# CHALLENGES TO THE ASSESSMENT OF BENTHIC POPULATIONS AND BIODIVERSITY AS A RESULT OF RHYTHMIC BEHAVIOUR: VIDEO SOLUTIONS FROM CABLED OBSERVATORIES

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**Abstract** All marine species studied thus far show rhythmic temporal patterns in their behavioural, physiological, and molecular functions, which are collectively known as biological rhythms. Biological rhythms are generated by biological clocks that time biological functions and are synchronised by geophysical cycles such as the solar light-dark cycle and tidal cycle. On continental margins, behavioural rhythms can be detected by diel (i.e., 24-h based) or seasonal periodical trawling as a consequence of massive inward and outward displacements of populations to and from the sample areas. As a result, significant errors in population/stock and biodiversity assessments performed by trawling may occur if timing of sampling is not taken into account. The increasing number of cabled and permanent multiparametric seafloor observatories now allows direct, continuous, and long-lasting monitoring of benthic ecosystems and analysis in relation to several habitat cycles. This review describes the adaptation of this technology to investigations of rhythmic behaviour by focusing on automated video-imaging. Diel fluctuations in the number of video-observed individuals can be used as a measure of average population rhythmic behaviour. The potential implementation of automated video image analysis in relation to animal tracking and classification procedures based on the combined use of morphometric tools and multivariate statistics is detailed in relation to populational and community studies. Based on video cameras mounted at multiparametric cabled observatories, an integrated time-series analysis protocol using chrono-biomedical procedures is proposed to place video-recorded bio-information in an oceanographic context.

**Keywords:** Behavioural rhythms, marine chronobiology, cabled multiparametric underwater observatories and networks, automated video-imaging, tracking, species classification, Elliptic Fourier Analysis (EFA), Fourier Descriptors (FD), Scale-

Invariant Feature Transform (SIFT), Red-Green-Blue (RGB) coordinates, bio- and habitat data integrated time-series analysis, internal tides, diel captures, nektobenthic movements

## The missing chronobiology framework in marine ecological studies

### *Chronobiology in deep water areas*

The evolution of life occurs within a framework of deterministic (predictable) habitat fluctuations (i.e., geophysical cycles; Aschoff 1981), such as light intensity or tidal cycles. As a result, biological processes are governed by internal oscillatory mechanisms, i.e., biological clocks, that have evolved as mechanisms to regulate the period and phase of rhythmic behaviour and physiology (Daan & Aschoff 2001, Carr et al. 2003). Because biological clocks are 'entrained' (or synchronised) to specific environmental cycles, the biological rhythms they generate are in tune with the cyclic temporal habitat of each species. Biological rhythms are collectively defined as fluctuations in biological functions at any level of complexity, from molecular, physiological, and behavioural processes in individuals, up to oscillations at the population and community level (Aguzzi & Company 2010). Chronobiology is a rapidly developing field that studies the generation and entrainment of biological rhythms using approaches that include reductionist strategies such as molecular biology up to holistic level ecology (Naylor 2010).

The definition of rhythmic activity is usually the first step in biological rhythm analysis, being the result of all underlying physiological fluctuations. Presently, rhythmic behaviour is mostly studied in terrestrial animals by conducting laboratory tests in which geophysical cycles are simulated or eliminated (i.e., constant conditions) to reveal intrinsic (endogenous) mechanisms underlying the functioning of biological clocks (Johnson et al. 2003). This laboratory-oriented research allowed the study of biological timing systems in controlled conditions, conditions that cannot be easily satisfied in field studies Laboratory research responds to the needs of biomedicine (e.g., chronopharmacology), which studies behaviour as a phenotypic expression of molecular clock mechanisms (Tosini & Aguzzi 2005, Hu et al. 2007, Bertolucci et al. 2008). For example, the neurophysiologic basis of circadian (24-h endogenously based) clocks and their light entrainment pathways are currently being studied in several mammalian and insect species (e.g., Golombek & Rosenstein 2010) to establish the complex arrangement of neural oscillator cells in the nervous system and their relationships with peripheral tissues (Welsh et al. 2010).

In marine animals, rhythmic behaviour occurs in the form of predictable variations in the rates of swimming, walking, and crawling, which are difficult to study in the laboratory given the three-dimensional character of the marine environment (i.e., the water column plus the seabed), where these activities take place (Aguzzi & Company 2010). Laboratory and field research on this topic has chiefly been developed using shallow or inland water fishes and decapod crustaceans of commercial interest (e.g., related to fisheries and aquaculture) or other invertebrates (e.g., the gastropods *Aplysia* or the American horseshoe crab; Lickey et al. 1976, Watson et al. 2008) based on the combination of pragmatism in sampling  (accessibility of animals for collection) and high rates of survival in controlled laboratory conditions (Reebs 2002, Naylor 2005). If from one side, terrestrial chronobiology is well studied and control processes deeply characterised, marine chronobiology has only been developed to a similar degree for the intertidal zone (Naylor 2010). Data on rhythmic behaviour for species inhabiting the continental margin areas of shelves and slopes, including the deep sea are currently scant (Aguzzi et al. 2004b). The paucity of data on rhythmic behaviour becomes dramatically evident as the depth range inhabited by species increases. Laboratory studies using deep-water continental margin species (i.e., those of the middle and lower shelves or slopes) are less common, with major animals studied being decapod crustacea such as the Norway and the American clawed lobsters (*Nephrops norvegicus* and *Homarus americanus*) (reviewed by Aguzzi & Sardà 2008; Aguzzi et al. 2008).

In a context where the behavioural habit is unknown for the vast majority of marine species, rhythmic displacement of individuals can fundamentally influence the perception of sampled population sizes and therefore communities' compositions according to the time of the day (Naylor 2005). Accordingly, biodiversity observations should be somehow linked down to individual behavioural performances in relation to changing habit conditions. This fact has been poorly considered to date, as day-night scheduled field sampling procedures are not often employed due to a lack of suitable technology or sufficient resources for repetition. The aim of this review is to describe the application of the newly developed video methods associated with permanent cabled observatory stations to the study of the behavioural rhythms of benthic species. This technology has the potential to provide important data for evaluation of the reliability of population, stock and biodiversity assessments in depth contexts where sampling repeatability and duration are strongly constrained.

## *Ecological implications of behavioural rhythms*

A number of biological traits, including rhythmic behaviour, are still unknown for the majority of benthic species, including many of commercial interest (Company & Sardà 1998, 2000, Company et al. 2003). Rhythmic movements related to the sea bed environment occur in a complex habitat scenario characterised by marked depth-dependent variations in key oceanographic parameters.

### *Behavioural rhythms as unknown displacement typologies in the complex environmental context of continental margins*

Light plays a central role in the regulation of the rhythmic behaviour of animals inhabiting bottom areas within the twilight zone range (Chiesa et al. 2010). Because light intensity decreases and spectral quality narrows with increasing depth (Herring 2002), day-night driven rhythms should be also weaker in slope species in comparison with shallower-dwelling shelf species (Aguzzi et al. 2009a). In the oligotrophic western Mediterranean Sea, the twilight zone ends on the middle slope (Margalef 1986), and light can potentially regulate the circadian system of benthic species down to depths of 1000 m (Tobar & Sardà 1992, Sardà et al. 2003). As light progressively fades with depth, the day-night control of behaviour decreases, and therefore, other geophysical variables and cycles are expected to control the behavioural rhythms of benthos.

