

New high-tech flexible networks for the monitoring of deep-sea ecosystems

Running Head: High-tech deep-sea monitoring networks

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43 **ABSTRACT**

44 Increasing interest in the acquisition of biotic and abiotic resources from within
45 the deep sea (e.g. fisheries, oil-gas extraction, and mining) urgently imposes the
46 development of novel monitoring technologies, beyond the traditional vessel-
47 assisted, time-consuming, high-cost sampling surveys. The implementation of
48 permanent networks of seabed and water-column cabled (fixed) and docked
49 mobile platforms is presently enforced, to cooperatively measure biological
50 features and environmental (physico-chemical) parameters. Video and acoustic
51 (i.e. optoacoustic) imaging are becoming central approaches for studying benthic
52 fauna (e.g. quantifying species presence, behaviour, and trophic interactions) in
53 a remote, continuous, and prolonged fashion. Imaging is also being
54 complemented by *in situ* environmental-DNA sequencing technologies, allowing
55 the traceability of a wide range of organisms (including prokaryotes) beyond the
56 reach of optoacoustic tools. Here, we describe the different fixed and mobile
57 platforms of those benthic and pelagic monitoring networks, proposing at the
58 same time an innovative roadmap for the automated computing of hierarchical
59 ecological information of deep-sea ecosystems (i.e. from single species'
60 abundance and life traits, to community composition, and overall biodiversity).

61

62 **KEYWORDS:** Deep sea, monitoring networks, cabled observatories, crawlers,
63 video and acoustic imaging, biological variables, ecosystem indicators

64

65 **1. Introduction**

66 Throughout the Anthropocene Era (Crutzen & Steffen, 2003) the human
67 footprint on the ecosystems of the global ocean has been increasing continuously
68 (Halpern et al., 2015). As this footprint is rapidly expanding toward great depths,
69 the need for a global observing effort in the deep ocean is crucial (Levin & Le
70 Bris, 2015). The accurate monitoring of our incursive impacts on marine
71 ecosystems, however, requires the development of novel and effective
72 technological solutions.

73 The deep-sea seafloor and overlying waters (below 200 m depth) form the
74 largest biome on Earth, although it remains poorly explored (Ramirez-Llodra et
75 al., 2010; Mora et al., 2011; Sutton et al., 2017). The monitoring of key ecosystem
76 features and functions has proven difficult, owing to the extreme environmental
77 conditions associated with these depths (e.g. high pressures, low temperatures,
78 corrosiveness and remoteness), coupled with limited sampling capabilities
79 offered by low numbers of adequately equipped research vessels (Woodall et al.,
80 2018).

81 In order to sustain correct management and protection actions a
82 spatiotemporally extended monitoring regime must be implemented to gather
83 data on species and their communities across the vast extent of the great global
84 ocean basins (Danovaro et al., 2017). Clear examples of shortfalls in current data
85 include the lack of knowledge on biomass, abundance, reproductive cycles,
86 population dynamics (i.e. growth and mortality), migrations and geographic
87 ranges (Danovaro et al., 2014). Furthermore, community biodiversity, food web
88 structures and the influence of organic matter transfer within ecosystem
89 compartments and across boundaries are also poorly studied in relation to the

90 neighboring shallower and coastal ecosystems (Snelgrove et al., 2018). All these
91 aspects have repercussions on penetration and propagation of human footprint
92 into marine ecosystems (e.g. pollutants and microplastics; Zhao et al., 2018).

93 To fill these knowledge gaps, the efficient integration of ongoing
94 technological developments into a strategic framework for deep-sea monitoring
95 is critical (e.g. Aguzzi et al., 2012). Such development should be capable of
96 producing tools for the spatiotemporal location and quantification of deep-sea
97 organisms across a wide range of body sizes, as well as their activity and
98 response to changing environmental conditions and anthropogenic stressors.

99

100 **1.1. Objectives**

101 In this study, we review the status and development of high-tech, interactive
102 networks of fixed and mobile platforms, currently used for spatiotemporally
103 flexible and appropriate monitoring of deep-sea ecosystems. We propose an
104 innovative roadmap for the hierarchical extraction of ecosystem indicators related
105 to assemblage structure, biodiversity and ecosystem functioning, as obtained
106 from biological variables encompassing species abundances, demographic
107 descriptors, and behavior. We center our analysis on ecosystem indicators
108 extracted from video and acoustic imaging of marine megafauna (i.e. organisms
109 of size from centimeters and above), representing the apical ecological
110 complexity component, that is fundamental in conditioning ecosystem
111 functioning, services, and health (Schoening et al., 2012).

112

113 **2. Growing high-tech cabled observatory networks**

114 The ongoing technological development in seafloor cabled observatories is
115 motivated by the growing awareness about the strategic value of acquiring
116 multidisciplinary biological and environmental data in a concomitant fashion, in
117 order to derive putative cause-effect relationships as drivers of ecosystem
118 changes (Favali et al., 2010; 2015; Lelievre et al., 2017). The successful
119 integration of such platforms equipped with camera systems, multi-parametric
120 biogeochemical, oceanographic, and biological sensors with seafloor power and
121 communication cables now allows the remote, continuous, high-frequency (> 1Hz
122 as real-time), long-term (up to decades) monitoring of the deep-sea biome (Ruhl
123 et al., 2011). In this highly integrated monitoring approach, megafauna
124 identification, tracking and counting through optoacoustic and new molecular
125 sensors should be a key focus, in relation to productivity and services (e.g.
126 fishery; Aguzzi et al., 2015).

127 Throughout the last two decades, cabled observatories have provided
128 relevant data, helping to fill the gaps in knowledge on species presence, behavior,
129 and associated changes in biodiversity and ecosystem function (**Table 1**).
130 Unfortunately, cabled systems are fixed and have limited spatial coverage when
131 the deep continental margins and ocean basins are considered as a whole
132 (Aguzzi et al., 2015; Danovaro et al., 2017). An attempt to overcome such a
133 limitation has occurred in some cases through the installation of a local network
134 of seabed platforms. Good examples are the Ocean Network Canada (ONC),
135 Deep-ocean Environmental Long-term Observatory System (DELOS) and
136 Lofoten Verlag observatory (LoVe), respectively in Juan de Fuca plate (NW
137 Pacific), off Angola (SE Atlantic) and in Norway (Barnes et al., 2007; Vardaro et
138 al., 2013; Bagley et al., 2015; Osterloff et al., 2016).

139 The deployment of observatory modules in clusters with separation
140 distances on the order of hundreds of meters or a few kilometres is presently
141 envisaged to maximize the ability to quantify species distributions and habitat
142 associations over multiple scales (see **Table 1**). Deploying multiple ecosystem
143 observatory clusters along environmental or habitat gradients would be effective
144 in elevating the system from examination of local habitats to ecosystem level
145 observation. Each node can acquire imaging and acoustic as well as
146 multiparametric environmental data in a temporally coordinated fashion.
147 Accordingly, temporal changes in species presence and abundance in an area of
148 the deep sea can be tracked through neighboring environmental niches (Doya et
149 al., 2017; Thomsen et al., 2017).

150

151 ***2.1. Permanent mobile platforms increase spatial monitoring capability***

152 Nevertheless, networks of cabled observatories are not enough to ensure
153 efficient monitoring across highly variable benthic seascapes (Aguzzi et al.,
154 2015). Presently, there is a drive to integrate mobile platforms through docking
155 stations into existing cabled observatory infrastructures, to provide extended
156 coverage at local, regional and basin-wide spatial scales, both on the seafloor
157 and within the water column (**Figure 1**). Benthic mobile platforms are represented
158 by crawlers: a new class of Internet Operated Vehicles (IOVs), tethered to cabled
159 observatories (Purser et al., 2013). These tracked vehicles are capable of real-
160 time navigation control and data collection *via* simple web browser interfaces
161 operable from anywhere. At the same time, a new class of rovers, non-tethered
162 benthic mobile crawlers are entering into active research, capable of
163 automatically returning to the docking station for charging, data transfer or

164 recovery (Flögel, 2015). To complement the seafloor monitoring capacities of
165 crawler systems, pelagic monitoring is presently achieved using tethered
166 Remotely Operated Vehicles (ROVs) and free swimming Autonomous
167 Underwater Vehicles (AUVs) some of which may, also dock with cabled stations
168 for energy recharge and data transmission (Bellingham, 2016). These also allow
169 monitoring of the water column at a high-frequency over extended periods and
170 across depth strata (e.g. Ludvigsen & Sorensen, 2016; Masmitjia et al., 2018).

