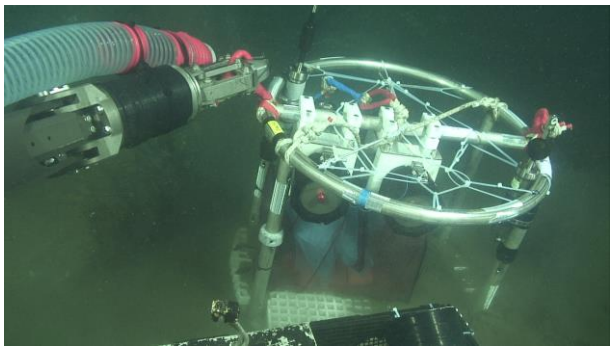
Magnification image of bacterial mat at a hydrothermal vent site during HD#2014



Magnification image of *Sebastiscus tertius* around a tubeworm site during HD#2017

5. Camera observatory deployment

A camera observatory was deployed beside a hydrothermal vent at a depth of 200 m. A total of 7.5-hour video clips was taken, which showed active bubbling from the vent. No creatures were seen, which was consistent with the ROV observation.

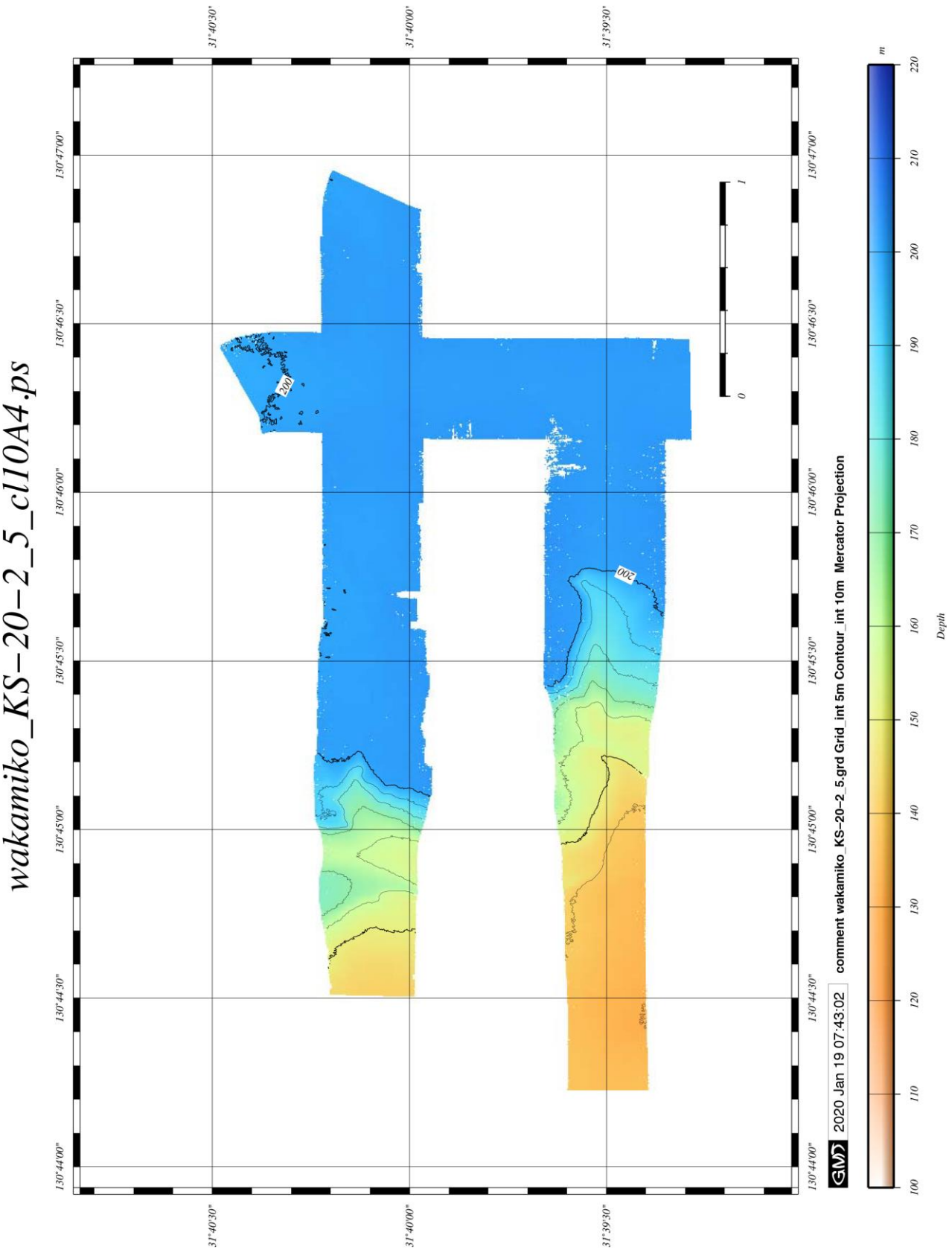


Camera observatory deployment beside a hydrothermal vent. ROV lights were turned on.

Camera observatory deployment beside a hydrothermal vent. ROV lights were turned off.

Image taken by the camera observatory. Active bubbling is seen from a hydrothermal vent.

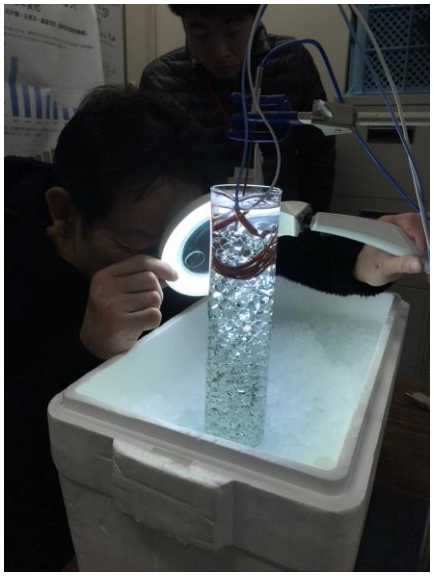
6. Geophysical survey results



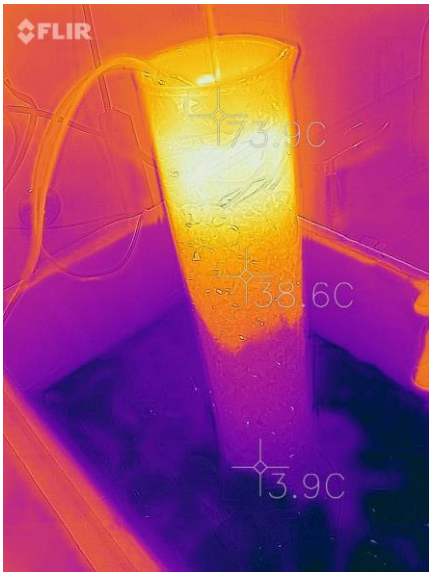
7. Scientific results

7-1. Thermo-gradient experiment

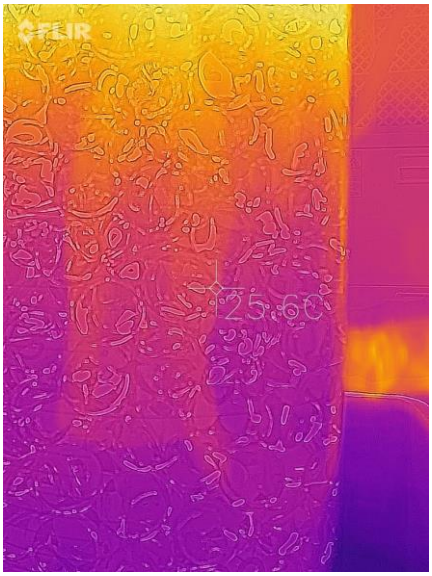
To elucidate the suitable temperature for *N. tagiri* collected from the tubeworm site, a thermo-gradient experiment was conducted. A one-litter graduated cylinder was filled with filtered seawater and grass beads, which placed in an ice-cold bath. The top water in the graduated cylinder was heated using a heating cable. The temperature gradient was from < 0 to > 70 °C, which was recorded using a thermography camera. Before heating, a hundred individuals of *N. tagiri* was introduced in the cylinder. Most *N. tagiri* individuals moved down and underwent asphyxia due to the cold temperature. Rest stayed close to the surface and were finally killed by the heat. Therefore, the suitable temperature for *N. tagiri* was not measured.