It is known that internal tides influence the rhythmic behaviour of fishes (e.g., zoarcids) inhabiting the aphotic deep sea (Aguzzi et al. 2010b). Wagner et al. (2007) show that deep-sea fishes living at continental rise and abyssal depths, beyond the reach of solar light, have an endogenous clock mediated through release of melatonin (Priede et al. 1999). Water flow variations based on a 12.4-h tidal cycle affect the rhythmic production of this hormone, the function of which is related to variations in metabolic activity in association with animal behavioural activation (Wagner et al. 2007). In areas where the tidal pull is negligible (e.g., the Mediterranean Sea), other cycles, such as weaker atmosphere-driven inertial currents, appear to be an effective means of geophysical control over the rhythmic behaviour and physiology of benthos (Aguzzi et al. 2009e). Anyway, the rhythmic behaviour of deep water species in relation to depth-variable light intensity regimes and geographically variable tidally or inertially driven current flows remains very poorly understood.

A traditional sampling methodology for the study of behavioural rhythms in marine species is the temporally scheduled use of pelagic and bottom trawling. Rhythmic catch patterns associated with these temporally modulated sampling methods are considered as a proxy for rhythmic displacements of populations into and out of seabed sampling areas (Aguzzi et al. 2009d).

The behavioural rhythms of many pelagic species are characterised by diel (i.e., 24-h based) vertical migrations (DVMs) that occur within the water column in response to defined light intensity variations (Naylor 2006, Gaten et al. 2008) typified by ascent towards the surface at night time and descent at dawn. Repetition of pelagic sampling at different depth strata at similar times has allowed the characterisation of migration extension ranges for different demographic components of micro- and macronekton (i.e., gender-related and ontogenetic modulations of behaviour) (Foxton 1970a,b, Franqueville 1971, Foxton & Roe 1974, Herring & Roe 1988, Frank & Widder 2002). Within the DVM displacement category, a diel cycle of occurrence of benthopelagic species is reported in seabed collections taken at different times (Aguzzi et al. 2006a). Animal catches increase during daytime when benthopelagic populations descend in the water column, hiding on the sediment surface from pelagic visual predators (Aguzzi et al. 2006a).

Similarly detailed knowledge regarding the behaviour of benthos is still a long way off. Several species apparently perform long-range migrations within the benthic boundary layer, following bathymetric gradients across continental shelves and slopes (Benoit-Bird & Au 2006). These movements can be broadly defined as nektobenthic (Cartes et al. 1993). Catches of populations of these species vary rhythmically at a certain depth stratum as the result of arrival and departure of schools (Aguzzi & Company 2010). The occurrence of this behaviour has been inferred in most of cases by combining direct observations, such as trawl catch patterns, with indirect ones, such as comparisons of morphological and metabolic adaptations (Company & Sardà 1998, Pulcini et al. 2008, Aguzzi et al. 2009b, Antonucci et al. 2009).

*The effects of behavioural rhythms on population/stock and biodiversity assessments*

Deep oceans represent one of the last frontiers for human exploration on Earth, with deep-sea marine biology being a relatively recent scientific discipline (Glover et al. 2010a). Ocean zones with water depths below 200 m cover 75% of the planet's surface, but very little is known about the species inhabiting these zones and their life histories (Waterman 2001). Deep-water ocean areas potentially host some of the ecosystems with the highest biodiversity levels on the planet (Ramirez-Llodra et al. 2010). Most of these deep-sea areas along continental margins are threatened by increasing anthropogenic pressures (Sheppard 2000) mainly related to searching for minerals, oil, and gas, with commercial fisheries apparently exerting the greatest impact (Benn et al. 2010). For example, a deep water fishery in the NE Atlantic Ocean trawling down to a maximum depth of 1500 m has been shown to deplete the demersal fish assemblage, affecting up to 77 species of fish in addition to the 3 main species targeted by the fishing vessels (Bailey et al. 2009, Priede et al. 2011). Furthermore, in these deep-sea areas anthropogenic changes in species composition should be distinguished from more long-term natural ones that can be associated with climate (Bailey et al. 2006, Billet et al. 2009).

Studies on biodiversity in deep-water areas should be linked to the concept of community functioning in the face of habitat changes through time (Smith et al. 2009). The management of new fisheries grounds, or any other area where human activity may represent a potential source of impacts, requires precise data on population demography and distribution as well as the evaluation of biodiversity (Pauly et al. 2003, Morato et al. 2006). Commercially targeted species represent the focus of management policies, and the preservation of their biomass for sustainable exploitation also requires investigation of all other surrounding species, from which data must be derived and integrated into increasingly holistic models (Roberts 2002). This requirement is one of the main justifications for biodiversity studies in deep-water areas and attempts to link community functioning with habitat changes through space and time (Danovaro et al. 2010).

One of the great limitations to improving the reliability of estimating species abundance estimates using trawl surveys is the overall variability of data collected at sea (Godø 1998). In trawl surveys, the time of the day is usually preserved as constant, or it may represent a random character due to technical factors (Raffaelli et al. 2003). Random sampling with respect to time produces unpredictable variability in the species composition of catches (Bahamon et al. 2009). Planning temporally scheduled sampling to standardise field data has been generally accepted in terrestrial ecology studies for quite some time (Park 1941) but this approach is still inconsistently applied in marine research (Naylor 2005).

Trawl timing is intuitively perceived as a biasing factor when sampling is not repetitively performed at a given depth. Changes in species abundances and composition are related to the rhythmic behaviour of individuals (Aguzzi & Company 2010). Therefore, sampling at a given depth should be repeated at different times of day and in different seasons to evaluate the biasing effects of time on the reported trawl data (Aguzzi & Bahamon 2009). Unfortunately, these factors are not often taken into account. There is a conflict between allocation of effort to sampling and space or time not only in deep-water sampling but also for coastal-water sampling (Azzurro et al. 2011). An increase in the surveyed area, as required for the assessment of population distributions, usually implies a concomitant reduction in the frequency of repetitive sampling within a certain location (Benoit & Swain 2003). Only temporally scheduled sampling within a fixed area can provide the data required for the characterisation of behaviour rhythms.

Within this framework, specifically planned day-night trawl surveys show the occurrence of broad diel variations in communities from different continental margin areas exposed to fluctuations in solar light intensity (Petrakis et al. 2001, Benoit & Swain 2003, Carpentieri et al. 2005, Benoit-Bird & Au 2006, Benoit-Bird et al. 2008, Aguzzi & Bahamon 2009). For example, in the western Mediterranean Sea, diel differences in catch composition are the product of the rhythmic appearance and disappearance of populations from trawl-hauling windows (Figure 1). This variation can also follow a seasonal pattern. Several decapod and fish species appear to modify their depth ranges from autumn-winter to spring-summer (Figure 2; Aguzzi et al. 2009a). In benthic species, the number of individuals collected at a given depth and location may vary over the year, not only because of the reproductive cycle (i.e., the recruitment of pelagic larvae into benthic adult cohorts; e.g., Company et al. 2003), but also due to the modulation of behavioural rhythms in response to changing photoperiod lengths. Some populations appear to move into darker, deep waters when the duration of the photophase increases, while others move to shallower depths, where they become exposed to brighter daytime environmental illumination levels (Aguzzi et al. 2009a, Aguzzi & Company 2010). Such seasonal bathymetric changes have also been observed in other continental margin and abyssal areas. For example, seasonal changes in the population size-frequency distribution of grenadier fishes (*Coryphaenoides* spp.) have been detected in both the Central North Pacific Ocean at a depth of 5800 m (Priede et al. 2003) and on the Porcupine abyssal plain of the North Atlantic Ocean at a depth of 4800 m (Smith et al. 1997). It was proposed that seasonal changes in size-frequency indicate the occurrence of basin-scale seasonal migrations of these fishes across the seafloor into and out of the study area.

Studies employing day-night scheduled trawling between 700 m and 1000 m depths have also reported diel variations in the composition of communities (Cartes et al. 1993, Sardà et al. 2003), indicating the effect exerted by the few remaining photons on the extremely sensitive visual organs of mid-slope species. Despite these observations, population, biomass and biodiversity studies making extensive use of oceanographic data very rarely consider solar light measurements as a potentially helpful tool for interpreting data (e.g., Hart et al. 2010).