171 Each of these mobile platforms provides a unique contribution to the
172 ecosystem observatory, as well as some task redundancy. The AUV equipped
173 with imaging or acoustic devices is ideally suited for habitat and biota distribution
174 mapping (Morris et al., 2014, Williams et al., 2016) and can be used to conduct
175 transects around the observatory and between observatories. The AUV provides
176 the highest mobility and flexibility in sampling design for mapping with impacts on
177 the benthic habitat by maritime activities (e.g. noise, substrate disturbance at
178 different scales and artificial light pollution effects). Although the ROV design
179 implies a tether, such a platform has also a high mobility and it can be used
180 similarly to AUVs (Robinson et al., 2017), with the advantage of having two way
181 real-time data transmission and manipulator arms to be used for management
182 and maintenance tasks within the monitoring infrastructure (e.g. manipulative
183 experiments or for placing autonomous recorders such as stand-alone
184 autonomous cameras). In addition, ROVs are the best option for collecting video
185 data on the development of the fouling community on the observatory
186 infrastructure and fauna association with the structure. The major drawback of
187 ROVs is that they must operate with thrusters, creating high levels of noise and
188 their limited ability to conduct sampling and observations at specific locations for

189 extended periods of time (Rountree & Juanes, 2010). Crawlers, on the other
190 hand, can be used to conduct census observations at specific locations (in
191 constant transect or stepping-stone fashion) for extended time periods (minutes
192 to hours). Crawlers can also share some infrastructure servicing tasks with the
193 ROVs and carry larger payloads. Drawbacks to crawlers include noise
194 production, but more importantly physical disturbance of the benthic habitat and
195 associated fauna along the movement tracks.

196

197 **2.2. Benthic networks growing in the pelagic realm**

198 The need to monitor energy fluxes between pelagic and benthic ecosystem
199 compartments (i.e. benthopelagic coupling) and their spatiotemporal changes
200 (e.g. Griffiths et al., 2017), requires the development of three-dimensional
201 monitoring networks of platforms, with cabled nodes and mobile platforms
202 operating in tandem (**Figure 2**). This ecologically integrated monitoring is
203 presently being facilitated by incorporating to the benthic data collection,
204 secondary data streams supplied by water column fixed (i.e. moored) and
205 superficial buoys, as well as satellites (Thomsen et al., 2017). Satellites are
206 optimal tools for gathering large-scale physicochemical data from superficial (i.e.
207 epipelagic) ecosystems, quantifying relevant biological variables from ocean
208 color (e.g. chlorophyll content, particulate matter, etc.). Unfortunately, satellite
209 sensors cannot penetrate much beyond the surface of global water mass, and
210 therefore pelagic buoys are more appropriate for the monitoring of sub-surface
211 oceanic strata.

212 In this scenario, benthopelagic monitoring capabilities are also being
213 potentiated *via* data collection from the routine operations of large astrophysical

214 experimental infrastructures, such as underwater neutrino telescopes (see
215 **Figure 1C**). These telescopes consist of arrays of vertically moored (up to 700
216 m), flexible, strings or towers of photon detectors (Photo-Multiplier Tubes; PMTs)
217 for neutrino particle quantification, placed at different altitudes above the seabed
218 and connected to shore *via* power and fiber-optic data cables (Adrián-Martínez
219 et al., 2016b).

220 Although the primary use of these platforms is within the high-energy
221 astrophysics domain (Adrián-Martínez et al., 2016a), their infrastructure provides
222 a network of subsea connection points and sensors usable for marine ecological
223 monitoring. Hydrophones for passive acoustic listening are connected to the
224 system to monitor position of the towers in relation to currents and to
225 simultaneously triangulate PMT location with the aid of acoustic beacons, so that
226 the trajectories of detected neutrinos can be properly computed. As a by-product,
227 this real-time acoustic monitoring produces useful oceanographic flow condition
228 data and information on anthropogenic marine noise, as well as cetacean
229 movement, population structure, and communication (Nosengo et al., 2009;
230 Sciacca et al., 2015; Viola et al., 2017). The PMT detectors themselves also
231 provide unique high-frequency and continuous data on bioluminescence, as
232 swimming animals luminesce when hitting the infrastructures (Aguzzi et al.,
233 2017). At time of writing, real-time and continuous data acquisition from these
234 telescope infrastructures as a whole is providing important information on
235 seasonal changes in gravity carbon fluxes and controlling oceanographic
236 processes (e.g. dense shelf water cascading and effects on deep-sea bacterial
237 productivity; Tamburini et al., 2013; Adrián-Martínez et al., 2016b; Durrieu de
238 Madron et al., 2017).

239

240 **3. A roadmap for the monitoring of ecosystem indicators**

241 The development of efficient deep-sea ecosystem monitoring is currently
242 based on the successful extraction and quantification of key ecosystem
243 characteristics (e.g. biogeochemistry, animal presence, abundance and
244 behavior, local and regional biodiversity, ecosystem functioning; see **Table 1**).
245 This monitoring development is being based on the combined use of optoacoustic
246 and molecular biological sensors which are being implemented in the framework
247 of cabled observatories. The capability to acquire temporally-related time series
248 of multiparametric habitat and biological data, allows researchers to envision
249 aspects such as, benthic primary production *via* chemosynthesis, deep-sea
250 species ecological niches and food web structure (Aguzzi et al., 2011; 2012;
251 2018). These datasets can be used to feed new numerical-based ecology
252 approaches centered on multivariate statistics, time series analysis and
253 ecosystem modeling (e.g. Borcard & Legendre, 2002, Matabos et al., 2014;
254 Puillat et al., 2014; Thomsen et al., 2017), in order to estimate the level of
255 significance for putative cause - effects relationships (i.e. environmental control
256 *versus* species and communities response) and provide an immediate vision of
257 complex ecological processes at a local scale (e.g. species tolerance to the
258 variation of key habitat drivers). This approach allows a transition from a still too
259 descriptive deep-water and deep-sea ecology into a more quantitative one, as
260 occurs in more directly accessible coastal areas and land.

261 To optimize the outcome quality from a highly-integrated deep-sea
262 monitoring strategy of this type, protocols for data collection and analysis should
263 be implemented to efficiently characterize local biodiversity along with those

264 processes that sustain it and determine the overall ecosystem functioning and
265 health status (Allen et al., 2008; Danovaro et al., 2016). From an operational point
266 of view, a bottom-up scheme of monitoring should be conceived with cabled
267 observatories and docked mobile platforms producing video and acoustic
268 imaging information on fauna within a wide range of sizes (e.g. from macro-
269 zooplankton to megafauna classification and counting, morphometric description,
270 and quantification of intra- and inter-specific interactions). Then, acquired
271 baseline biological data can be directly related to multiparametric environmental
272 information obtained *via* the concomitant collection of geochemical and
273 oceanographic data (Aguzzi et al., 2012; 2015; Ferrari et al., 2016).

274

275 **3.1. The central role of optoacoustic technologies for monitoring**

276 High-definition still and video image data (e.g. 2D, 3D, hyperspectral) and
277 active acoustic imaging (i.e. multi-beam cameras; Juanes, 2018) to date
278 represent key approaches for the optoacoustic monitoring of remote deep-sea
279 ecosystems (Danovaro et al., 2017). Outputs of optoacoustic monitoring provide
280 relevant data for management in key human activities, as for example fisheries
281 or jellyfish blooms (Samhuri et al., 2014, Bicknell et al., 2016, Corgnati et al.,
282 2016; Marini et al., 2018). Moreover, species distribution and habitat use can be
283 studied over extended spatial scales by mosaicking high-resolution imagery,
284 captured by mobile platforms operating in the regions surrounding the cabled
285 infrastructure stations (Purser et al., 2013) or by integrating laser-scanning
286 systems into the mobile platforms, to create high-resolution 3D full-color surface
287 models (GE Reports Canada, 2017). Further development of similar methods that
288 expand the spatial coverage of (stereo)-imaging data, can help with the

289 quantification of other biological components and fauna sizes of high ecological
290 relevance which are more difficult to quantify remotely over extensive areas of
291 the deep sea. Fixed cameras (Aguzzi et al., 2011) and mobile platforms
292 (Valentine et al., 2016) can be used to assess epibenthic bacterial mat coverage
293 in combination with customized molecular and chemical microsensors, providing
294 *in situ* analysis of microbial communities (see Section 3.3 below), a proxy for
295 chemosynthetic production at reducing sites (e.g. cold-seeps, hydrothermal
296 vents; Purser et al., 2013; Russ et al., 2013).