Lab setting for thermo-gradient experiment



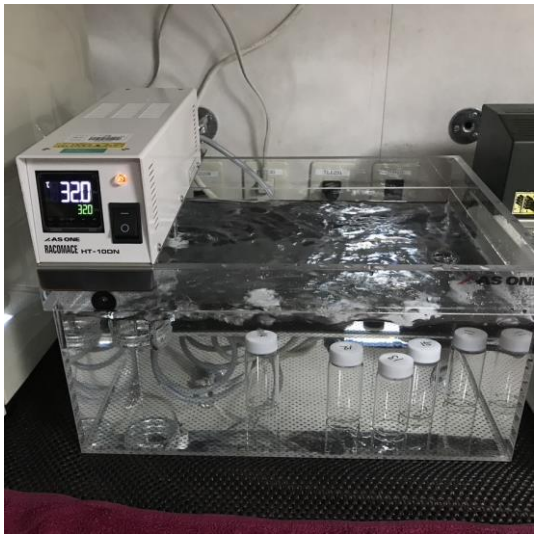
Thermo-gradient in a graduated cylinder



Nebalia tagiri located between grass beads at temperature of 25.7 °C. Left: light image; Right: thermography image.

7-2. Thermo-tolerance experiment

Thermal tolerance of *N. tagiri* was tested using a thermal-controlled water bath. A temperature gradient started at * °C and ended at * °C. Three time series were prepared (two-, three- and four-hour periods for the total experiments). The temperature was increased step-by-step in one-degree increment. Ten individuals were placed in a 15-mL vials filled with filtered seawater. Five vials (50 individuals in total) were applied for each series. Most individuals survived up to * °C in each experiment period, and the survival rates showed a sharp decline at * °C and higher. All the individuals died at * °C in the all experimental periods.



Lab setting for thermo-tolerance experiment

*Specific numbers not shown

GRAPH NOT SHOWN

Thermal tolerance of *Nebalia tagiri*

7-3. Development of tubeworm eggs using pressure vessel

-Introduction and Purpose

It has been known that eggs of *L. satsuma* fertilize in female body. Therefore, removed eggs develop and grow to larvae in the sea water kept at 10-17℃ without artificial fertilization. However, it is rare that eggs grow and reach to trochophore larvae. According to previous record, removed eggs have positive buoyancy and develop to trochophore larvae in about 7 days.

In this study, the experiment and observation were conducted to confirm whether a ratio of normal development of removed eggs of *L. satsuma* under pressured condition is different from atmosphere condition or not by using a handy pressure vessel.

-Materials and Methods

The eggs used this experiment were removed from the individuals of *L. satsuma* collected at No. HPD2106 and 2107 dives. The eggs removed from the 13 individuals collected at HPD2106 were incubated in a handy pressure vessel compressed at 0.7 Mpa (Fig. 1). The eggs removed from the 4 individuals collected at HPD2107 were incubated in a 3L plastic vessel at atmosphere pressure over 6 days. Before sealing the vessels, sea water in them were bubbled by oxygen for keeping dissolved oxygen higher. After this treatment, put eggs into the vessels and sealed without air. The vessels, after sealing, were put into an aquaria temperature of 17℃. After the incubation over 6 days, both of incubated eggs were removed from vessels and observed.

-Results and Discussion

After incubation of eggs over 6 days, normal development of *L. satsuma* was not observed neither pressure vessel nor sample bottle. Although bubbling of oxygen before sealing vessels, dissolved oxygen of sea water in them immediately after incubation over 6 days was 6.4 ml l^{-1} which is slightly lower than normal sea water.

The 6 days incubation term in this study was adopted because of to avoid that eggs touch the water surface and air by positive buoyancy, and to keep eggs compressed during developing. However, the results suggest that 6 days is too long for normal development without changing water.

In the further studies, I would like to plan to change the term of incubation shorter or to try incubate embryo which is after eggs developing to blastula.

Fig. 1 The handy pressure vessel(left) and the eggs of *L. satsuma* in the sealed pressure vessel(right).

8. Proposals for the future studies

Thermal tolerance of the hydrothermal vent nebaliid *Nebalia tagiri*
Authors: Fujiwara, Y., Tsuchida, S., Kuraku, S., Kikuchi, T. et al.

VENT SCOPE: *in situ* endoscope for observations of infauna in deep sea.
Authors: Fujiwara, Y., Masuda, K., Tsuchida, S. et al.

Development and reproduction of *Lamellibrachia satsuma*.
Authors: Ayuta Yamaki and coworkers

Development and reproduction of *Periclimenes thermohydrophilus*.
Authors: Ayuta Yamaki and coworkers

Rearing and Exhibition method of *Nebalia Tagiri*.
Authors: Ayuta Yamaki and coworkers

Mineralogical and lithological study of hydrothermal precipitation and lithified sediment around hydrothermal and gas emitting areas in Wakamiko Crater, Kagoshima Bay, Japan
Authors: Yamanaka, T. and students of TUMSAT

Research title: Organic geochemical study of hydrothermal altered sediments around hydrothermal and gas emitting areas in Wakamiko Crater, Kagoshima Bay, Japan
Authors: Yamanaka, T. and students of TUMSAT

Appendix

I. Sample list

I-1. Macro organisms

On board ID	Dive No.	Sample Name	No.	Sampling Method	Fixation	Preservation	Locality	Lat.(N)		Lon.(E)		Depth	Date Collected				Remarks
								Deg.	Min.	Deg.	Min.	[m]	yyyy	mm	dd	hh:mm	
2105-1	2105	Lamellibrachi satsuma	many	Manupulator	Live	Live	Tubeworm site	31	39.7550	130	48.0634	90.5	2020	1	20	10:45	
2105-2	2105	Periclimenes thermohydrophilus	112	Slurp gun	Live	Live	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-3	2105	Periclimenes thermohydrophilus	72	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-4	2105	Gastropoda	6	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-5	2105	Ascidiacea	4	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-6	2105	Solemyidae	2	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-7	2105	Porifera	15	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-8	2105	Amphipoda	14	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	

On board ID	Dive No.	Sample Name	No.	Sampling Method	Fixation	Preservation	Locality	Lat.(N)		Lon.(E)		Depth	Date Collected				Remarks
								Deg.	Min.	Deg.	Min.	[m]	yyyy	mm	dd	hh:mm	
2105-9	2105	Nebalia tagiri	1	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-10	2105	Gastropoda	4	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-11	2105	Acrocirrus validus	3	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-12	2105	Plychaete	2	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-13	2105	Plychaete	many	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-14	2105	Ophiurida	6	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-15	2105	Tanaidacea	many	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-16	2105	Ostracoda	many	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-17	2105	Actiniaria	4	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	
2105-18	2105	Nebalia tagiri	5	Slurp gun	Live	Live	Tubeworm site	31	39.7560	130	48.0633	91.2	2020	1	20	9:26	

On board ID	Dive No.	Sample Name	No.	Sampling Method	Fixation	Preservation	Locality	Lat.(N)		Lon.(E)		Depth	Date Collected				Remarks
								Deg.	Min.	Deg.	Min.	[m]	yyyy	mm	dd	hh:mm	
2106-1	2106	Lamellibrachi satsuma	30	Manupulator	Live	-	Tubeworm site	31	39.7568	130	48.0641	90.4	2020	1	20	15:52	解剖、卵のみ持ち帰り
2106-2	2106	Periclimenes thermohydrophilus	179	Slurp gun	Live	Live	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-3	2106	Gastropoda	5	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-4	2106	Ostracoda	51	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-5	2106	Amphipoda	8	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-6	2106	Periclimenes thermohydrophilus	50	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-7	2106	Ascidiacea	6	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-8	2106	Gastropoda	18	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-9	2106	Tanaidacea	17	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-10	2106	Plychaete	many	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	

On board ID	Dive No.	Sample Name	No.	Sampling Method	Fixation	Preservation	Locality	Lat.(N)		Lon.(E)		Depth	Date Collected				Remarks
								Deg.	Min.	Deg.	Min.	[m]	yyyy	mm	dd	hh:mm	
2106-11	2106	Nihonotrypaea sp.	1	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-12	2106	Porifera	23	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-13	2106	Solemyidae	6	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-14	2106	Aphroditiformia	3	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-15	2106	Plychaete	many	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-16	2106	Hydrozoa	1	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	エフィラ？
2106-17	2106	Plychaete	many	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	タケフシゴカイ？
2106-18	2106	Plychaete	4	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-19	2106	Acrocirrus validus	5	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	
2106-20	2106	Nebalia tagiri	27	Slurp gun	Live	Live	Tubeworm site	31	39.7557	130	48.0619	90.5	2020	1	20	14:30	

On board ID	Dive No.	Sample Name	No.	Sampling Method	Fixation	Preservation	Locality	Lat.(N)		Lon.(E)		Depth	Date Collected				Remarks
								Deg.	Min.	Deg.	Min.	[m]	yyyy	mm	dd	hh:mm	
2107-1	2107	Nebalia tagiri	35	Slurp gun	Frozen	Frozen	Tubeworm site	31	39.7588	130	48.0655	91.5	2020	1	21	10:39	
2107-2	2107	Tanaidacea	3	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-3	2107	Actiniaria	2	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-4	2107	Oligochaeta	21	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-5	2107	Solemyidae	6	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-6	2107	Echiura	1	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-7	2107	Ostracoda	1	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-8	2107	Gastropoda	36	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-9	2107	Amphipoda	21	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-10	2107	Nebalia tagiri	11	Bait trap	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	10:39	