### *The needs of a new observational technology*

Changes in communities over a 24-h or a seasonal period within a benthic sampling area represent the sum of all species rhythmic displacements that result from synchronic movement of all individuals of a population within different depths of the water column and the continental margin. In this sense, investigations of behavioural rhythms in populations of different species in an area can be placed within the broad framework of studies on ecosystem functioning (Kronfeld-Schor & Dayan 2003).

In this context, sampling methodologies and their applicability represent a major bottleneck in understanding the biology of species inhabiting deep-water continental margins and the deep sea. Trawling is still one of the most effective and more economically feasible methods of sampling (Raffaelli et al. 2003, Sardà et al. 2004). Trawling studies are broadly conducted over large seabed areas for the assessment of the distribution and demography of populations (e.g., stock assessment), as well as for overall biodiversity evaluations (Coll et al. 2010). Pluriannual surveys are common on several of the world's continental margins for these purposes, for example, in the Mediterranean (Relini & Piccinetti 1994, Bertrand et al. 2002), parts of the North Atlantic, such as the Gulf of St. Lawrence (Bailey & Elner 1989, Hurlbut & Clay 1990), the Bering Sea (Bakkala 1993), and the Pacific Ocean (Knuckey & Dichmon 2009).

## Automated video-imaging from cabled observatories for the study of behavioural rhythms

The lack of tools for obtaining direct ethological observations leads to major knowledge gaps regarding the behavioural mechanism underlying trawl sampling variability. Technological limitations in direct observation capabilities are at the base of the presently scarce modelling capacity regarding population/stock and biodiversity assessments as well as ecosystem functioning in continental margin areas. Improvement in this field requires a new observational technology to monitor community changes produced by the synergic sum of behavioural rhythms of all constituting species. This technology must be i) continuous and long-lasting with respect to the frequency and duration of data collection; ii) automated in relation to the real-time processing of biological data; and iii) multiparametric in terms of the integrated acquisition and processing of biological and habitat data (see Section on Multiparametric measurements and chronobiological analytic protocols for the characterisation of ecosystem functioning).

### *Cabled seafloor observatories for multiparametric video and oceanographic monitoring*

Behavioural rhythms of species within a community represent the core of ecosystem temporal functioning having still poorly known implications on results of assessment surveys in continental margin areas and the deep-sea. The study of rhythmic behaviour at diel and seasonal scales requires a technological development mostly directed towards coupled acquisition and analysis of diversified and enlarged datasets over periods of time larger than one year (Matabos et al. 2011).

*The state of art in video sampling technology and cabled observatories as innovative technology*

In the last 30 years, the development of video-imaging in association with multisensor measurements for habitat characterisation has been applied to understanding patterns of biomass and biodiversity variations at different spatial and temporal scales (reviewed by Glover et al. 2010b). Technological effort has been applied to development of observational tools for remote exploration and monitoring of the sea. Remotely Operated Vehicles (ROVs), Autonomous Underwater Vehicles (AUVs), and non-permanent camera stations have provided glimpses of the continental margin and, more generally, of deep-sea ecosystem functioning over a range of temporal windows (Allen et al. 1978, Stoner et al. 2008). However, none of these technologies can currently be used to produce continuous time series of integrated biological, geological, and oceanographic data over consecutive years, as is required for reliable monitoring of benthic ecosystems in relation to present challenges of management and conservation.

Cabled multiparametric seafloor observatories connected to the shore for power and real-time data transmission represent a substantial innovation in this respect. In principle, the data provided by such platforms satisfies the above needs. Cabled observatories use a vast array of instruments on submarine platforms, including seismometers, acoustic current profilers, hydrophones, sonar, echo-sounders, conductivity and temperature sensors, and pressure sensors (Aguzzi et al. 2011b). In addition to imaging cameras, the biological tools available for these experiments include plankton samplers, sediment traps capturing falling particulates to the deep-sea floor, turbidity sensors, and benthic flow simulation chambers. Chemical and gas sensors for measuring carbon dioxide, oxygen, methane, and nitrates are also available (Matabos et al. 2011).

Sensors deployed over the seabed are only a part of the relatively complex instrument arrays that can be set up in cabled observatories. Profiler instrument packages installed at scientific nodes can provide multi-parametric measurements of ecological processes within the water column above. An example of that is represented by the Regional Scale Nodes associated with the US Ocean Observatory Initiative (OOI) (see Section on Networking of seafloor cabled observatories as a tool for future predictive and informative studies); these are sensors attached to profilers that vertically traverse the ocean from just above the seafloor to the subsurface. CTDs, dissolved oxygen sensors, current meters, and fluorometers are expected to be installed in these profiler instrument packages to provide temporal and spatial measurements over almost the entire oceanic depth range.

### *A complex management scenario for data acquisition*

Data acquisition still represents a critical step depending on sensor typologies and specifications (Majumder et al. 2002). In the three-dimensional context of the seabed including the overlying water column, multiparametric coordinated data collection requires establishment of acquisition and management protocols to optimise these processes (Bahamon et al. 2011). For example, even a single habitat parameter, such as the temperature from CTDs, yields highly complex information when measured repeatedly over time at different depths, including the seabed (Figure. 3).

Interest in database technology is growing as new tools become available, such as Structured Query Language (SQL) applications that are capable of storing and managing large volumes of spatial and time series of data, though they fail to support multi-dimensional arrays as a core data type (Aguzzi et al. 2011b). Relational mapping methods (Barry & Stanienda 1998) are gaining growing interest as an alternative database technology to develop an object-relational approach to effectively create a new interface for an existing database management system, thus allowing object-oriented data and relational data to be combined in a single framework.

Data fusion is a fairly new informatics discipline, as it represents the capacity to integrate data from different instruments (Klein 2004, Mitchell 2007). Technologies should be adopted if they can be constantly calibrated and in which inter-instrumental transfer of data is possible. A good example of this type of technology, though from a different field, is provided by meteorological stations that can be coupled with networks used to forecast weather conditions in relation to animal health and production (Menesatti et al. 2009b). Thus, local dairy cow housing environments can be monitored by sensors of different types integrated into the same managing system. Within these networks, only a few stations for which calibrated instruments are available can be used for forecasting purposes.

### *Video-imaging as novel bio-sensor approach*

Geophysical and oceanographic sensors are able to directly measure parameters driving processes of interest. In contrast, one of the difficulties associated with marine biology research is the lack of sensors capable of life functions directly at the level of individuals, species and communities. Most bio-sensors are indirect, since they measure products of life processes in terms of chemical derivates (e.g., dissolved oxygen, chlorophyll or nitrates; Aguzzi et al. 2011b).

Cabled observatories often have video cameras (Favali & Beranzoli 2006, Favali et al. 2010). In relation to benthic ecosystems, these cameras potentially represent the primary effective bio-sensor at individual animal, population, and species levels, provided that automated image analysis is sufficiently developed to classify animals based on differences in shape, texture and colour. Video cameras may thus be used to efficiently detect fluctuations in the numbers of individuals of different species over time as a proxy of population behavioural rhythms (Aguzzi et al. 2009c, 2010b). When image time series are coupled with other physical parameters collected synchronously, information on rhythmic activities can be related to environmental fluctuations within the habitat under investigation (Matabos et al. 2011). This could be helpful in searching for the geophysical cycles and processes ultimately controlling the behaviour of individuals and populations of different species as one of the major driving forces of ecosystem functioning (see Section on Multiparametric measurements and chronobiological analytic protocols for the characterisation of ecosystem functioning).