297 Video imaging at depth requires continuous illumination which carries a
298 poorly understood potential for harmful effects on deep-sea fauna (Herring et al.,
299 1999; Irwin, 2018). However, digital still time-lapse cameras may collect *in situ*
300 images with triggered flash illumination, limiting the exposure to light of these
301 perpetually dark deep-sea ecosystems. At the same time, red or infra-red lighting,
302 at wavelengths not detectable by deep-sea animals, has been used with some
303 success (Widder et al., 2005) but those wavelengths are rapidly attenuated in
304 water and the resulting monochrome images contain much less information than
305 equivalent color images (Priede, 2017).

306 Classic high-definition video monitoring approaches are being integrated
307 with novel acoustic imaging systems (Rountree, 2008; Juanes, 2018) with an
308 increasing level of complementarity in deep-water areas (**Figure 3**). Acoustic
309 cameras, such as high-frequency multi-beam imaging Dual-frequency
310 Identification Sonar (DIDSON) and Adaptive Resolution Imaging Sonar (ARIS)
311 can visualize fish and invertebrate shapes, and track the movement of individuals
312 at distances greater than those which may be achieved by visual systems
313 equipped with artificial lighting solutions (Martignac et al., 2015).

314 A limitation of acoustic camera use for monitoring fauna however is related
315 to animal identification, which with acoustic systems must be solely based on
316 morphology, since no colorimetric and limited texture information is captured by
317 acoustic camera devices. Spatial resolution of acoustic cameras is also
318 insufficient to resolve important details for species identification. However,
319 acoustic cameras can effectively 'see in the dark', thus avoiding photic
320 contamination, allowing investigation of how artificial lights may influence animal
321 behavior in the deep sea. In order to verify identifications, acoustic cameras must
322 be deployed simultaneously with new prototype low-light high resolution optical
323 imaging equipment (e.g. Barbier et al., 2012).

324 The space sampled around an observatory can be also increased by
325 mounting an imaging sonar on a rotating head (see **Figure 3B**). At present such
326 devices are installed on the ONC cabled observatory in Barkley canyon. These
327 sonars allow internet connected operators to qualitatively discern the presence
328 and abundance of benthic fauna and any associated bioturbation over surfaces
329 larger than in any single fixed image (Robert & Juniper, 2012). Similar rotating
330 side or upward facing sonar packages are undergoing initial deployments on
331 other cabled infrastructures, capable of being used to identify animals, when they
332 are not too densely grouped, at distances of up to ~1 km (Godø et al., 2014).

333 Currently, automation in image processing for animal tracking,
334 classification, counting, the extraction of morphological features (e.g. size, shape,
335 color patterns), and characterization of behavioral aspects (e.g. crawling,
336 walking, swimming, burying, and territoriality; *sensu* Aguzzi & Company, 2010),
337 is becoming a relevant tool in biological data provision from cabled observatories
338 and their associated mobile platforms. More automated routines are urgently

339 required as the volume of image data collected by these systems increases in
340 line with technological developments. Such routines will enable researchers to
341 overcome the human analysis-dependent bottleneck of manual processing
342 (Aguzzi et al., 2012), whilst also reducing observer bias (Schoening et al., 2012).
343 By developing Artificial Intelligence (AI; in the form of learning algorithms) in
344 computer vision, cameras may be transformed into the equivalent of a calibrated
345 sensor, automatically providing time series quantitative data on key fauna, to
346 augment the qualitative data represented by the images themselves (Cognati et
347 al., 2016, Marini et al., 2018). Despite the difficulties inherent in converting the
348 expert knowledge into useful algorithms, calibration and tuning *via* sufficiently
349 extensive feedback can result in operational performances comparable to those
350 of expert researchers (MacLeod et al., 2010).

351

352 **3.2. Passive Acoustic Monitoring (PAM) to support image-based monitoring**

353 PAM monitoring of fish and invertebrate sounds increase species
354 monitoring capability well beyond the reach of optoacoustic technologies. Such a
355 technological application has become an important tool in fisheries and
356 conservation research (Rountree et al., 2006; Luczkovich et al., 2008). The use
357 of PAM assets provides a long-range monitoring capability in remote locations
358 where traditional sampling methods are difficult or impossible to implement (ACT,
359 2007), as for example in the case of sponge reefs (Archer et al., 2018) or
360 seamounts (Riera et al., 2016). Furthermore, combining acoustic localization with
361 video and other forms of observation can be used to identify sound producing
362 species as well as document their soniferous behavior (Mouy et al., 2018). This

363 approach is finding an increasing use in the collection of long-term data for
364 integrated biodiversity assessment (Pieretti et al., 2017).

365 Unfortunately, the application of PAM is limited by the paucity of archived
366 data on fish sounds (Rountree et al., 2002; Rountree et al., 2018a). For example,
367 of the approximately 400 fish species in British Columbia waters, only 22 have
368 been reported to “vocalize” in large part because sound production has been
369 investigated in so few species (Wall et al., 2014). This is especially true in the
370 deep sea, where fish sounds have rarely been studied despite the fact that many
371 species possess sonic muscles presumably used in vocalization (Rountree et al.,
372 2012; Parmentier et al., 2018).

373 Although many fishes and invertebrates do not produce purposeful sounds,
374 it is important to understand that incidental sound production may occur upon
375 physiological and behavioral activity (e.g. specific swimming and feeding mode
376 sounds). Those acoustic marks can be used to assess the presence of individuals
377 for a certain species and are therefore being incorporated into PAM monitoring
378 procedures (Rountree et al., 2006; Rountree et al., 2018b).

379 The aforementioned PAM applications, combined with other observation
380 technologies (e.g. video, acoustic imaging and sonar) improving the
381 documentation of organism sound production and associated behavior, will add
382 further ecological value to the integrated monitoring framework of ocean
383 observatories (Rountree, 2008).

384

385 ***3.3. Molecular sensing as benchmark for species traceability***

386 Molecular tools have diverse applications in marine ecological studies and
387 biological monitoring. Substantial contributions have been provided by several

388 DNA barcode initiatives generating and implementing databases, along with the
389 development of metabarcoding protocols to recover community diversities from
390 unsorted samples (Stefanni et al., 2018). The latest revolution in bio-monitoring
391 is linked to the collection and analysis of genetic material obtained directly from
392 environmental samples, namely environmental DNA (eDNA). This protocol
393 enables tracing of the presence of species from skin cells, fish scales, gametes
394 and food left overs, without the need to isolate any target organisms (Taberlet et
395 al., 2012). Direct sequencing of eDNA has been shown to provide several
396 advantages over traditional techniques, improving the capacity to unravel the
397 “hidden” biodiversity (e.g. detect rare, cryptic, elusive and non-indigenous
398 species in the early stages of invasion) and enabling global census of species in
399 near real-time (Stat et al., 2017).

400 However, eDNA tracing presents some limitations such for example, the
401 detection of false positives (when target species is absent but its DNA is
402 recovered), and false negatives (i.e. species undetected where they are present)
403 which have to be carefully evaluated and avoided (Taberlet et al., 2012; Cristescu
404 & Hebert, 2018). Major difficulties encountered in deep-sea ecosystems for
405 studies involving molecular analysis of diversity are: the general lack of taxonomic
406 knowledge as well as the absence of appropriate databases of species-specific
407 marker sequences (Carugati et al., 2015; Dell’Anno et al., 2015; Sinniger et al.,
408 2016). When these molecular markers are identified (Barnes & Turner, 2016), *in*
409 *situ* hybridization techniques may be used with great success when targeting
410 expected taxa within monitoring programmes (Scholin, 2010; Berry et al., 2019).

411 Recent technical improvements concern the development of “Eco-genomic”
412 sensors capable of autonomously collect biological samples and perform

413 molecular analyses (Ottesen, 2016). These sensors allow the characterization of
414 marine community composition as a whole, regardless of the faunal size classes
415 involved (McQuillan & Robidart, 2017). One example is the Environmental
416 Sample Processor (ESP Scholin et al., 2009), designed to autonomously collect
417 discrete water samples, concentrate microorganisms, and automate the
418 application of molecular probe technologies.