On board ID	Dive No.	Sample Name	No.	Sampling Method	Fixation	Preservation	Locality	Lat.(N)		Lon.(E)		Depth [m]	Date Collected				Remarks
								Deg.	Min.	Deg.	Min.		yyyy	mm	dd	hh:mm	
2107-11	2107	Plychaete	20	Slurp gun	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	9:46	
2107-12	2107	Nebalia tagiri	many	Bait trap	Live	Live	Tubeworm site	31	39.7588	130	48.0655	91.5	2020	1	21	10:39	
2107-13	2107	Nebalia tagiri	50	Bait trap	99.5% EtOH	99.5% EtOH	Tubeworm site	31	39.7577	130	48.0648	90.1	2020	1	21	10:39	
2107-14	2107	Nebalia tagiri	many	Bait trap	Live	Live	Tubeworm site	31	39.7588	130	48.0655	91.5	2020	1	21	10:39	
2107-15	2107	Lamellibrachi satsuma	9	Manupulator	Live	Live	Tubeworm site	31	39.7555	130	48.0623	92.2	2020	1	21	12:59	解剖、卵のみ持ち帰り
2017-16	2107	Nebalia tagiri	20	Slurp gun	70.0% EtOH	70.0% EtOH	Tubeworm site	31	39.7555	130	48.0623	90.1	2020	1	21	9:46	
2017-17	2107	Nebalia tagiri	70	Bait trap	70.0% EtOH	70.0% EtOH	Tubeworm site	31	39.7555	130	48.0623	91.5	2020	1	21	10:39	

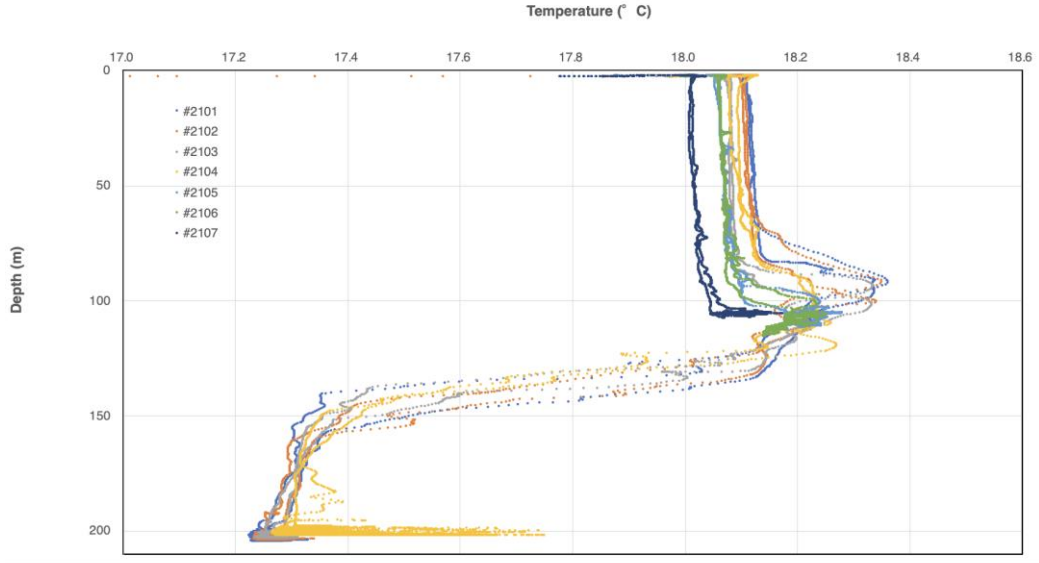
I-2. Sediments

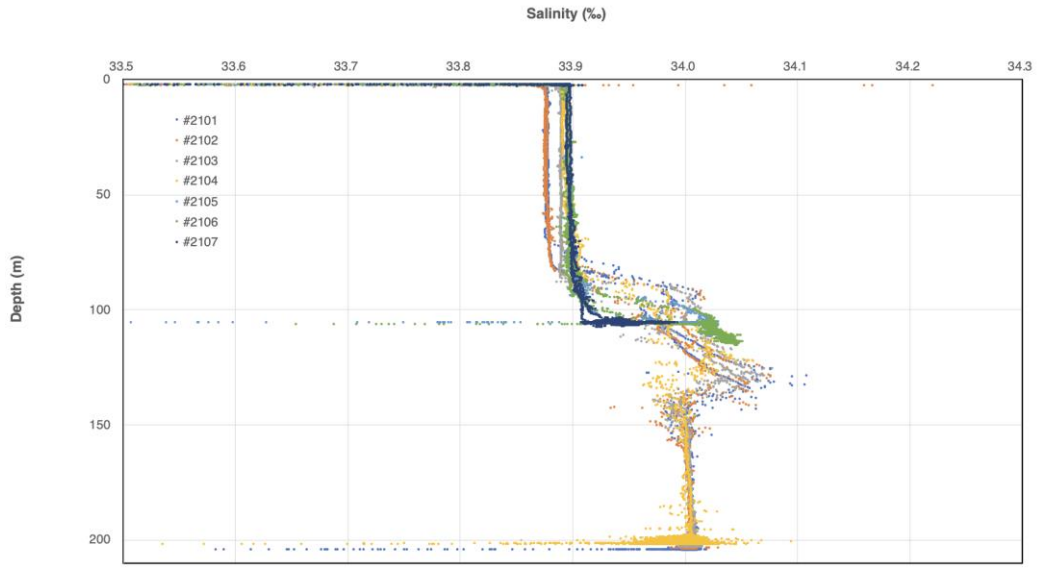
Dive #	On board ID	Sample Category	Sampling Method	Preservation	Date Collected				Latitude (N)		Longitude (E)		Depth	Area	Locality	Remarks	Distribution/Purpose
					yyyy	mm	dd	hh:mm	Deg.	Min.	Deg.	Min.	(m)				
2101	HPD#2101B	Mud	Manipulator & Kumade	4°C&Frozen	2020	1	18	12:32	31	39.7444	130	46.2944	201	Kagoshima Bay	Wakamiko Crater	Box (large)	Yamanaka/Mineral&rock analysis
	HPD#2101S	Mud	Suction sampler	4°C&Frozen	2020	1	18	13:04	31	39.7444	130	46.2944	201	Kagosima Bay	Wakamiko Crater	multiple canisters	Yamanaka/Mineral&rock analysis
2104	HPD#2104B1	Mud	Manipulator & Kumade	4°C&Frozen	2020	1	19	14:11	31	40.1077	130	45.6782	198	Kagoshima Bay	Wakamiko Crater	Box (large)	Yamanaka/Mineral&rock analysis
	HPD#2104B2	Mud	Manipulator & Kumade	4°C&Frozen	2020	1	19	14:40	31	40.0720	130	45.6839	198	Kagoshima Bay	Wakamiko Crater	Box (middle)	Yamanaka/Mineral&rock analysis