Previous time-lapse imagery studies carried out with semi-autonomous observatories (e.g., landers) have indicated the effectiveness of cameras for the characterisation of ecosystem functioning. Kemp et al. (2008) described the DOBO system (Deep Ocean Benthic Observatory), which is capable of remaining on the seafloor for up to 12 months releasing bait at regular intervals. Time series observations were obtained for squat lobsters (*Munidopsis* spp.) and macrourid and zoarcid fishes at a depth of 3664 m in the Atlantic Ocean. This type of system is capable of providing information during winter months when sampling using surface vessels is not feasible due to adverse weather conditions. DOBO is also equipped with multiple sensors for conductivity, temperature and pressure and an Acoustic Doppler Current profiler.

### *Investigation of biological rhythms of marine populations through time lapse video imaging*

Video cameras on cabled seafloor observatories are often installed as an accessory to the primary purpose of acquisition of geological, chemical, and physical data. Hence these video sources are rarely used for continuous, long-term ecosystem surveying. One limitation on their use is the lack of an acceptable level of automation of image processing. (Costa et al. 2011). Human inspection of videos is time-consuming and represents an important bottleneck for the extraction of quantitative information from video footage (Walther et al. 2004, Cline et al. 2007, Glover et al. 2010b).

During the past two decades automated video-imaging techniques have been increasingly used to extract quantitative biological information for a wide variety of land and marine species (Table 1). This information includes not only the shape and the colour of organisms for the purpose of achieving suitable levels of automatic discrimination of species (i.e., classification) once identified in frames, but also the ability to follow and quantify animal behaviour (i.e., tracking).

Implementing procedures for animal tracking and classification is crucial for automated video-image analysis related to investigations of the behavioural rhythms of marine populations. Tracking is the process of identifying the same animal as it displaces within a set of temporally consecutive frames (Voss & Zeil 1995, Lipton et al. 1998, Chraskova et al. 1999, Edgington et al. 2004, Walther et al. 2004). Classification is the grouping of each newly identified animal within a pre-established category, which is usually the species (Strachan & Nesvadba 1990, Lipton et al. 1998, Dah-Jye et al. 2008). While tracking is critical for characterisation of behavioural rhythms (i.e., the counting of individuals over time), classification is necessary for the characterisation of communities at a local scale.

Tracking procedures should be considered at different temporal scales in relation to two major aspects of animal behaviour (Aguzzi et al. 2009c,d, Menesatti et al. 2009a): i) the average temporal presence of an organism in the Field Of View (FOV), according to its speed of displacement; and ii), the average time interval required by an organism to re-occur in the FOV, according to the periodicity of its behaviour rhythm. In relation to the first aspect, frame collection should be set at frequencies 4-5 times higher than the average probability of spotting a targeted organism in the FOV (Nyquist-Shannon frequency; Shannon 1949). Regarding the second aspect, video acquisition has to be carried out over a temporal window encompassing several cycles (equivalent to the periodicity) in the fluctuation of a certain habitat driver to allow consistent and reliable time-series analysis (see Section on Multiparametric measurements and chronobiological analytic protocols for the characterisation of ecosystem functioning). This requirement is particularly strong in field studies in which the measured rhythms are noisy due to the variable level of synchronism in the activities of all individuals constituting the population (Aguzzi et al. 2010a,b, Azzurro et al. 2011).

Depending on frame acquisition frequency, video-imaging techniques can be broadly subdivided in two major groups: ‘single frame’ (i.e., a collection of temporally isolated and still images, as in the case of ‘time-lapse photography’ (Sherman & Smith 2009) and ‘motion vision’, the continuous acquisition of frames at ≥ 24 fps conventionally known as “video footage” (Aguzzi et al. 2010b). In the first mode, the frequency of acquisition tends to be lower than the movement rate of animals, which implies that tracking cannot be performed, and only classification is achievable. In the motion vision mode, the frequency of image acquisition is usually higher than displacement velocity, and tracking of animals through consecutive frames can be carried out together with the computation of their trajectories of displacement. Frame interval should be chosen to match the expected movement rate of the species.

Estimation of local population abundances by video-imaging is hindered by the problem of repeat counting of individuals as they move around in the FOV. (Trenkel et al. 2004). One way of avoiding this is by estimating displacement trajectories and subsequently eliminating all initially counted individuals within the same frame set (Smith et al. 1993). Video studies on behavioural rhythms of populations may produce biases in the estimation of counted individuals when trajectories are not considered. However, because those biases are constant throughout the sampling period, they do not mask inherent fluctuations in the individuals counted as a result of behaviour rhythms (Aguzzi et al. 2010b).

Having identified displacing animals in single frames, the problem reduces to tracking their displacement across a set of temporally consecutive frames. Assuming that an animal is already tracked in a temporally continuous group of frames, certain algorithms can be used to match the identified animal in a new frame, hence connecting it to the precedent trajectory. This assignation can be done by comparing an object's position with its expected position for each event, extrapolated from its positions in the past with each object being assigned to the event that it matches best (Edgington et al. 2004). Computation of trajectories (spatial tracking) can be implemented using Kalman Filters (Cline et al. 2007) as a group of mathematical equations providing an efficient and recursive solution to the method of minimum squares (Lauritzen 1981). This solution allows computing an optimum statistical spatial estimation of an object at any time point. For this purpose, the available information at time t - 1 is updated with the information during the following t moment. Another algorithmic approach includes recursive Bayesian filters (Betke et al. 2007). This approach solves the problem of tracking a single object in an image sequence recursively by estimating the state (s) of the object in the current frame (t), defined as s(t), based on its state in the previous frame, s(t - 1), and by filtering measurement x(t) in the current frame.

### *Methods for the automated classification of tracked animals*

Identification of individuals to species or classification is fundamental to the study of behavioural rhythms using automated video image analysis. The automated counting of individuals can be efficiently carried out only after a suitable level of efficiency in the pattern recognition of targeted species is achieved. Classification can be carried out by considering the outlines of animals (i.e., the profile given by the silhouette) or their colour and physical properties.

The ‘Pattern Recognition problem’ consists of determining a procedure that can assign each entity to its proper class on the basis of its attributes. Automated systems for pattern recognition and classification are based on a supervised approach and, for this reason, should be tuned in their efficiency by a training process. This programming procedure consists of a ‘visual census’ performed by trained operators on a subset of data for the entities considered, with this subset being referred to as the training set. Thus, a training set consists of entities characterised by a set of attributes that belong to known classes (Nieddu & Patrizi 2000). In the case of imaging products from cabled observatories, training sets are represented by a collection of selected images depicting the targeted species viewed from different angles. (Aguzzi et al. 2011b).

### *Statistical tools for classification*

Multivariate classification analyses can be generally divided into two main categories: unsupervised and supervised (Costa et al. 2011). For unsupervised techniques, grouping or clustering methods for multivariate elements (x-block) are based on functional relationships among these elements (i.e. distances or variances). They do not require *a priori* knowledge of the class categories. By contrast, in supervised techniques, class attribution is provided by single or multiple variables (y-block). Thus, multivariate methods are forced to cluster into *a priori* established classes. Unsupervised methods are mainly applied in an exploratory sense, when the aim is to analyse or visualise non-forced aggregating relationships (unsupervised) among elements.

Concerning supervised techniques, it is possible to distinguish two main analytical approaches: classification and modelling. Classification analysis requires a decision rule, referred to as the ‘classification criterion’, to sort objects into classes on the basis of selected quantitative features (Jayas et al. 2000). By contrast, in modelling, it is possible to attribute objects not only into one or more classes but also to none (i.e., in this case, the object is an outlier). Modelling techniques calculate the 'prediction probability' using a classification threshold for each modelled class (Costa et al. 2011). The modelling efficiency is indicated by statistical parameters, such as 'sensitivity' and 'specificity'. Sensitivity represents the percentage of the objects of a category accepted by the modelled class. Specificity is the percentage of objects different from the modelled classes that were rejected by the classification criterion. However, for classification, a matrix of correct classifications can be used (Costa et al. 2008b).