419 In parallel, recent advances in high-throughput sequencing technologies are
420 allowing the processing of huge amounts of genomic data using small portable
421 devices (i.e. miniaturized sequencers such as produced by Oxford Nanopore
422 Technologies having the size of a USB stick). This kind of devices, together with
423 advances in bioinformatics could represent the most important revolutionary
424 breakthrough technology in ecological networks monitoring. Challenges related
425 to the taxonomic assignments of genomic sequences and their interpretation
426 (incompleteness of databases) may be solved applying machine learning
427 algorithms (Cordier et al., 2017). Such approaches can maximize ecologically
428 meaningful insights and provide a list of highly informative sequences ecosystem
429 indicators that could provide the basis for hybridization chips (i.e. micro-arrays)
430 for denser, mobile and cheaper *in situ* devices that can be scaled up appropriate
431 spatiotemporal resolutions (Cordier et al., 2018).

432 AI approaches are gaining relevance in the metabarcoding analysis and
433 provide a fast and cost-effective way for assessing the quality status of
434 ecosystems (Cordier et al., 2018; 2019). Recent examples in -omics analysis
435 were based on Random Forest (Breiman, 2001; Fernández-Delgado et al., 2018)
436 and Self Organizing Maps (Kamimura, 2019). These were used for identifying
437 biotic indices for the foraminiferal metabarcoding. Similarly, Gerhard & Gunsch

438 (2019) used a random forest based approach for selecting the relevant
439 biomarkers for classification of ocean, harbor and ballast water samples. LeCun,
440 (2015) used a deep recurrent neural network (approach for a base calling
441 application on portable sequencing machines (Merelli et al., 2018), where
442 meaningful results were sent to a cloud service through an Internet of Things
443 framework for further analysis (Čolaković & Hadžialić, 2018).

444 Along with molecular based monitoring tools, other chemical sensing
445 applications may complement DNA probing (Ishida et al., 2012) and sequencing.
446 An example is provided by *in situ* mass spectrometry, originally developed for
447 targeting xenobiotic compounds in marine water micro-samples, which has been
448 successfully used for identifying species presence based on their physiological
449 by-products (Wollschlager et al., 2016).

450

451 **3.4. Ecosystem indicators**

452 In the near future, the integration of advanced genomic and chemical
453 approaches for *in situ* detection of organisms (e.g. Cordier et al., 2017) and
454 quantification of their biochemical activity (Goodridge & Valentine, 2016) will
455 greatly enhance the performance of ecological monitoring networks, adding to
456 the detection capacity of optoacoustic imaging and passive acoustic approaches
457 alone.

458 Stitched imaging products (e.g. mosaicking) can provide valuable
459 information on species distribution and habitat use at more extended scales. *In*
460 *situ* molecular methods can detect the presence of taxa otherwise undetected by
461 imaging outside a small temporal window or too small for morphological
462 recognition, while acoustics expand the spatial scales of deep-sea biological

463 monitoring, enabling the integration of horizontal (nekto-benthic displacement;
464 Aguzzi & Company, 2010) and vertical (i.e. benthopelagic coupling; Griffiths et
465 al., 2017) biomass and energy fluxes. With the use of such combined datasets,
466 a series of biological variables can be measured and ecosystem indicators
467 extracted, as essential elements for the accurate assessment of the health of
468 benthic ecosystems and cover the complete range from benthic (e.g.
469 chemosynthetic) primary production, individual characteristics, population
470 dynamics, species and community dynamics to finally the ecosystem functioning
471 level.

472 As a result of these ever-growing demands, the need for automation in data
473 collection, analysis and interpretation procedures is paramount. Integration of
474 cabled observatories and associated mobile systems equipped with AI for real-
475 time content extraction from imaging systems, hydrophones and e-DNA
476 samplers, would allow the monitoring of ecosystem indicators and representation
477 of ecosystem functioning over extended spatiotemporal scales (from square
478 meters to kilometers, over days, months, seasons, and decades). To date, no
479 such integrated system exists in the deep sea to verify the concept (Danovaro et
480 al., 2017). At present there are major shortfalls in automation of image and sound
481 processing and producing an efficient, long-term *in situ* e-DNA extraction and
482 sequencing device. However, many of these systems are integrated into the ONC
483 cabled observatory infrastructure in the NE Pacific, with data being collected in
484 real-time at a number of nodes and returned to a central repository (i.e. Ocean
485 2.0 data bank system). Similarly, real-time, interactive tools such as the Scripps
486 Plankton Camera System (<http://spc.ucsd.edu/>) facilitate quick access to visual
487 data and a statistical overview. The implementation of these types of data

488 repositories can allow environmental comparisons to be made among
489 neighboring and more distantly arrayed platforms in an attempt to scale local
490 results over a larger networked area (**Figure 4**). This endeavor is providing the
491 guidelines for future development of spatiotemporally integrated monitoring
492 protocols.

493 Autonomous monitoring of biological variables and derived ecosystem
494 indicators by cabled observatories and their integrated mobile platforms should
495 be implemented following a general and standardized common operational
496 protocol: *i.* all multiparametric readings from optoacoustic imaging, PAM,
497 molecular, biogeochemical, and oceanographic sensors should be acquired
498 synchronously by all cabled and mobile platforms; *ii.* such data acquisition should
499 occur in a high-frequency and time-lapse mode, where the image content should
500 be automatically analyzed by AI algorithms and classified on board of the device,
501 (saving storage PAM and transmission bandwidth space), while preserving the
502 observation time georeferenced stamp; and finally, *iii.* all mobile platforms should
503 constantly survey the same benthic and pelagic areas (subdivided into specific
504 stations) among cabled observatories and their moored vertical projections. Such
505 an automated and spatiotemporally coordinated and standardized protocol for
506 data acquisition will make data treatment, transmission, and storage easier,
507 whilst simultaneously facilitating more straightforward repeatability/reproducibility
508 of observations at the same location and comparison of measurements made
509 with other networks, allowing regional/global level analysis.

510 The measurement of biological variables needed for the hierarchical
511 computation of ecosystem indicators, should be carried out through a series of
512 sequential automated steps (**Table 2**). *i.* all imaging outputs initially processed for

513 the classification, counting and tracking of fauna and quantification of bacterial
514 mat coverage and activity; *ii.* animals measured (e.g. by stereovision, acoustic
515 scaling or laser scanning) to obtain class-size frequency distribution and sex-ratio
516 (when morphology allows individual discrimination); *iii.* total species counts from
517 all seabed and water column areas summed and standardized for the imaged
518 volume, to obtain an overall abundance (i.e. density) and biomass estimation; *iv.*
519 species counts computed for each station analyzed by mapping procedures (e.g.
520 percentage of presence/occupation per quadrant), to derive information on
521 habitat use as well as displacement routes through different zones (i.e. corridors);
522 *v.* a species richness list and biodiversity obtained at each platform (alpha
523 diversity), between platforms (beta diversity), and the level of the whole network
524 (gamma diversity), to assess habitat heterogeneity influences on species
525 distribution, community composition, and overall ecosystem boundaries; finally,
526 *vi.* density and biomass for each species related to carbon inputs from
527 benthopelagic fluxes in chlorophyll-*a* and turbidity (as proxy for transported
528 organic and inorganic matter), as well as from geochemical fluxes, when relevant
529 (i.e. carrying the reduced chemicals as for example, methane, hydrogen, sulfide,
530 that fuel chemosynthetic microbes), to calculate ecosystem functioning and
531 productivity performances. All automated analysis stages need to be verified by
532 human researchers to ensure accuracy of the algorithm functioning, while the
533 nature of specified ecological interpretation must be cross-checked against
534 published results from conventional methods such as analysis of stomach
535 contents, stable isotopes and fatty acids markers (Choy et al., 2017).

536

537 **4. Perspectives and Outlook**

538 Autonomous flexible networks of cabled observatories and mobile platforms can
539 allow extensive monitoring of marine life at different levels of biological
540 organization and at unprecedented spatial and temporal resolution. Although
541 integrated monitoring actions such as those outlined herein are yet to attain full
542 operational readiness, and therefore proofs of some of the concepts discussed
543 are missing, the technological developments are ongoing. Progress to date
544 already allows researchers to utilize services-oriented ecological monitoring of
545 some isolated deep-sea ecosystems. It is important, however, that future
546 observatories are designed from the ground up for ecosystem monitoring and
547 data integration, rather than being developed on an *ad hoc*, and somewhat
548 haphazard basis, as funding for individual projects becomes available.