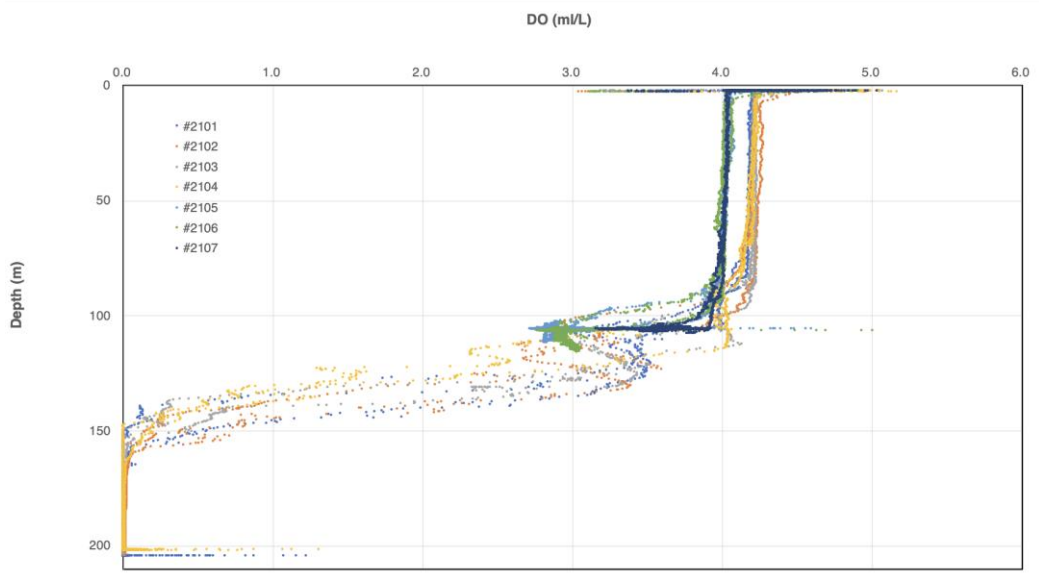
I-3. Water

Dive #	On board ID	Sample Category	Sampling Method	Preservation	Date Collected				Latitude (N)		Longitude (E)		Depth [m]	Area	Locality	Remarks	Distribution /Purpose
					yyyy	mm	dd	hh:mm	Deg.	Min.	Deg.	Min.					
2101	HPD#2101V	Seawater	Vacuum sampler	4°C	2020	1	18	10:42	31	39.7459	130	46.2943	200	Kagoshima Bay	Wakamiko Crater	Filtered (0.45 µm)	Yamanaka/Ion analysis
	HPD#2101N1	Seawater	Niskin bottle	4°C	2020	1	18	12:02	31	39.7444	130	46.2944	201	Kagoshima Bay	Wakamiko Crater	Green/Filtered (0.45 µm)	Yamanaka/Ion analysis
	HPD#2101N2	Seawater	Niskin bottle	4°C	2020	1	18	12:04	31	39.7444	130	46.2944	201	Kagoshima Bay	Wakamiko Crater	Red/Filtered (0.45 µm)	Yamanaka/Ion analysis
2104	HPD#2104V	Seawater	Vacuum sampler	4°C	2020	1	19	12:46	31	40.1077	130	45.6782	198	Kagoshima Bay	Wakamiko Crater	Filtered (0.45 µm)	Yamanaka/Ion analysis
2105	HPD#2105N1	Seawater	Niskin bottle	RT	2020	1	20	8:55	31	39.7562	130	48.0648	102	Kagoshima Bay	Wakamiko Crater	Green	Fujiwara/onboard experiments
	HPD#2105N2	Seawater	Niskin bottle	RT	2020	1	20	8:55	31	39.7562	130	48.0648	102	Kagoshima Bay	Wakamiko Crater	Red	Fujiwara/onboard experiments
	HPD#2106N1	Seawater	Niskin bottle	RT	2020	1	20	14:26	31	39.7560	130	48.0633	103	Kagoshima Bay	Wakamiko Crater	Green	Fujiwara/onboard experiments
	HPD#2106N2	Seawater	Niskin bottle	RT	2020	1	20	14:26	31	39.7560	130	48.0633	103	Kagoshima Bay	Wakamiko Crater	Red	Fujiwara/onboard experiments
	HPD#2107N1	Seawater	Niskin bottle	RT	2020	1	21	8:55	31	39.7580	130	48.0646	103	Kagoshima Bay	Wakamiko Crater	Green	Fujiwara/onboard experiments
	HPD#2107N2	Seawater	Niskin bottle	RT	2020	1	21	8:55	31	39.7580	130	48.0646	103	Kagoshima Bay	Wakamiko Crater	Red	Fujiwara/onboard experiments