The choice of suitable statistics to treat the different categories of data (i.e., morphometric or colorimetric descriptors) represents an important step in the development of automated video image analysis. The many different algorithms that can be used for classification can be summarised into the following categories:

1. Probabilistic-based:
    - Parametric - Linear Discriminant Analysis (Li & Yuan 2004) and Quadratic Discriminant Analysis (Wu et al. 1996).
    - Non parametric - Potential Function (Pei & Xie 1999).
2. Distance-based: k-nearest neighbour (Findik et al. 2010), Soft Independent Modelling of Class Analogies (Aguzzi et al. 2009b), and Partial Least Squared Discriminant Analysis (Costa et al. 2008b).
3. Experience-based: Support Vector Machine (Amit & Boaz 2005) and Artificial Neural Networks (Costa et al. 2009c).

As stated by Mitchie et al. (1994) "…Machine Learning aims to generate classifying expressions simple enough to be understood easily by humans. They must mimic human reasoning sufficiently well to provide insight into the decision process…". Machine-learning approaches can be implemented by combining video-acquired morphological descriptors and multivariate statistics. Existing methods are based on random forest classifiers (i.e., an ensemble of decision trees, where each tree is a computer-generated structure analogous to a dichotomous key) taken directly from descriptors (reviewed by Lytle et al. 2010). In a random forest, each tree is constructed using a process that incorporates a random component to introduce variation amongst the trees.

### *The fuzzy logic approach in animals' classification*

In extreme environments such as the deep sea, where the wide range of animal diversity is often currently unexplained, the use of a fuzzy logic approach within object classification could return an interesting way of classifying new (not yet classified) objects in a trained guild. A fuzzy set is a set in which the elements have degrees of membership. An element of a fuzzy set can be full member, or a partial membership value can be assigned to an element that is no longer restricted to only two values; 0 or 1 but also any value in between (Bahamon et al. 2009). Fuzzy systems have the capability of representing classification decisions explicitly in the form of fuzzy 'if-then' rules. Fuzzy sets allow the assignment of partial- and multiple-value memberships. Fuzzy systems use vague, imprecise or uncertain information to generate simpler, more suitable models that are easier to handle and more familiar to human thinking. Concerning quantitative features for the automated classification of marine organisms, these classifications can be based on shape, colour, texture or a Scale-Invariant Feature Transform (SIFT) algorithm (see below).

### *Morphometric and colorimetric descriptors for automated animals' classification*

For quantitative image analysis (i.e., chromatic or hyperspectral derived) different elaborative steps finalised altogether to enhance the chromatic properties (contrast, equalisation, amplification, denoising, smoothing, and etc.) and to extract the sensible part of the image can be used to differentiate objects from the background (segmentation and thresholding). This procedure can be used to measure sensible objects by morphometric, optical densing or texture mapping (Bharati 2004, Li 2008, Lillholm 2003, Huang 2003). Among the different features to be extracted and processed with image analysis tools, shape and colour are some of the most important and easy to be extracted.

The shape of a given animal can be automatically classified and assigned to a species by many different methods, including univariate techniques, such as fractal indices or ratios, or multivariate techniques, such as Fourier methods (Rohlf & Archie 1984). These tools allow the recognition of an organism through the fitting of its profile with a set of harmonic functions.

In Elliptic Fourier Analysis (EFA), the animals' shape can be studied by profile digitisation (Figure 4; Costa et al. 2009a, 2010, 2011). A set of points is automatically positioned along the outline in an equiangular or equidistant fashion. The contour is transformed into an incremental harmonic function when a set of *x,y* coordinates is computed for each point. That function is then fitted with a set of different ellipses to approximate its variation with the highest precision. At the end of the fitting process, each biological sample is represented by a set of ellipses, each of which has four coefficients. As a result, a matrix comprising all individuals with their respective ellipse coefficients can be obtained. This matrix is the input required for multivariate statistical analysis (Costa et al. 2011).

Fourier Descriptors (FD) are also employed for the automated recognition of tracked animals during the study of behavioural rhythms (Toth & Aach 2003, Veeraraghavan et al. 2005). FD can be utilised to describe the shape outline of a biological form in terms of its frequency variability (Aguzzi et al. 2009c). This description is obtained by fitting a set of circular harmonic functions, each with its own coefficients (the FDs), onto the outline of a newly tracked animal. By choosing the correct number of harmonics, it is possible to tune the precision of the outline approximation.

SIFT is an algorithm employed in computer imaging used to detect and describe local features in images (Lowe 1999). Interesting points of a particular organism can be extracted to provide a 'feature description' of the object. This description, extracted from a training image, can then be used to identify the object in a test image containing many other objects (Figure 5; Aguzzi et al. 2011a). A significant requirement of this process is that the set of features extracted from the training image must be robust enough to allow efficient identification, despite changes in image scale, noise, illumination, and local geometric distortion. This method robustly identifies objects even under conditions of clutter and partial occlusion because the SIFT feature descriptor is invariant to scale, orientation, and affine distortion and is partially invariant to illumination changes (Lytle et al. 2010).

### *Flowcharts of image processing and animal classification for the study of population behavioural rhythms*

Different automated video-imaging trials have been elaborated in the past few years when working with imaging products from cabled observatories in different continental margin areas. The procedure of image treatment at each video station necessarily requires the implementation of specific algorithms, due to different light levels in relation to artificial lighting conditions, as well as turbidity, fouling and substrate texture/heterogeneity. Based on tracking and classification processes, general image treatment flowcharts can be proposed for the automated processing of time series of images collected by cabled observatories (e.g., the Canadian VENUS in Saanich Inlet or the Japanese Sagami Bay observatories at 103 and 1100 m depth, respectively; see on Networking of seafloor cabled observatories as a tool for future predictive and informative studies), to study population behavioural rhythms.

Colour features, numerically defined by coordinates in n-dimensional colour spaces, can be important descriptors for animal classification (Bruno & Svoronos 2005). For example, in combination with shape analysis, the RGB (Red-Green-Blue) content of organisms can be added to increase recognition efficiency. Morphological classification can be then based on EFA, FD, SIFT, and FD + RGB semi-automated procedures (Aguzzi et al. 2009c, 2010b, 2011a). Each tracked animal can be recognised on the basis of its set of FDs and its average colour content coordinates (Figure 5; Aguzzi et al. 2011a). A library of manually supervised and classified images is required for each target species (Storbeck & Daan 2001, Culverhouse et al. 2003, Edgington et al. 2006). A subset of frames is chosen, and manual classification of animals is performed by a human observer through interactive scripts. Images of animals from different angles can be saved in a binary format to allow later morphometric comparisons and recognition. Moving animals are identified and assigned to species as pre-established categories by using multivariate supervised statistics, which allow constructing colorimetric and morphological models of reference (see the next section).

The acquired frames are processed for grey-scale transformation and segmentation (i.e., binarisation of the coloured image in black and white) (Figure 6A, B). Subsequent subtraction of consecutive frames is then applied to detect all moving objects, and areal thresholding permits selecting object sizes of interest that correspond to the general body size of animals (Figure 6C). The profile of identified objects can then be superimposed on the original image according to an area that corresponds to the animal’s body with its peculiar RGB coordinates (Figure 6D). Both the animals’ profiles and their RGB contents can be subsequently classified by multivariate supervised statistics (i.e., K-Nearest Neighbour, KNN or Partial Least Square Discriminant Analysis, PLSDA; reviewed by Costa et al. 2011), according to morphological models of reference extracted from libraries of manually classified images (i.e., the training set).