549 Bio-imaging technologies already play a central role in ecosystem
550 exploration and monitoring. Increasing levels of automation in image processing
551 are transforming cameras into true sensors, delivering time series data for a
552 number of biological variables and derived ecosystem indicators. Visual data are
553 being increasingly complemented by *in situ* passive acoustic listening sensors
554 and new e-DNA sequencing technologies for species traceability. All these
555 initially disparate data sources can be combined to form a detailed and high-
556 resolution monitoring approach applicable to the benthic and pelagic components
557 of a deep-sea ecosystem. The output from such a monitoring regime will support
558 decisions of policy makers, allowing them to assess the impacts of increased
559 industrial activities and pressures on deep-sea ecosystems (e.g. oil or gas
560 extraction and mining or trawl fishing), including a better assessment of already
561 evident but poorly quantified climate change impacts at great depths. The
562 obtained data will be of paramount importance for the accurate assessment of

563 the health status of ecosystems, the physical damage to habitats and to efficiently
564 monitor their resilience and the efficacy of restoration actions. The compiling of
565 multiannual time series monitoring data sets (continuously updated in real-time)
566 will allow the identification of shifting environmental baselines and rapidly
567 highlight the onset of any negative environmental impacts which may develop,
568 potentially unpredictably, from human activities in these remote deep-sea
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570

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581

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1085 **Table 1. Biological studies from permanent, autonomous platforms.** Worldwide applications of monitoring of deep-water and
 1086 deep-sea ecosystems by cabled observatories and crawlers, at different ecologically-ranked levels (i.e. individual, population,
 1087 community and ecosystem).

Hierarchical step	Monitored biological variable or ecosystem indicator	Cabled platform	Publications
1. (Semi-) Automated detection, counting and classification by optoelectronic methods (imaging)	Cold-seep fauna abundance	JAMSTEC Cabled Observatory (1100 m; NW Pacific)	Aguzzi et al. (2009)
			Aguzzi et al. (2010)
	Macrofaunal abundance	LoVe Ocean Observatory (250 m; Norwegian Sea)	Osterloff et al. (2016)
	Zooplankton abundance	NEPTUNE Cabled Observatory (ONC; Barkley Canyon; 400-1000 m; NE Pacific)	De Leo et al. (2018)
	Benthic fauna abundance, bacterial mat coverage	VENUS Cabled Observatory (ONC; Saanich Inlet; 100 m; NE Pacific)	Aguzzi et al. (2011)
	Biological scattering layers classification		Ross et al. (2013)
2. (Semi-) Automated detection, counting and classification by passive acoustic monitoring (PAM)	Cetacean abundance	ALOHA Cabled Observatory (4700 m; Central Pacific)	Oswald et al. (2011)
		ANTARES Neutrino Telescope (2500 m; Western Mediterranean)	André et al. (2017)
		MARS Cabled Observatory (MBARI; 900 m; NE Pacific)	Ryan et al. (2016)
		NEMO-SN1 (OnDE; EMSO; KM3NeT-It; 2100 m; Central Mediterranean)	Caruso et al. (2015)
		NEPTUNE Cabled Observatory (ONC; Endeavour; 2200 m; NE Pacific)	Weirathmueller et al. (2017a)
		NEPTUNE Cabled Observatory (ONC; Cascadia Basin; 2700 m; NE Pacific)	Weirathmueller et al. (2017b)
		NEPTUNE Cabled Observatory (ONC; Barkley Canyon slope; 400-1000 m; NE Pacific)	Kanes et al. (2017)
Zooplankton abundance		De Leo et al. (2018)	
3. Behavioral and life traits (e.g. morphology, rhythms, motility, trophic interactions, territoriality, etc.)	Cetacean diel activity	ALOHA Cabled Observatory (4700 m; Central Pacific)	Oswald et al. (2011)
		ANTARES Neutrino Telescope (2500 m; Western Mediterranean)	André et al. (2017)

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1090 **Table 1. Biological studies from cabled observatories.** Continued from previous page.

3. Behavioral and life traits (e.g. morphology, rhythms, motility, trophic interactions, territoriality, etc.)	Cold-seep fauna movement, behavior and diel activity		Aguzzi et al. (2009)
	Cold-seep fauna diel and tidal rhythms	JAMSTEC Cabled Observatory (1100 m; NW Pacific)	Aguzzi et al. (2010)
	Environmental variability and carcass decomposition		Aguzzi et al. (2012)
	Cetacean diel activity	MARS Cabled Observatory (MBARI; 900 m; NE Pacific)	Ryan et al. (2016)
		NEMO-SN1 (OnDE; EMSO; KM3NeT-It; 2100 m; Central Mediterranean)	Caruso et al. (2017)
	Deep-sea inertial bioluminescence rhythms	NEMO Phase-2 tower (KM3NeT-It; 2500 m; Central Mediterranean)	Aguzzi et al. (2017)
	Benthic megafauna movement	NEPTUNE Cabled Observatory (ONC; Barkley Canyon shelf-break; 400 m; NE Pacific)	Robert & Juniper (2012)
	Benthic fauna movement		Chauvet et al. (in Press)
	Benthic fauna behavior and diel activity	NEPTUNE Cabled Observatory (ONC; Barkley Canyon slope; 600-1000 m; NE Pacific)	Doya et al. (2014)
	Benthic fauna diel activity		Matabos et al. (2014)
	Cetacean diel activity		Kanes et al. (2017)
	Hydrothermal vent fauna behavior and diel activity		Cuvelier et al. (2014)
	Hydrothermal vent fauna diel activity	NEPTUNE Cabled Observatory (ONC; Endeavour; 2200 m; NE Pacific)	Cuvelier et al. (2017)
	Hydrothermal vent macrofauna rhythms		Lelièvre et al. (2017)
	Cold-seep fauna diel activity	NEPTUNE Cabled Observatory (ONC; Barkley Canyon hydrates; 900 m; NE Pacific)	Chatzievangelou et al. (2016)
	Cold-seep fauna behavior		Doya et al. (2017)
	Environmental variability and carcass decomposition	VENUS Cabled Observatory (ONC; Strait of Georgia; 300 m; NE Pacific)	Anderson and Bell (2016)
			Anderson and Bell (2014)
	Benthic fauna behavior		Doya et al. (2016)
	Benthic fauna diel activity	VENUS Cabled Observatory (ONC; Saanich Inlet; 100 m; NE Pacific)	Matabos et al. (2011)
Benthic fauna behavior, diel and tidal rhythms		Matabos et al. (2015)	
Biological scattering layers classification		Ross et al. (2013)	

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1092 **Table 1. Biological studies from cabled observatories.** Continued from previous page.

	Cetacean seasonality	ALOHA Cabled Observatory (4700 m; Central Pacific)	Oswald et al. (2011)
	Deep-sea episodic bioluminescence blooms	ANTARES Neutrino Telescope (2500 m; Western Mediterranean)	Tamburini et al. (2013)
	Cold-seep bivalve spawning and fecundity	JAMSTEC Cabled Observatory (1200 m; NW Pacific)	Fujikura et al. (2007)
	Macrofaunal spatial distribution	LoVe Ocean Observatory (250 m; Norwegian Sea)	Osterloff et al. (2016)
		MARS Cabled Observatory (MBARI; 900 m; NE Pacific)	Ryan et al. (2016)
	Cetacean seasonality		Caruso et al. (2017)
		NEMO-SN1 (OnDE; EMSO; KM3NeT-It; 2100 m; Central Mediterranean)	Sciacca et al. (2015)
	Cetacean size estimation		Caruso et al. (2015)
		NEPTUNE Cabled Observatory (ONC; Barkley Canyon shelf; 400 m; NE Pacific)	Robert & Juniper (2012)
	Benthic megafauna abundance and size		
		DELOS observatories (1400 m, SW Atlantic off Angola)	Vardaro et al. (2013)
	Benthic fauna seasonal and inter-annual trends		Chauvet et al. in press
	Zooplankton seasonal and interannual trends		De Leo et al. (2018)
	Benthic fauna size classes and migrations	NEPTUNE Cabled Observatory (ONC; Barkley Canyon slope; 400-1000 m; NE Pacific)	Doya et al. (2014)
			Juniper et al. (2013)
	Benthic fauna seasonality		Vardaro et al. (2013)
		DELOS observatories (1400 m, SW Atlantic off Angola)	
	Cold-seep fauna spatial distribution		Chatzievangelou et al. (2017)
	Cold-seep fauna seasonality and reproductive cycles	NEPTUNE Cabled Observatory (ONC; Barkley Canyon hydrates; 900 m; NE Pacific)	Doya et al. (2017)
	Cold-seep fauna seasonality		Thomsen et al. (2017)
			Cuvelier et al. (2017)
	Hydrothermal vent fauna microhabitat use		Cuvelier et al. (2014)
		NEPTUNE Cabled Observatory (ONC; Endeavour; 2200 m; NE Pacific)	
	Cetacean seasonality, inter-annual trends and spatial distribution		Weirathmueller et al. (2017a)
	Benthic fauna size classes, seasonality and microhabitat use		Doya et al. (2016)
	Benthic fauna size classes and seasonality	VENUS Cabled Observatory (ONC; Saanich Inlet; 100 m; NE Pacific)	Matabos et al. (2012)
	Biological scattering layers classification		Ross et al. (2013)

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1096 **Table 1. Biological studies from cabled observatories.** Continued from previous page.