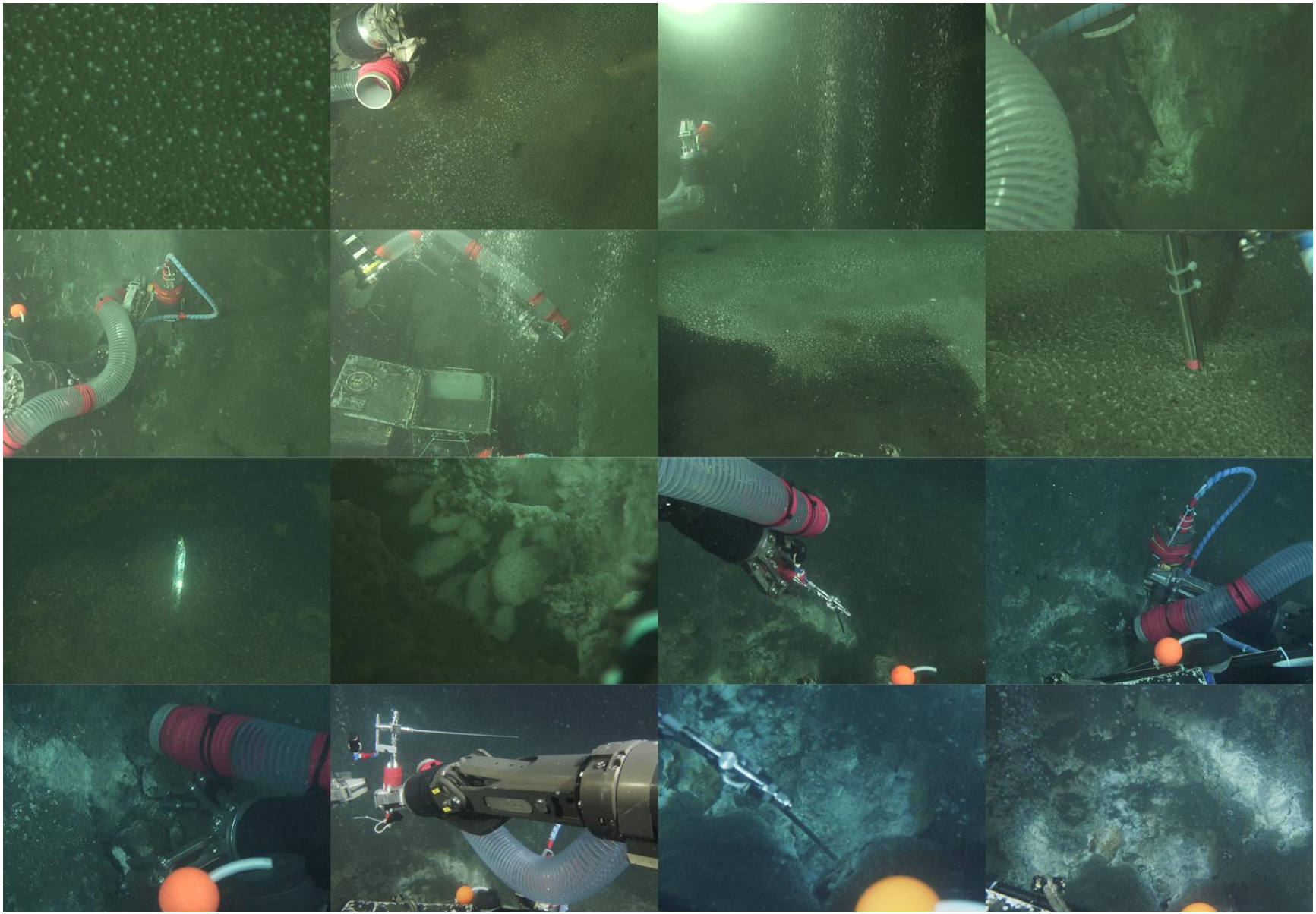
II. CTD/DO profiles



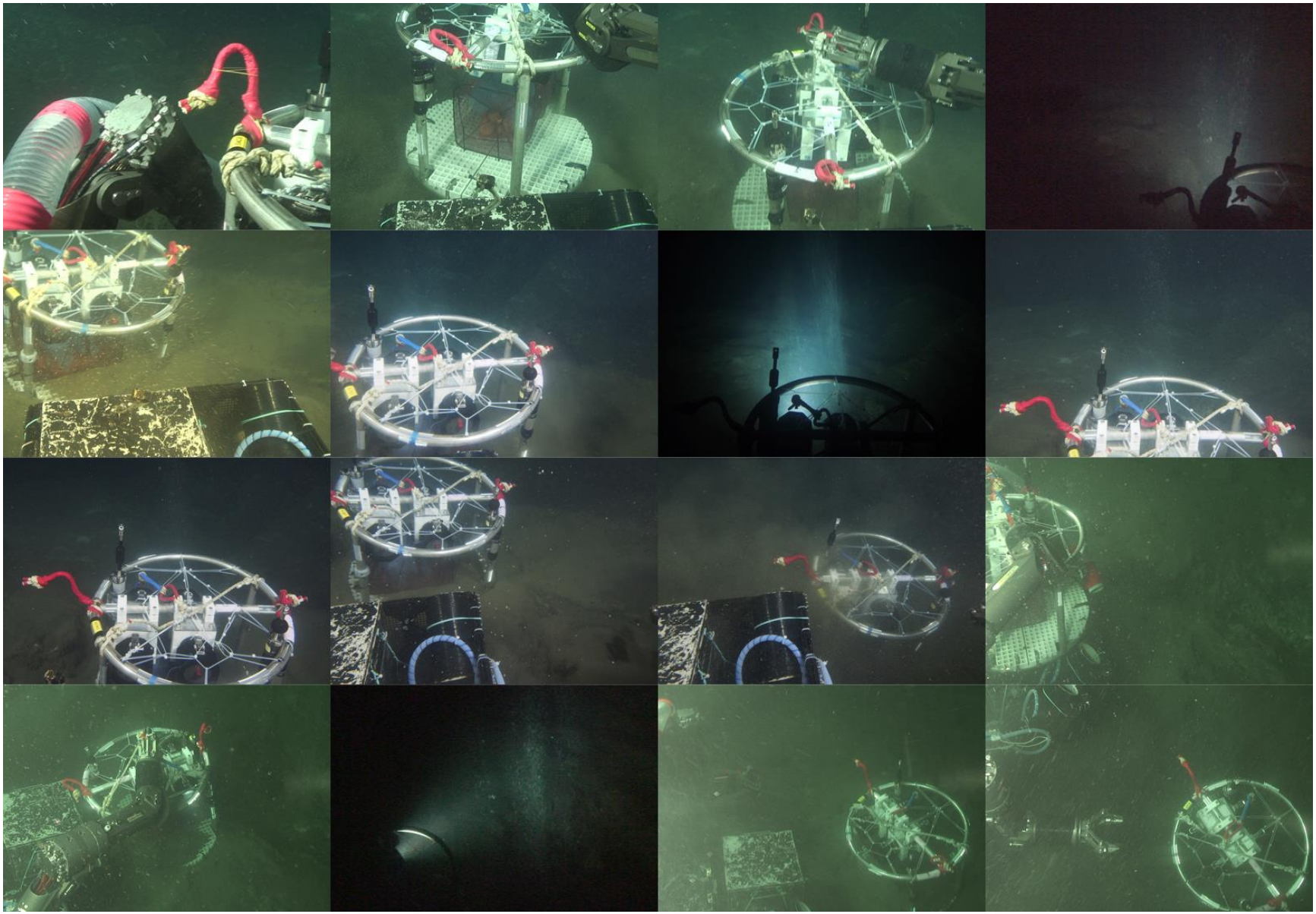




III. Still images from each dive: *HD#2101*



HD#2102



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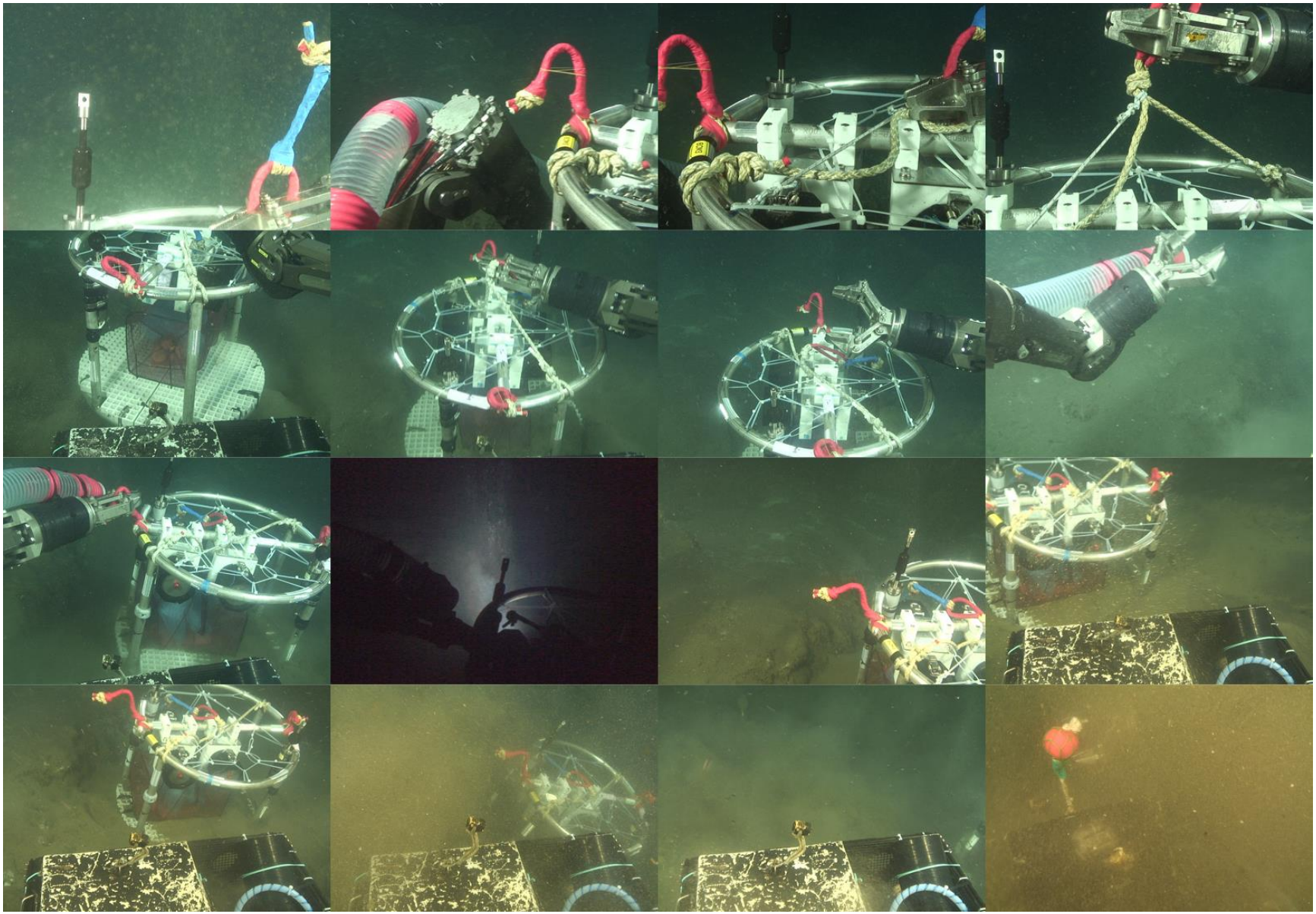

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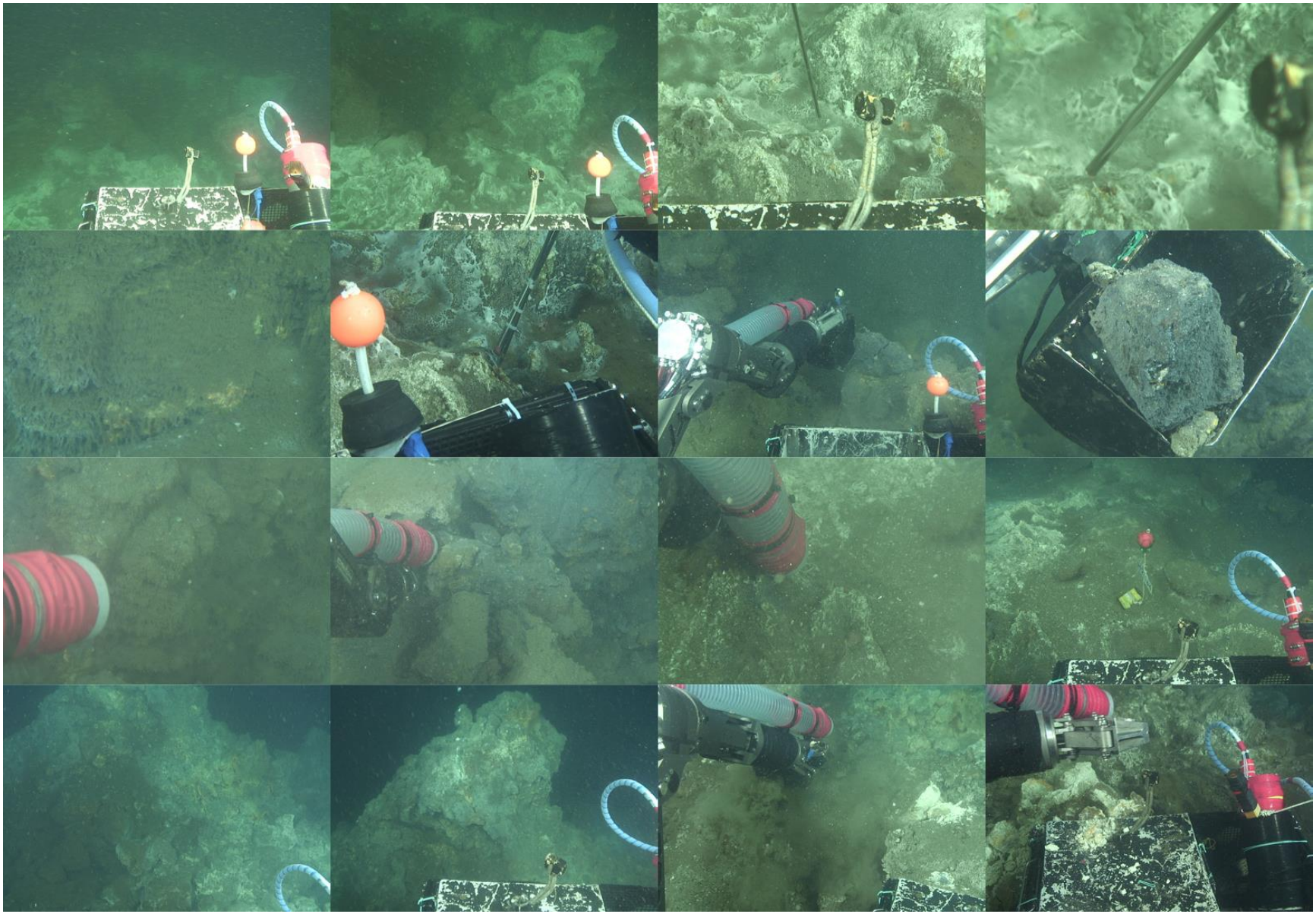