### *Acoustic tracking as a complementary method to automated video-imaging in investigations of the behaviour of marine species*

A concern related to use of video-imaging is that the lights necessary to acquire the images may influence the behaviour of the organisms being observed. Widder et al. (2005) used red light to illuminate a scene and demonstrated differences in the fauna observed. The use of acoustic imaging may solve problems of photic contamination (i.e., lighting at moments of frame/footage acquisition) at depths below the twilight zone.

Active sonar sensors oriented vertically upwards from a sea floor observatory seem to present a scope similar to video cameras in relation to population behavioural rhythms. These instruments allow depiction of zooplankton and pelagic fish displacements within the pelagic environment (reviewed by Lemon et al. 2007, Kaartvedt et al. 2009, Borstad et al. 2010, Cox & Borchers 2011). Horne et al. (2010) deployed the Deepwater Echo Integrating Marine Observatory System (DEIMOS) with Simrad 38 kHz upward-looking sonar at a depth of 890 m in Monterey Bay off the coast of California and recorded seasonal variability in diel migrations. Borstad et al. (2010) recorded a 2-year time series using a 200 kHz system deployed in Saanich Inlet, British Columbia and made major progress in the analysis and presentation of this type of large dataset, which was derived from a 0.3 Hz ping rate. The Deep-sea Long-term Observatory System (DELOS) at 1500 m depth off Angola is not presently cabled, being retrieved yearly for data downloading (see Section on Networking of seafloor cabled observatories as a tool for future predictive and informative studies). This multiparametric platform is also equipped with Kongsberg high resolution 675 kHz scanning sonar oriented horizontally outward to a range of 150 m. This positioning enables tracking movements of fishes and other targets around the observatory. Appropriately configured arrays can track whales in three dimensions, identifying different types of behaviours and feeding events (Nosal & Fraser 2007).

Acoustic technology in general, does not allow ultimate species classification, although multiple emissions frequency and resulting echo-trace analysis (using frequency responses) can distinguish the sizes of moving animals, thus allowing some distinctions to be made (Petitgas et al. 2003, Jech & Michaels 2006, Reier Knudsen & Larsson 2009). Acoustic video cameras could be used in the benthic environment to create high-resolution images of moving animals, similarly to what can be done using video-imaging systems (Mueller et al. 2006). However, the problem of species classification on acoustic-image products still remains. Acoustic imaging could efficiently replace video cameras only in cases when targeted taxa show very different morphologies (i.e., without the requirement of distinction based on colour and its patterning) (Boswell et al. 2008). Recent methodological improvements reached an acceptable level of acoustic classification with a reduced group of small fishes under laboratory controlled conditions (Roberts et al. 2011).

An alternative to optical methods for observing the behaviour of organisms is acoustic passive and active tracking, together with the use of tags or transponders (Sarriá et al. 2001). As an example of this, the DELOS platform is equipped with passive listening devices (Bagley et al. 2011). These sensors can detect sounds produced by crustaceans, fishes, and cetaceans. Individual fishes can be tracked using ingestible transponders that are tracked by sonar mounted on an observatory platform (Priede & Bagley 2000). Using ingestible acoustic transmitters, Priede et al. (1994)

detected seasonal changes in the swimming speeds of abyssal grenadier fishes in the NE Pacific Ocean. The global Ocean Tracking Network (OTN; O`Dor et al. 2010) uses a chain of acoustic observatories around the world to detect fishes with surgically-implanted acoustic identity tags (O’Dor & Stokesbury 2009). Using multiple observatories, OTN will be able to track mobile species throughout their annual or multi-annual migration patterns. The data and power capabilities of cabled observatories are enabling great advances to be made in the application of acoustic methods to long-term time series research.

## Multiparametric measurements and chronobiological analytic protocols for the characterisation of ecosystem functioning

The temporal functioning of continental margin and deep sea communities according to habitat cycles at diel and seasonal scales is essentially unknown at present (Barans et al. 2005, Wagner et al. 2007). Reports on species presence and abundance from the deep ocean are too limited in both space and time mainly because of observational technological limitations (see Section on The missing chronobiology framework in marine ecological studies). Marine species display rhythmic behaviours in response to geophysical cycles, and these responses affect observable populations during field sampling. This phenomenon influences population/stock and biodiversity assessments.

### *From descriptive to more cause-effect-based deep water ecology*

The integrated processing of biological and habitat information is a pending issue in continental margin ecology and represents one of the most promising ways to move beyond the current, chiefly descriptive, approach. The transition from descriptive to a more cause-effect-based marine research could be achieved by implementing the conceptual framework of integrated time-series analysis (Dickey & Bidigare 2005, Kelly et al. 2010). Inspiration can be obtained, for example, from chronobiology, which compares and integrates very different datasets involving behavioural, physiological, and molecular levels of complexity to study circadian clocks (Yamada & Forger 2010).

The temporal regulation of physiological processes within a whole organism involves the clock-coordinated activities of oscillators in the central nervous system and in several peripheral tissues (Dibner et al. 2010, Harrington 2010). The centralised control of biological clocks over all biological rhythms in an animal's body can be studied by characterising the direction of flow of regulating information (Yamazaki et al. 2000). This characterisation can be methodologically approached by obtaining synchronic time series for different physiological or molecular indicators in different tissues and organs and by detecting the peaks of rhythms as markers of the activity of the oscillator (Fukuhara et al. 2005). This type of comparison seeks anticipations and delays in the peaks of each oscillator in relation to each other, which could yield information on the direction of the flow of controlling information (Tosini and Aguzzi 2005). Similarly, in the case of multiparametric data acquisition from seafloor stations, time series of several habitat parameters should be compared and cross-checked to portray which incremental combination of such parameters causes a subsequent biological response (i.e., peaks in the counted individuals of a given population).

The statistical tools required for such analyses are already available in commercial chronobiological software applications which are used to statistically describe rhythms at molecular, physiological and behavioural level (e.g., Aguzzi & Chiesa 2005, Aguzzi et al. 2006b; Tosini et al. 2007). However, further development is needed for these tools to become more user-friendly, to fully satisfy the needs of biological and habitat data multiparametric analyses in field study of populations, i.e., in terms of applying automated sequential steps for data pre-processing (i.e., filtering; see below) and consequent time series analysis.

### *Integrated waveform analysis as a protocol for multiparametric data treatment*

A general protocol for the automatic and integrated treatment of bio- and habitat data can be proposed (Figure 7) according to the principle of anticipation and delay as a marker of cause-effect relationships between habitat fluctuations (i.e., the cause) and consequent population rhythms (i.e., the effect). Firstly, time series of biological observations (i.e., the number of video-counted individuals for each selected species) should be acquired with a frequency that corresponds to the timing of the recording of concomitant habitat parameters (Figure 7A). Multiparametric data banks should then be implemented to store such diversified information in columns (i.e., for all species and selected habitat parameters), all starting with the same reference time. Secondly, time series should be pre-processed for data filtering to eliminate excess noise (e.g., moving average and binning or outlier removal; see review in Levine et al. 2002) (Figure 7B). Thirdly, pre-processed time series should then be screened for inherent periodicity with periodogram functions (Figure 7C).

Waveform computing represents the fourth step in the proposed data analysis protocol, which, when successfully carried out in an integrated fashion, could lead to the discovery of interesting hints in order to discuss any potential cause-effect relationships between habitat fluctuations and the behavioural response of species (Aguzzi et al. 2004a,c). Although phase-relationship associations in visual counting of species (i.e., by video-imaging) with the light-dark or tidal cycles do not demonstrate *per se* a causal relationship, integrated waveform analysis provide a temporal linkage between the circadian system of species and several potential fluctuating environmental cues (i.e., zeitgebers). In the laboratory, the phase relationship of behavioural rhythms associated with the light-dark cycle accounts for the diurnal or nocturnal niche of species, demonstrating under controlled conditions a causal relationship between behaviour, underlying physiology, and light intensity variation (Aguzzi et al. 2005, Chiesa et al. 2010). The same correlation between behavioural rhythms and physical variables does not necessarily imply entrainment in the field. However, this represents a good attempt at description of ecosystem dynamism based on behavioural changes of individuals constituting the community, within the framework of surrounding variable habitat conditions.