5. Biodiversity (e.g. composition, richness, alpha / beta / gamma diversity, etc.)	Cold-seep fauna diversity	NEPTUNE Cabled Observatory (ONC; Barkley Canyon hydrates; 900 m; NE Pacific)	Chatzievangelou et al. (2017)
	Benthic fauna diversity	NEPTUNE Cabled Observatory (ONC; Barkley Canyon slope; 900-1000 m; NE Pacific)	Chauvet et al. (in Press)
	Benthic community composition	DELOS observatories (1400 m, SW Atlantic off Angola)	Juniper et al. (2013)
	Hydrothermal vent fauna community composition	NEPTUNE Cabled Observatory (ONC; Endeavour; 2200 m; NE Pacific)	Matabos et al. (2014)
	Benthic community composition	VENUS Cabled Observatory (ONC; Saanich Inlet; 100 m; NE Pacific)	Vardaro et al. (2013)
	Benthic community dynamics		Cuvelier et al. (2017)
			Matabos et al. (2012)
6. Ecosystem functioning (food-web structure, carbon flux, bioturbation /remineralization)	Seabed bioturbation by benthic megafauna	NEPTUNE Cabled Observatory (ONC; Barkley Canyon shelf-break; 400 m; NE Pacific)	Matabos et al. (2015)
	Zooplankton carbon fluxes	NEPTUNE Cabled Observatory (ONC; Barkley Canyon slope; 400-1000 m; NE Pacific)	Robert & Juniper (2012)
	Seasonal carbon fluxes	NEPTUNE Cabled Observatory (ONC; Barkley Canyon hydrates; 900 m; NE Pacific)	De Leo et al. (2018)
			Thomsen et al. (2017)

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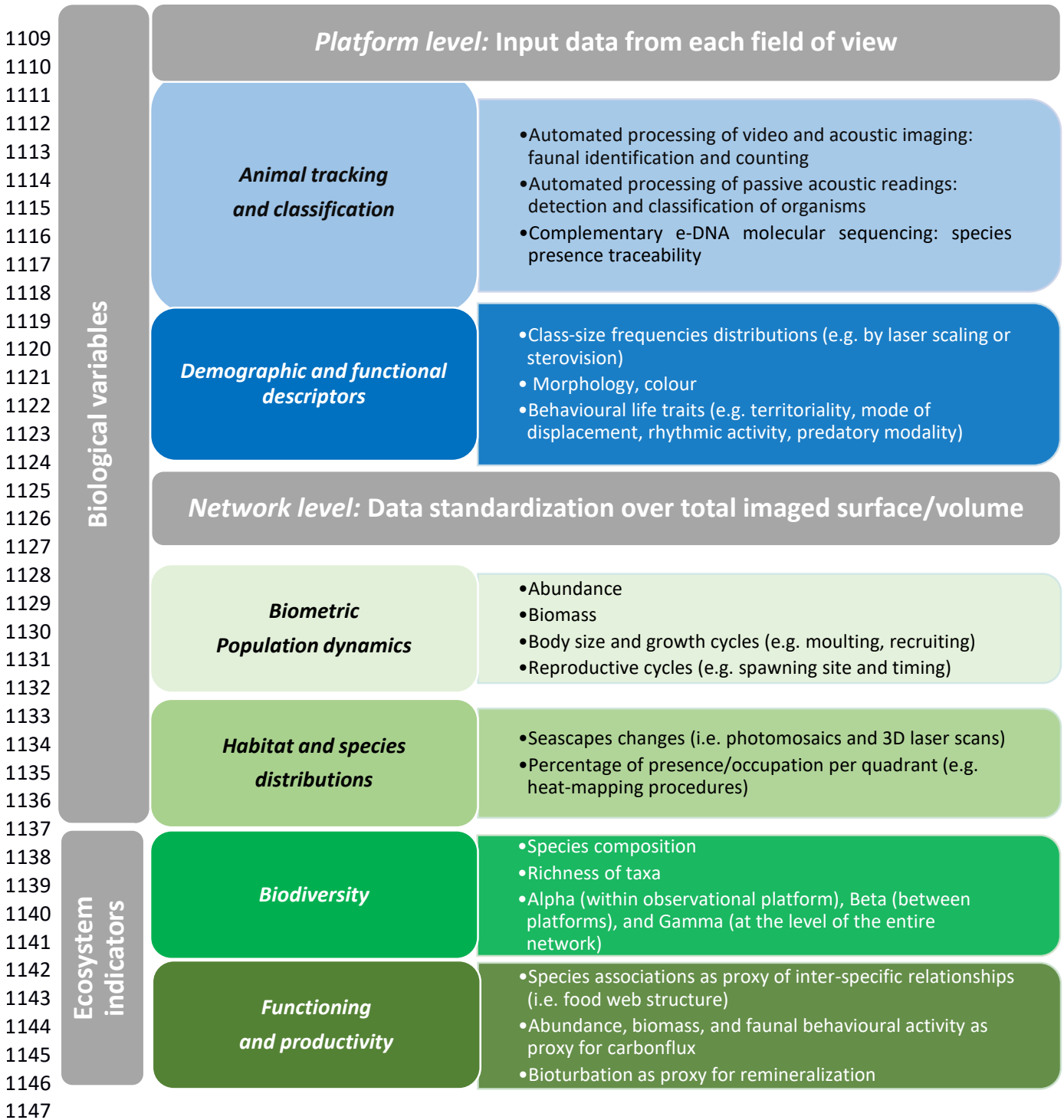
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1104 **Table 2. Indicators extraction roadmap.** Consecutive automatable steps for the
 1105 hierarchical computing of ecosystem indicators from input biological variables,
 1106 obtained by bio-imaging and other sensing technologies, installed on spatially
 1107 distributed autonomous networks of cabled observatories and their connected
 1108 mobile benthic and pelagic platforms.



1148 **Figure legends**

1149

1150 **Figure 1.** The fixed (i.e. cabled) and mobile docked platforms constituting a
1151 spatial network for the integrated benthic and pelagic ecosystem monitoring. (A)
1152 Video-cabled multiparametric observatory platform, acting as a docking station
1153 for a pelagic Remotely Operated Vehicle (ROV) and a tethered mobile benthic
1154 crawler (Courtesy of Dr. O. Godø & Dr. T. Torkelsen); (B) rover (MANSIO-
1155 VIATOR) similar to crawlers but not tethered, docked to a vessel-assisted
1156 repositioning station; (C) Architecture of ANTARES (the Astronomy with a
1157 Neutrino Telescope and Abyss environmental RESearch detector) with a line of
1158 Photo-Multiplier Tubes (PMTs) and a tethered crawler.

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1160 **Figure 2.** Illustration of a variety of cabled observatories providing the sea bed
1161 infrastructure to control and coordinate mobile benthic and pelagic platforms such
1162 as docked crawlers, rovers, and AUVs. Platform monitoring is assisted by vessels
1163 and satellite-based technologies. Neut. Telescope – is an array of vertical moored
1164 lines of Photo-Multiplier Tubes (PMTs) deployed in the deep sea. Seabed
1165 infrastructures providing power and data transfer may be aided by connection
1166 with industrial or telecommunication cables, as reliable low-cost means for
1167 network deployment into vast abyssal areas (Danovaro et al., 2017).

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1169 **Figure 3.** Different video and acoustic imaging data outputs obtained by fixed-
1170 point and crawler platforms connected through the Ocean Networks Canada
1171 (ONC). (A, B) Commercially exploited sablefish (*Anoplopoma fimbria*) imaged
1172 from cabled observatory HD video (A) and from an imaging rotary sonar (B), at

1173 970 m depth in Barkley Canyon (NE Pacific); (C) A spiny dogfish (*Squalus*
1174 *achantias*) imaged with the ARIS acoustic camera at 120 m depth from a cabled
1175 observatory in the Strait of Georgia. The color scale bar indicates raw backscatter
1176 reflectivity amplitude (in decibels, dB) (courtesy of X. Mouy); (D, E) Photomosaic
1177 panoramas obtained by the crawler in Barkley Canyon (870 m; NE Pacific,
1178 Canada), depicting egg towers of *Neptunea* sp. snails (D), and a range of benthic
1179 species occupying a methane seep habitat patch; (F,G) 3D photomosaics of a
1180 methane hydrate mound at the same location, depicting mound area/volume
1181 changes over time due to uplift/growth in hydrates (areas in yellow) and slumping
1182 (areas in red; courtesy of Dr. T. Kwasnitschka).