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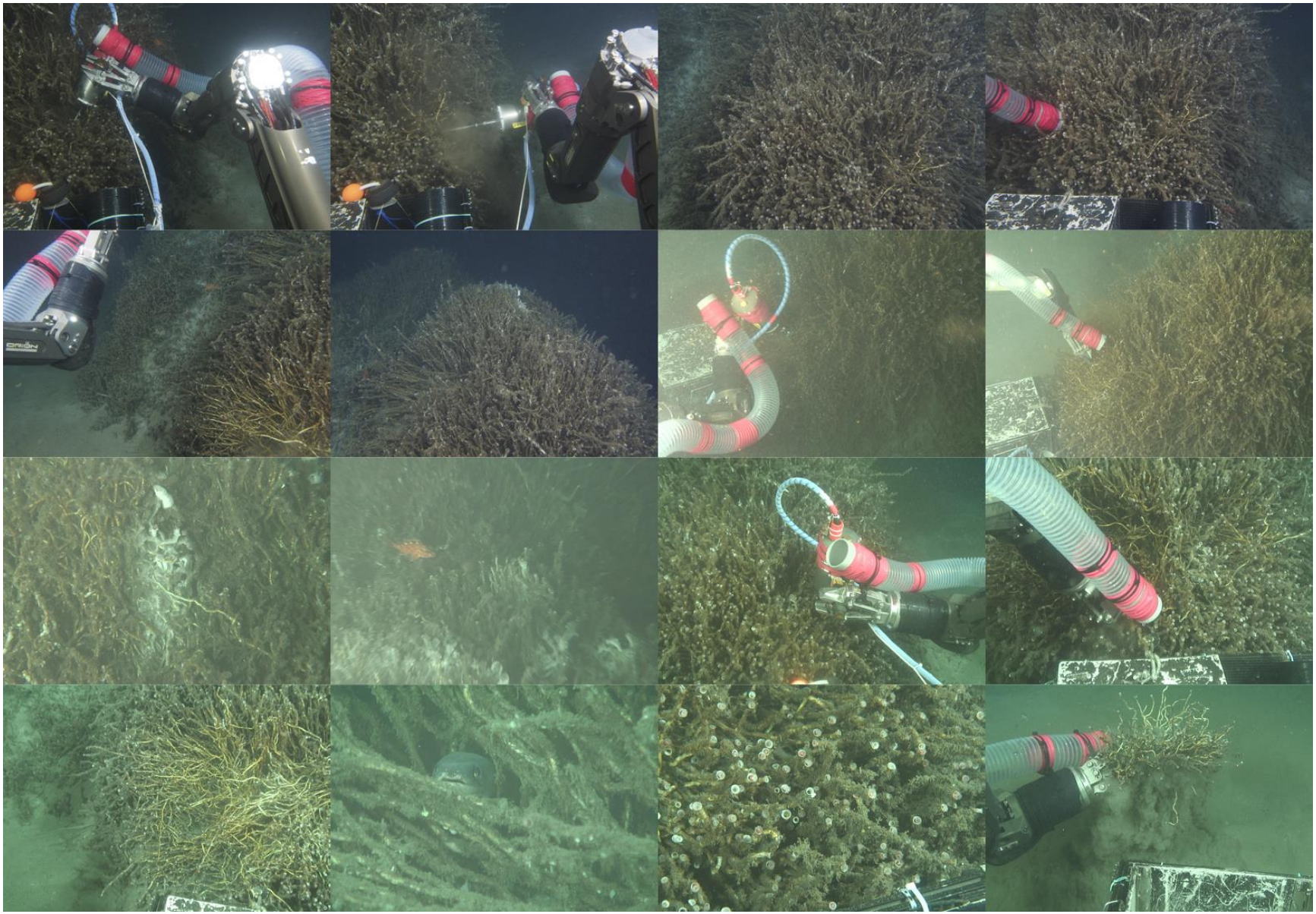

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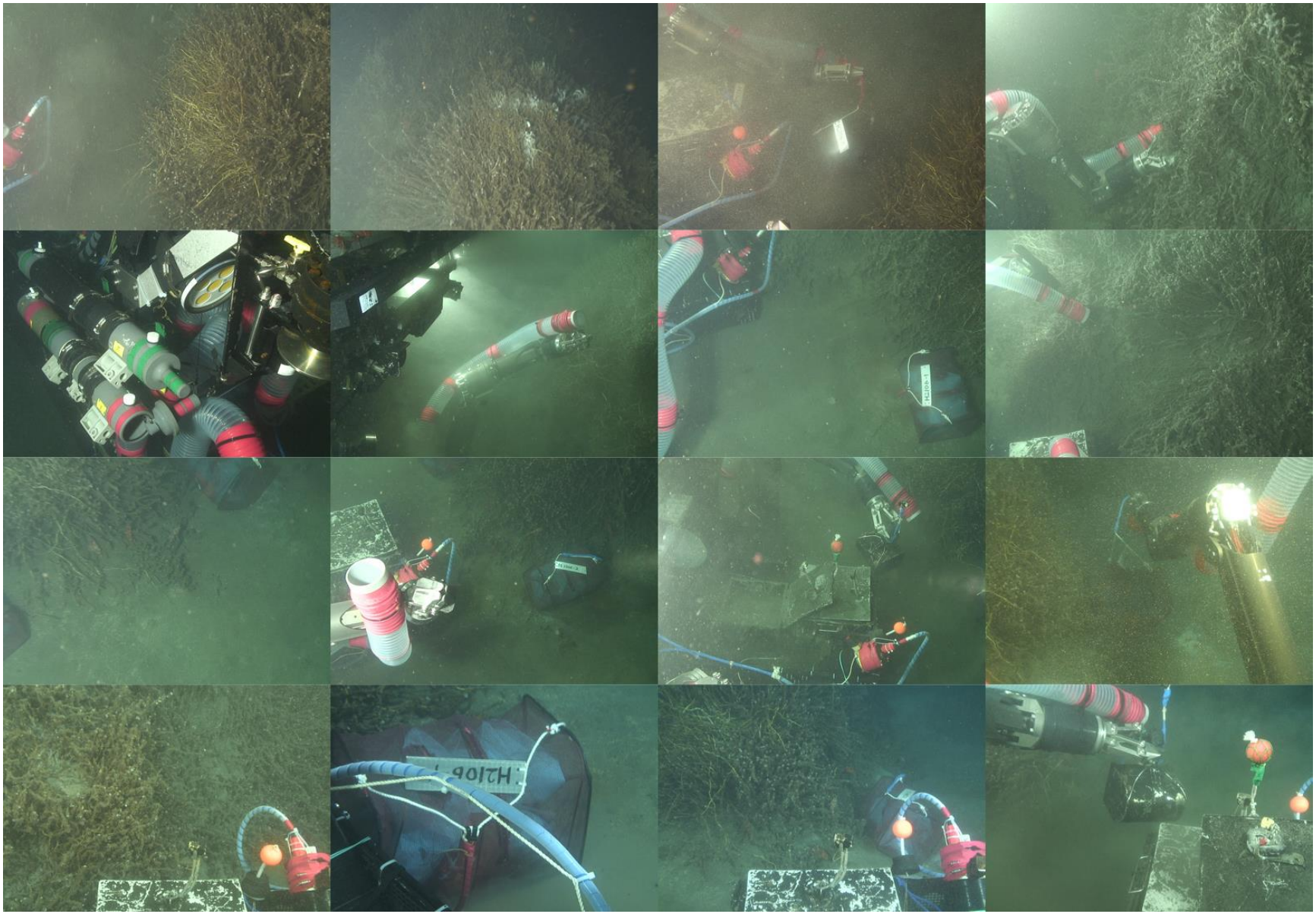