Integrated waveform analysis can be performed as follows. Time series of both behavioural and environmental variables can be subdivided into sub-sets with a length corresponding to the periodicity identified in periodogram analysis (for example, of 24-h length for the day-night cycle or of 12.4-h length for the tidal cycle; Figure 7D). Values from all subsets of bio- and habitat data should then be averaged for corresponding data bins, thus obtaining a consensus average curve (Figure 7E). Significant increments (i.e., onset and offset as phase markers of rhythm) can then be identified from values above the Midline Estimating Statistic of Rhythm (MESOR; Aguzzi et al. 2003a,b). The MESOR is conceptually equivalent to a daily mean as threshold used for significance: all average estimates of the waveform are re-averaged, and the resulting value is plotted onto the consensus curve as a threshold horizontal line. Above-MESOR values represent the peak (i.e. as significant increment in the biological fluctuation), the temporal amplitude of which can be represented as a continuous line in time. The temporal limits of the activity/rest intervals associated with behavioural rhythms in relation to measured habitat parameters can be assessed, and the indications that they provide about peaks in temporal relationships as markers of a cause-effect relationship are of particular interest (Aguzzi et al. 2005, 2006a,b, Costa et al. 2008a). Onset of peaks of different biological and habitat waveforms can then be compared over a standard 24-h cycle. Thus, the existence over consecutive cycles of significant increases in behavioural activity prior to the increase in the state of fluctuation of other habitat variables could represent the phenomenon of anticipation, as a marker of rhythm entrainment (Figure 7F). Additionally, the waveform area calculated at a given time interval can be used to estimate the amount of activity related to any endogenous or exogenous component regulating behavioural rhythms (Chiesa et al. 2005, Anglès-Pujolrès et al. 2006).

## Networking of seafloor cabled observatories as a tool for future predictive and informative studies

Seafloor cabled observatory video studies on the behavioural rhythms of animal populations provide an important source of data for modelling variability in sampling related to these rhythms responding to changeable habitat conditions (Aguzzi et al. 2010a). Regardless of their quantification efficiency, local direct population and biodiversity estimations using video methods should be undertaken very cautiously. Derived animal counts should not be extrapolated to population demography given the overly geographically localised character of the observations. Additionally, a lack of records for a given species does not imply its absence within a geographical area (Barans et al. 2005), as such records are challenged by remote video observations using food (i.e., bait) attractors (e.g., Cappo et al. 2004). For example, abyssal demersal fishes can be observed using free-fall lander technology, as they can be actively attracted to bait placed within view of seafloor cameras (Priede & Bagley 2000).

Accordingly, data from a permanent video cabled observatory can only assist in understanding how temporal biases in population/stock and biodiversity assessments may occur if the timing of sampling is not considered. In the future, increasing knowledge about the behavioural rhythms of deep-water species and their modulation based on geophysical cycles will be of great value to researchers in modelling such biases in other sampling methods, such as trawling (Barans et al. 2005).

### *Spatial coverage of existing or planned cabled observatory networks*

Presently, insufficient spatial coverage greatly biases any potential estimate of population demography and local biodiversity in continental margin areas. (Coll et al. 2010). The easiest solution to this problem would be increasing the number of observational sites and the length of monitoring periods in a given area. An interesting opportunity for extending the currently limited underwater monitoring capability in terms of both space and time is presented by the creation of networks of permanent multisensor cabled seafloor observatories, deployed from coastal areas to abyssal plains. Although at the present state of technological development, this objective is not realistic in the short term, the increase in the number of these platforms represents a real future opportunity to perform prolonged observations in a wide range of habitat conditions (Beranzoli et al. 2002, Favali & Beranzoli 2006, Favali et al. 2006a,b, Frugoni et al. 2006, Manuel et al. 2010).

A sustained stream of high quality interdisciplinary data on the marine environment associated with appropriate sampling is being acquired to study biological,, geological and oceanographic interlinked phenomena (Lampitt et al. 2010). Network configurations are particularly suitable for disciplines that require real-time integration of different submarine nodes deployed over large areas. Several countries and consortia have started long-term multiparametric monitoring initiatives by installing individual or networks of permanent and integrated cabled observatories (Table 2 lists marine multiparametric cabled seafloor observatories bearing video cameras) for a variety of purposes over large geographic areas (Favali et al. 2006a, Favali & Beranzoli 2009, Favali et al. 2010). Although ecological discovery and ecosystem bio-monitoring are not prime targets of these observatories, they often include associate science components that could quite easily host the type of research under consideration here.

Countries currently driving large international programmes aimed at creating underwater permanent cabled installations networks are Japan, Europe, the United States, and Canada.

Japan is presently working on the development of alert systems for earthquakes and associated tsunamis, which is currently one of the major requirements in seismically active areas (reviewed by Priede et al. 2004, Favali & Beranzoli 2006, Favali et al. 2006a, Mikada et al. 2010). The Dense Oceanfloor Network system for Earthquakes and Tsunamis (DONET 2011) consists of a network of seismic observational nodes along several locations in the Japan Trench (i.e., off-Tokachi-Kushiro, Suruga, Boso, Izu Peninsula, Sanriku, and in Sagami Bay).

The European Seafloor Observatory Network (ESONET) EC project indicated in its scientific objectives a number of observatory locations planned for different continental margin depths and latitudes including the Arctic (Svalbard), the Norwegian margin, the Nordic Seas (Faroese), Irish waters (Porcupine/Celtic; CELTNET), the Azores, the Iberian area of the Gulf of Cadiz, the Ligurian Sea, the Western Ionian Sea (Eastern Sicily), the Hellenic area, the Black Sea, the Marmara Sea, and the Canary Islands (PLOCAN) (Favali et al. 2010). The large-scale research infrastructure European Multidisciplinary Seafloor Observatory (EMSO 2011), forms part of the European Strategy Forum on Research Infrastructures roadmap (ESFRI 2011) and intends to develop permanent nodes of the underwater network at these sites. Within the Central Mediterranean, aims are related to geophysical and oceanographic applications and to the capture of neutrinos crossing the Earth by means of photomultiplier tubes (Amram et al. 2000, Priede et al. 2008, Ageron et al. 2009, Aguilar et al. 2010, Favali et al. 2011). In particular, the Western Ionian Sea cabled node of EMSO manages a variety of different instruments covering the scientific fields of geophysics, physical oceanography and bio-acoustics (Favali et al. 2006a, 2011) acquiring the data in real time. In the future it is planned to host video cameras at these locations.

Off the coast of the United States of America video cabled platforms are fully operative in the deep-water Californian margin (e.g. the Eye-in-the-Sea ,EITS, http://www.mbari.org/earth/mar_tech/EITS/eits_live.html from the Monterey Accelerated Research System - MARS; see Table 2). Other platforms that could also be easily equipped with cameras are the shallow-water Martha’s Vineyard Coastal Observatory (MVCO 2011) of Massachusetts’ Katama Air Park and the Long-term Environmental Observatory (LEO 2011) of New Jersey. The USA is also promoting the complex Ocean Observatories Initiative (OOI 2011) based initially on the deployment of permanent oceanographic buoys in the years from 2010 to 2014, which could be used in future for the deployment of cabled observatories.