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1184 **Figure 4.** Schematic representation of current seafloor monitoring infrastructure
1185 ONC in Barkley Canyon, where a power node distributes energy and data
1186 transmission capability to serve fixed multiparametric imaging-platforms and a
1187 crawler. The mobile platforms communication and coordinated function makes
1188 this area the first cooperative network (shown in the bubble) for the deep-sea
1189 ecological monitoring. As an example of the power of ongoing multiparametric
1190 monitoring, time series from several environmental sensors for the crawler are
1191 presented over consecutive years (data plotted at 1-h frequency). When gaps in
1192 data acquisition occur, data can be supplied by nearby cabled platform (as
1193 interpolated to cover maintenance periods) Shaded green areas indicates
1194 moments at which the environmental monitoring by the crawler has been
1195 accompanied by image collection, the processing of which is still manual, while
1196 automated scripts for animal tracking and species classification are under
1197 development.

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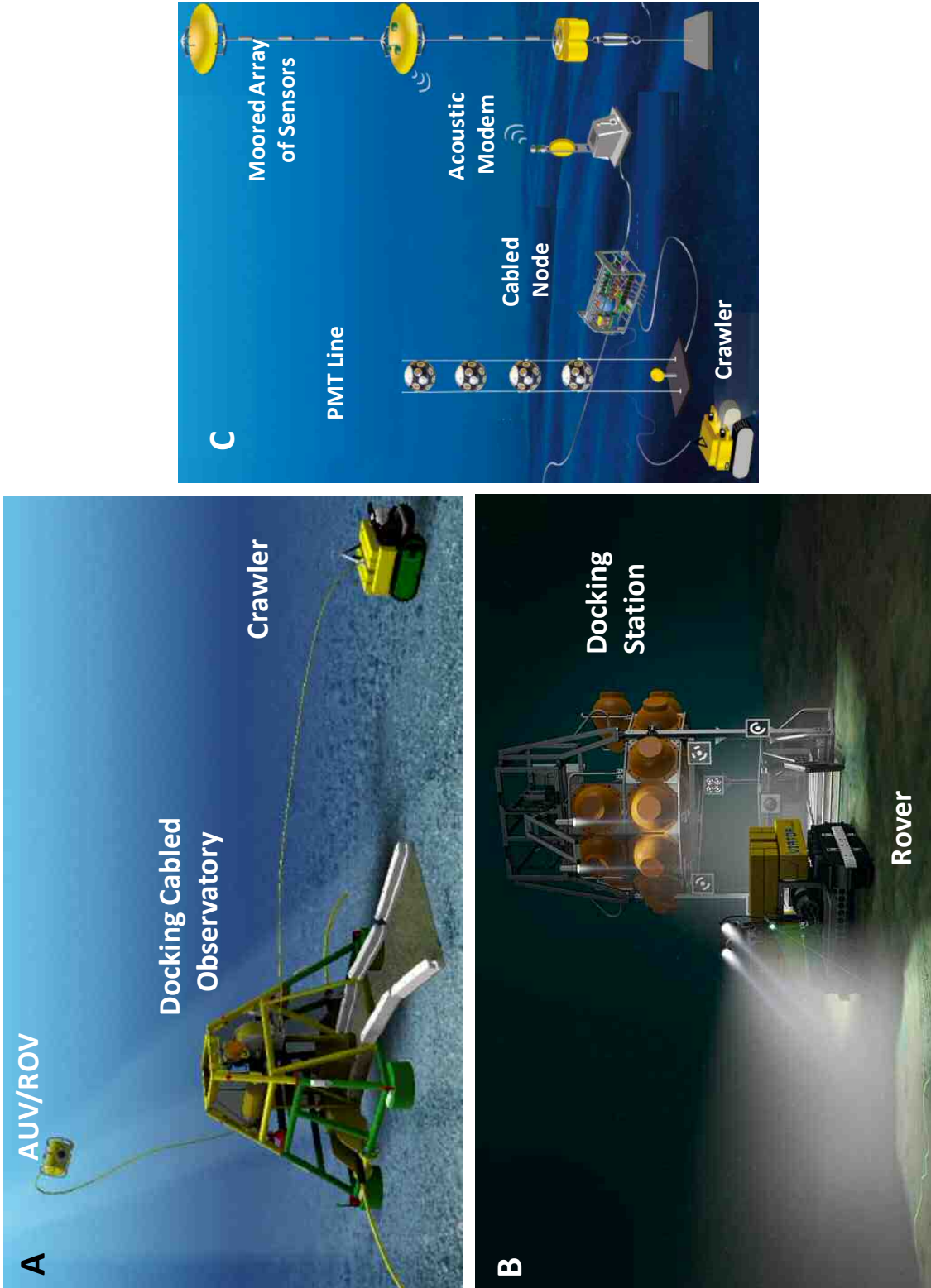
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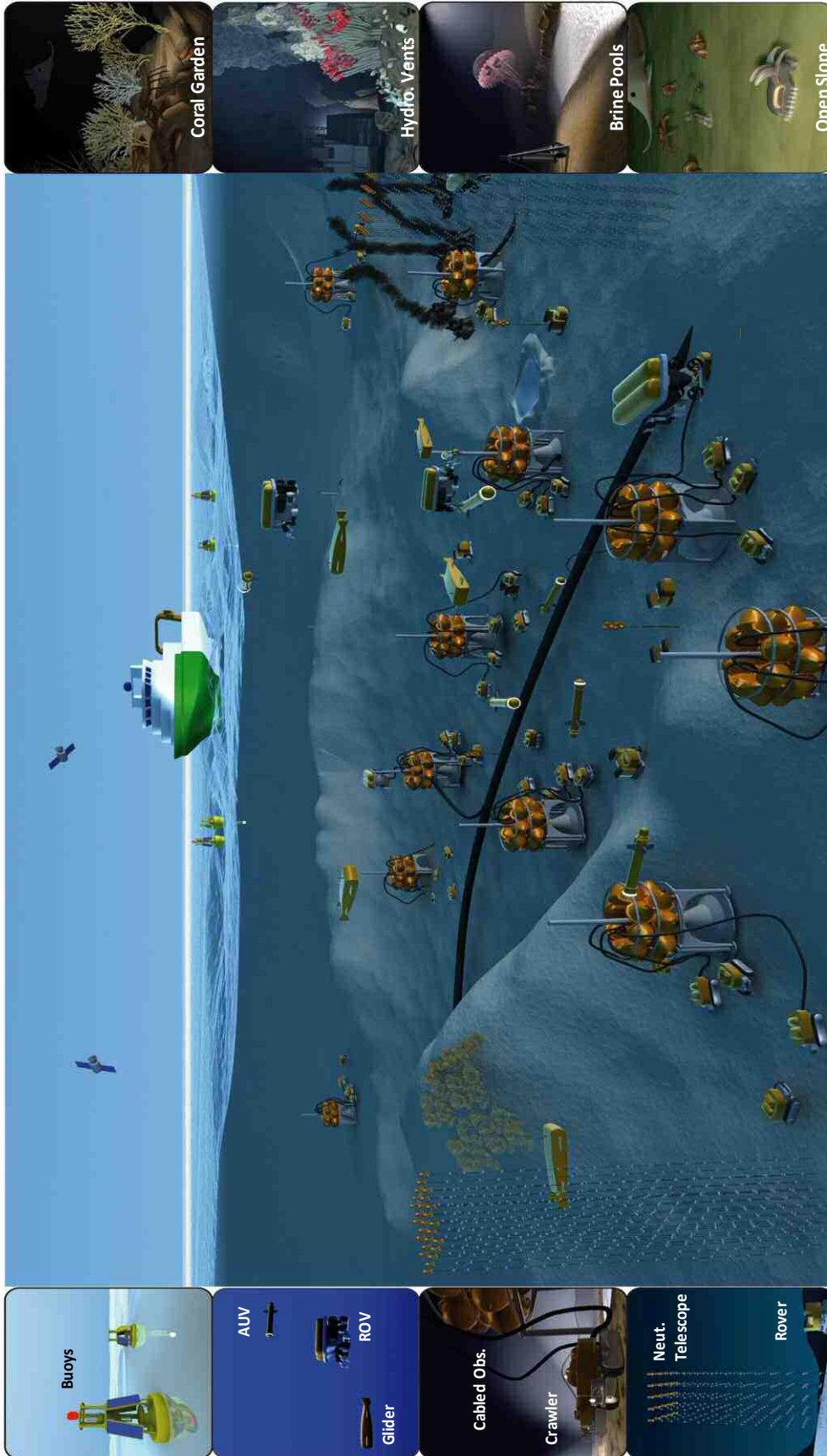
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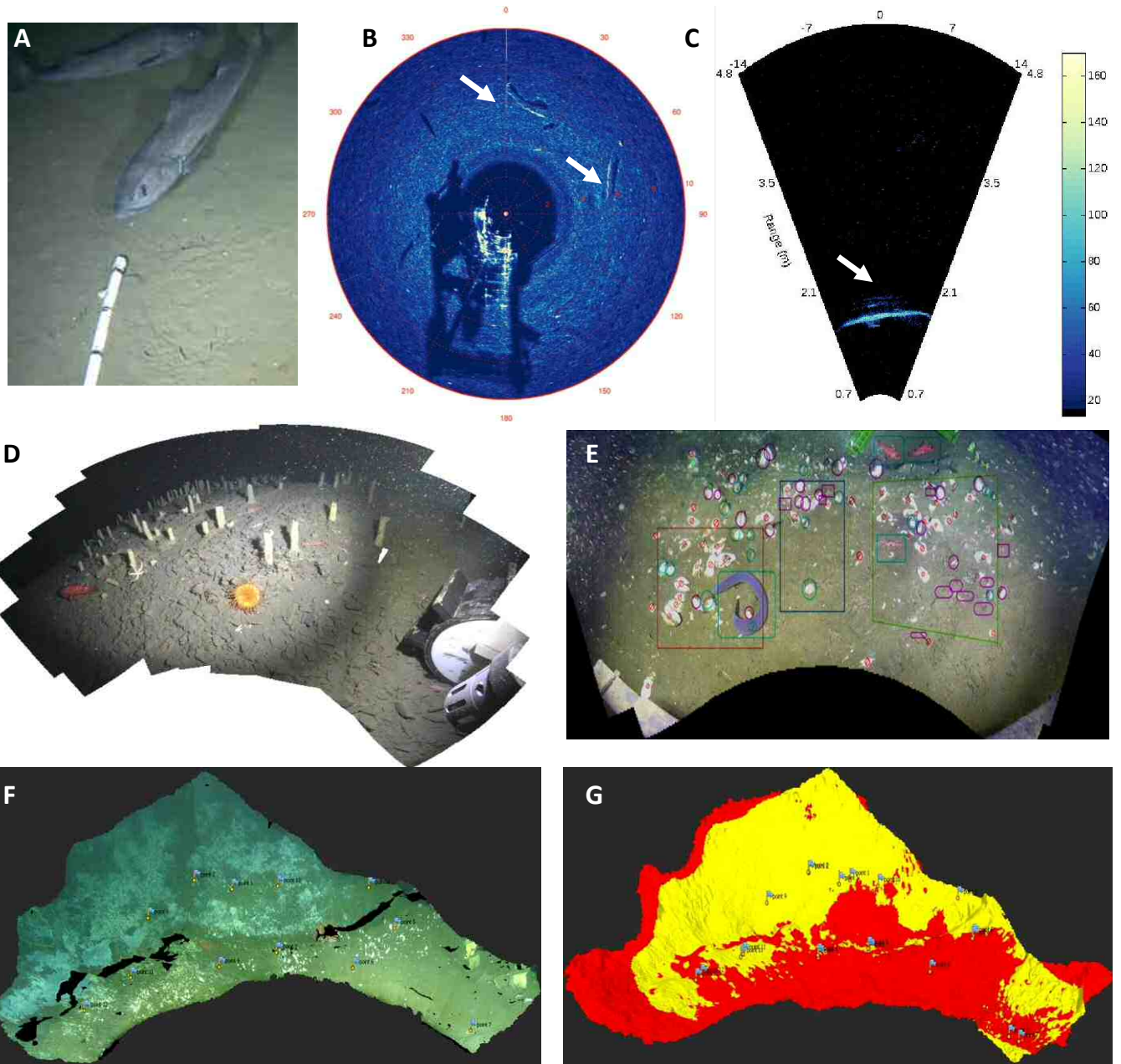
Aguzzi et al. Figure 1