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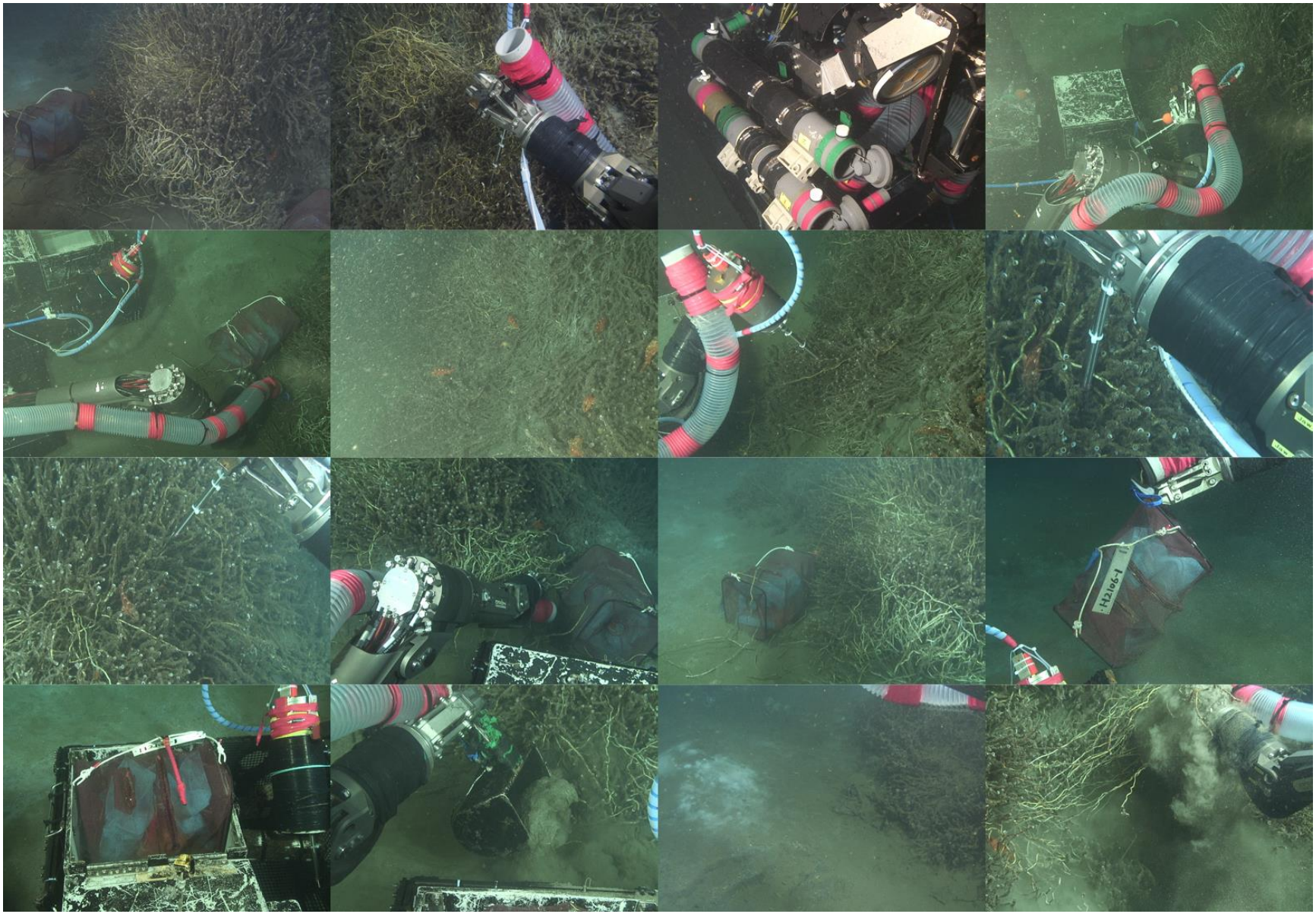


IV. Video file list

Dive No.	Date	Camera	Start	End	File Name
HD#2101	2020 1.18	Main	8:34:00	13:12:00	20200118_083400_131200_HD2101Main.mov
HD#2101	2020 1.18	Sub	8:47:00	13:13:00	20200118_084700_131300_HD2101Sub.mov
HD#2102	2020 1.18	Main	15:20:00	16:14:00	20200118_152000_161400_HD2103Main.mov
HD#2102	2020 1.18	Sub	15:21:00	16:15:00	20200118_152100_161500_HD2102Sub.mov
HD#2103	2020 1.19	Main	8:18:00	8:54:00	20200119_081800_085400_HD2103Main.mov
HD#2103	2020 1.19	Sub	8:19:00	8:55:00	20200119_081900_085500_HD2103Sub.mov
HD#2104	2020 1.19	Main	11:25:00	16:10:00	20200119_112500_161000_HD2104Main.mov
HD#2104	2020 1.19	Sub	11:26:00	16:11:00	20200119_112600_161100_HD2104Sub.mov
HD#2105	2020 1.20	Main	8:18:00	11:55:00	20200120_081800_115500_HD2105Main.mov
HD#2105	2020 1.20	Sub	8:19:00	11:56:00	20200120_081900_115600_HD2105Sub.mov
HD#2106	2020 1.20	Main	13:25:00	16:08:01	20200120_132500_160801_HD2105Main.mov
HD#2106	2020 1.20	Sub	13:26:00	16:09:01	20200120_132600_160901_HD2106Sub.mov
HD#2107	2020 1.21	Main	8:24:00	13:07:00	20200121_082400_130700_HD2107Main.mov
HD#2107	2020 1.21	Sub	8:25:00	13:09:00	20200121_082500_130900_HD2107Sub.mov

V. Shipboard log & ship track

Shipboard log

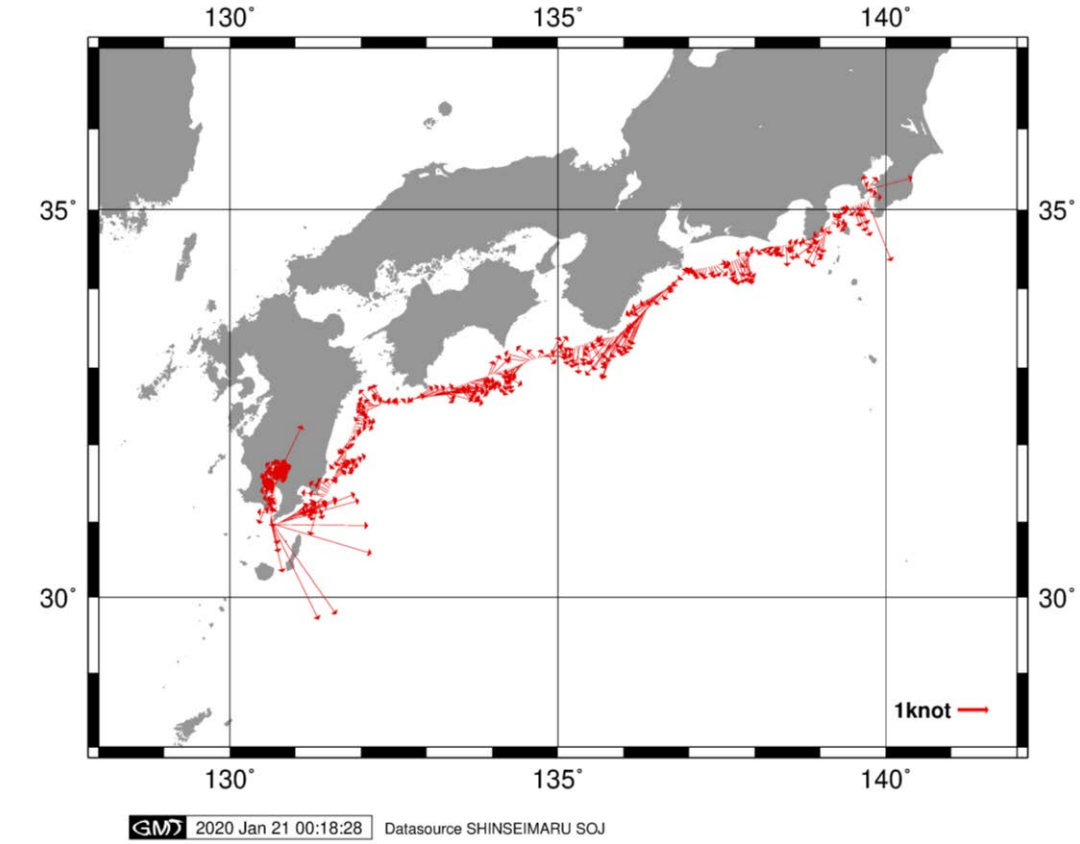
Date / Time	Descriptions	Weather, Wind and Sea condition
2020/1/15		
Wed.	**Noon Position:35-19.2N,139-39.0E(YOKOSUKA Port, JAMSTEC)**	
13:00	Onboarded	
14:00	Let go all shore lines and left JAMSTEC for KAGOSHIMA Bay	
15:00-15:30	Carried out education and training for scientists	
x16:00-16:30	Scientists meeting	
18:00-18:30	Scientists meeting	
2020/1/16		
Thu.	**Noon Position:33-23.5N,135-49.0E(Off SHIONOMISAKI)**	**c/NNE-2/2**
18:00-18:30	Scientists meeting	
2020/1/17		
Fri.	**Noon Position:31-24.5N,131-30.5E(Off TOINOMISAKI)**	**r/Morth-5/4**
18:00-18:30	Scientists meeting	
2020/1/18		
Sat.	**Noon Position:31-40.0N,130-46.5E(KAGOSHIMA Bay)**	**bc/NNW-3/2**
7:20	Arrived at research area KAGOSHIMA Bay	
7:28	Released XBT (31-39.5'N, 130-46.3'E)	
7:30	Arrived at dive point V1	
8:17	Hoisted up "Hyper-Dolphin"	
8:21	Launched "Hyper-Dolphin"	
8:33	"Hyper-Dolphin" dove & com'ced her operation #Dive NO. 2101 (WAKAMIKO Caldera site)	