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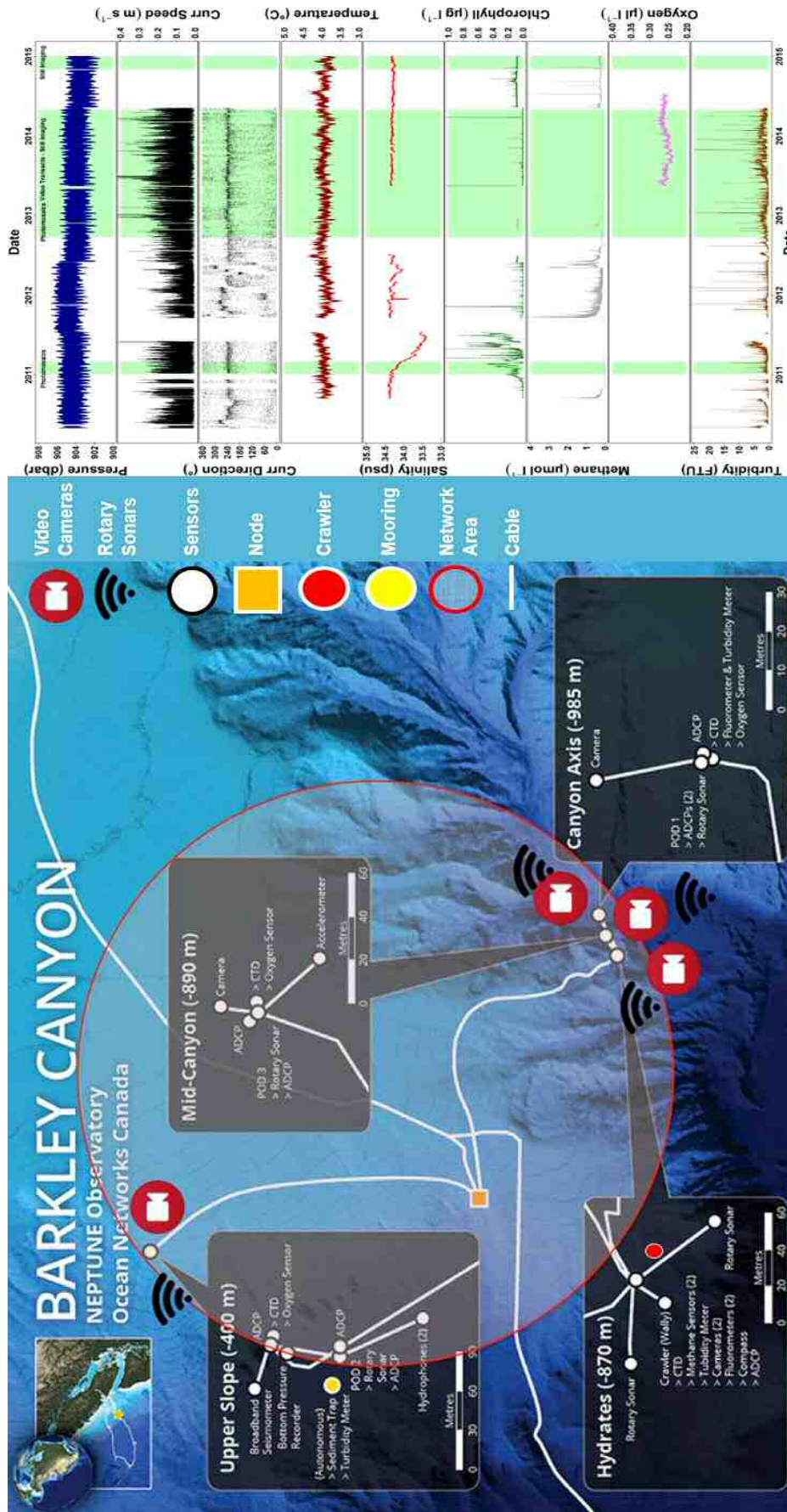
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Aguzzi et al. Figure 3

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Aguzzi et al. Figure 4