Date / Time	Descriptions	Weather, Wind and Sea condition
8:44	"Hyper-Dolphin" landed on the sea bottom (D: 199m)	
13:08	"Hyper-Dolphin" left the sea bottom (D: 201m)	
13:24	Hoisted up "Hyper-Dolphin"	
13:29	Recovered "Hyper-Dolphin" & finished her operation	
15:07	Hoisted up "Hyper-Dolphin"	
15:11	Launched "Hyper-Dolphin"	
15:19	"Hyper-Dolphin" dove & com'ced her operation #Dive NO. 2102 (WAKAMIKO Caldera site)	
15:30	"Hyper-Dolphin" landed on the sea bottom (D: 200m)	
16:08	"Hyper-Dolphin" left the sea bottom (D: 200m)	
16:17	Hoisted up "Hyper-Dolphin"	
16:28	Recovered "Hyper-Dolphin" & finished her operation	
16:43-17:25	Carried out MBES mapping survey	
18:00-18:30	Scientists meeting	
2020/1/19 Sun.	**Noon Position:31-40.0N,130-45.5E(KAGOSHIMA Bay)**	**c/west-1/1**
7:45	Arrived at dive point	
8:11	Hoisted up "Hyper-Dolphin"	
8:15	Launched "Hyper-Dolphin"	
8:24	"Hyper-Dolphin" dove & com'ced her operation #Dive NO. 2103 (WAKAMIKO Caldera site)	
8:35	"Hyper-Dolphin" landed on the sea bottom (D: 200m)	
8:50	"Hyper-Dolphin" left the sea bottom (D: 200m)	
9:02	Hoisted up "Hyper-Dolphin"	
9:13	Recovered "Hyper-Dolphin" & finished her operation	
11:11	Hoisted up "Hyper-Dolphin"	
11:15	Launched "Hyper-Dolphin"	

Date / Time	Descriptions	Weather, Wind and Sea condition
11:24	"Hyper-Dolphin" dove & com'ced her operation #Dive NO. 2104 (WAKAMIKO Caldera site)	
11:34	"Hyper-Dolphin" landed on the sea bottom (D: 199m)	
16:06	"Hyper-Dolphin" left the sea bottom (D: 198m)	
16:17	Hoisted up "Hyper-Dolphin"	
16:27	Recovered "Hyper-Dolphin" & finished her operation	
19:00-19:15	Scientists meeting	
2020/1/20 Mon.	**Noon Position:31-39.5N,130-48.0E(KAGOSHIMA bay)**	**c/NW-1/1**
8:13	Hoisted up "Hyper-Dolphin"	
8:16	Launched "Hyper-Dolphin"	
8:24	"Hyper-Dolphin" dove & com'ced her operation #Dive NO. 2105 (HAORIMUSHI site)	
8:37	"Hyper-Dolphin" landed on the sea bottom (D: 108m)	
10:51	"Hyper-Dolphin" left the sea bottom (D: 102m)	
11:03	Hoisted up "Hyper-Dolphin"	
11:12	Recovered "Hyper-Dolphin" & finished her operation	
13:14	Hoisted up "Hyper-Dolphin"	
13:18	Launched "Hyper-Dolphin"	
13:27	"Hyper-Dolphin" dove & com'ced her operation #Dive NO. 2106 (HAORIMUSHI site)	
13:36	"Hyper-Dolphin" landed on the sea bottom (D: 108m)	
16:03	"Hyper-Dolphin" left the sea bottom (D: 103m)	
16:18	Hoisted up "Hyper-Dolphin"	
16:27	Recovered "Hyper-Dolphin" & finished her operation	
18:45-19:15	Scientists meeting	

Date / Time	Descriptions	Weather, Wind and Sea condition
2020/1/21		
Tue.	**Noon Position:**	**c/NW-1/1**
8:17	Hoisted up "Hyper-Dolphin"	
8:20	Launched "Hyper-Dolphin"	
	"Hyper-Dolphin" dove & com'ced her operation #Dive NO. 2107	
8:32	(HAORIMUSHI site)	
8:44	"Hyper-Dolphin" landed on the sea bottom (D:105m)	
13:00	"Hyper-Dolphin" left the sea bottom (D: 103m)	
13:20	Hoisted up "Hyper-Dolphin"	
13:24	Recovered "Hyper-Dolphin" & finished her operation	
13:30	departure from HAORIMUSHI site for KAGISHIMA port	
15:20	Let go port anchor, arrived at KAGISHIMA port off	
18:00-		
19:00	Scientists meeting	
	Sent out 1st shore line, arrived at KAGOSHIMA port, then completed voy. No.	
10:00	KS-20-2	
	Disembarked, then completed voyage No. KS-20-2	

Ship track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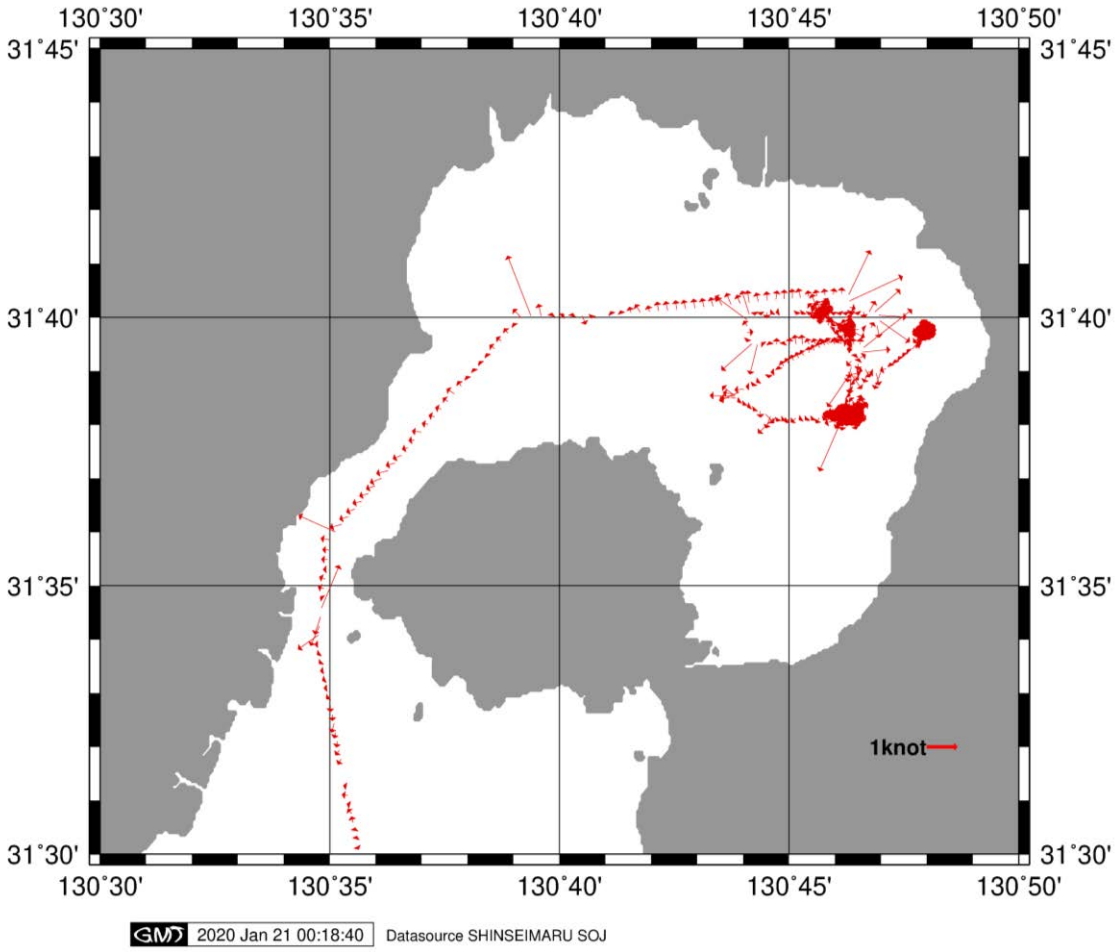
135°
140°
35°
30°
1knot
2020 Jan 21 00:18:28
Datasource SHINSEIMARU SOJ


130°30'
130°35'
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130°50'
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31°40'
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31°30'
1knot
2020 Jan 21 00:18:40
Datasource SHINSEIMARU SOJ

VI. Group portrait

VII. Miscellaneous photographs

VIII. Acknowledgements

We thank the captain and crew of the R/V *Shinsei Maru*, and the operation team of ROV *Hyper-Dolphin* for conducting the diving research and sampling. We also thank the Operations Department of vessels for support of cruise coordination.

January 22, 2020

Chief scientist of KS-20-2 cruise

FUJIWARA, Yoshihiro

& onboard researchers