NEPTUNE Canada (NC; see Table 2) is an operating cabled regional network across the Juan de Fuca plate in the Northeast Pacific off Vancouver Island, British Columbia (Barnes et al. 2008), which is part of the Ocean Networks Canada Observatory. The system now provides the continuous power and bandwidth to collect integrated data on physical, chemical, geological, and biological gradients at temporal resolutions relevant to the dynamics of the earth-ocean system. The location of this system over an entire tectonic plate provides a unique setting in which to study the influence of geological, physical, and tectonic processes on species dynamics and biodiversity in a comprehensive manner at temporal scales ranging from minutes to decades. Furthermore, that network encompasses a high diversity of ecosystems, including a coastal embayment (with both hardground and soft-bottom environments), the continental slope, a marine canyon, methane hydrate fields, and an abyssal plain with hydrothermal vents. The scientific outputs of the network will be easily applicable to other locations in the World Ocean. The NEPTUNE Canada regional network is currently equipped with six video camera systems located at depths ranging from 20-980 m, and two additional systems will be deployed in 2011 at hydrothermal vents on the Endeavour segment of the Juan de Fuca Ridge, at a depth of ca. 2190 m. These cameras are operated through a web interface available anywhere on the planet, and videos are freely accessible and available for download through the NC website (NEPTUNE 2011).

Although, in several cases, the primary target of observatory deployment is not image acquisition, presently installed nodes potentially represent a powerful laboratory for investigating the modulation of behavioural rhythms of local animal populations correlated with geophysical cycles (and events), such as those involving annual formations of water masses, light intensity fluctuations, and internal tides or inertial currents. Because of differences in geographic location, detected rhythms ultimately respond to local latitude, oceanographic setting, water depth, seafloor morphology, and the likely effects of climate change associated with global warming (Canals et al. 2006, Somot et al. 2006).

The acquisition of biological data by the use of video-imaging systems will likely become more widespread with the development of seafloor observatory technology and networking. Multidisciplinary data acquisition is not currently being conducted using standardised protocols of reference; this lack of standardisation inhibits interdisciplinary analysis, as will be required, for example, to improve geo-hazard warning and to evaluate the influences of climate change and anthropogenic impacts (Ruhl et al. 2011). Consequently the scientific community is in urgent need of standard protocols to acquire biological and habitat data with these platforms in different areas of the planet, to provide a global understanding of the dynamics of marine ecosystems and predict their evolution in the context of changing oceans.

### *Future issues to address in the use of cable observatory technology*

With the increase in, the number of cabled video observatories, the efficiency of automated image processing (i.e., biodata), and the integration of biological and habitat data analysis, it will be possible to undertake a series of studies of general interest within the context of novel ocean observing framework topics (IFSOO 2011).

#### *Ecosystem dynamic functioning in areas with geophysical cycles of conflicting periodicity*

In the near future, the use of video observation networks should allow for the study of the oceanographic modulation of behavioural rhythms in species with a wide distribution range, which are, thus, subjected to different combinations of day-night, internal tidal or inertial current cycles. Within the twilight zone, the light intensity cycle varies as a function of depth and latitude, the latter acting through the poleward decrease in angle of elevation of the sun (Fraenkel 1986).

Day-night shifts in seabed-living or demersal communities are found at continental margin depths within the twilight zone stratum at all latitudes from the tropical to polar regions such as off Antarctica (Kaufmann et al. 2003). Studies based on temporally scheduled trawling along continental slopes, rises, and abyssal plains, show that day-night variations in catches resulting from species behavioural rhythms are widespread, showing great potential for installation of video observatories (Figure 8A), At the same time, complex propagation patterns of internal tidal waves may occur due to the effect of seafloor topography (Figure 8B) (Garret 2003, Garret & Kunze 2007), which would lead to the occurrence of semidiurnal or inertial behavioural rhythms in populations (Wagner et al. 2007, Aguzzi et al. 2010b).

From a global perspective, the deployment of an integrated network of permanent video observatories is fully justified for the purpose of understanding geographic differences in community responses under mixed day/night, internal tidal, and inertial regimes. Observatories located in shelf and slope areas, where productivity is generally low and transparency high, are particularly well suited for the study of diel and seasonal behavioural rhythms. Other observatories in the almost tideless, oligotrophic Mediterranean Sea represent excellent platforms for the study of day-night-based community rhythms (reviewed by Aguzzi et al. 2009e).

By contrast, remote monitoring by observatory sites within coastal turbid areas (including estuaries) may not be as useful because of inherent difficulties in tracking animals and classifying them in poor visibility or when fouling occurs (Aguzzi et al. 2009c). Other observatories located in ocean areas associated with strong tidal motion could be used to identify community responses to water speed increases, irrespective of the level of sunlight exposure. Furthermore, deeper observatories in the Mediterranean Basin may facilitate the investigation of behavioural rhythms correlated with geophysical cycles involving water displacement that are much weaker than those produced by internal tides, such as atmospherically driven deep water inertial currents with an 18-h periodicity (reviewed by Aguzzi et al. 2009d).

### *The role of large diel nektobenthic and benthopelagic migrations in the indirect day-night regulation of deep-sea ecosystems*

The implementation of integrated permanent video observation networks should be useful for studying how the behavioural rhythms of continental margin species act as regulators of ecosystem functioning in different depth strata. Species receiving day-night-based light intensity entrainment in superficial depth strata may influence the functioning of deeper ecosystems in aphotic continental margin areas. Nektobenthic migrants likely perform large (i.e., few kilometres) diel horizontal migrations (DHMs) by analogy with diel vertical migrations (DVMs; see Section The missing chronobiology framework in marine ecological studies), the extension of which is still poorly described. Moreover, the occurrence and nektobenthic typology of rhythmic behaviour in the majority of continental margin species is currently poorly described. In contrast to the pelagic environment, where vertical population displacements have been sampled by by temporally coordinated net tows at different depths, such a sampling design has not often been used in shelf and slope trawl surveys (Aguzzi & Company 2010).

The relevance of DHMs is currently largely underestimated by marine ecology, despite their potential to involve a number of different fish and decapod species (Suetsugu & Otha 2005, Benoit-Bird & Au 2006). The ecological and adaptive implications of DHM movements could be similar to those already proposed for DVMs in the pelagic environment (Zaret & Suffern 1976, Aksens & Giske 1993, De Robertis 2002, Naylor 2006). The activation/suppression induced by DHMs with respect to the behaviour of seabed deep-sea species might follow a pattern similar to the one proposed in the scattering layer-interception hypothesis (i.e., diel changes in behavioural activation are the product of predator/ prey interactions; reviewed by Fock et al. 2002).

Video-image acquisition from bathymetrically coupled video stations, functioning continuously for 24-h in a synchronized manner (Figure 9), could be used to identify which species are DHM movers and which adopt a benthopelagic behaviour (i.e., as a sub- category within DVM; reviewed by Aguzzi and Company 2010). Unveiling temporal shifts in the number of video-counted individuals on continental shelves and slopes for a given species at equivalent time points could be considered as markers of DHM displacements. Conversely, the presence of fluctuations in the number of video-counted individuals only at a single station, usually the deeper, may indicate the occurrence of benthopelagic DVMs (Aguzzi et al. 2006c). It is likely that slope-positioned, cabled observatories will be able to detect DVMs in shrimps shifting between the seabed and the overlying benthic boundary layer (i.e. benthopelagic movements). These diel displacements may rhythmically bring animals within the camera FOV during certain parts of the day-night cycle (usually the night), similar to results from temporized bottom trawling (Aguzzi et al. 2006c). This phenomenon is likely to be more evident on slopes than shelf regions because of the greater depth range available for vertical movements. In addition to intrinsic behaviour, predator diel migrations are also influenced by movements of macro zooplankton or micronekton prey species in relation to environmental light and its effect on visual predation (Pusc et al. 2002).

Both DVM and DHM movements affect the presence of predators and prey over 24-h periods in deep water areas below the twilight zone. These movements may represent an indirect signal for the behavioural synchronisation to the day-night cycle of species residing in deep-sea areas. The NEPTUNE platforms located in Barkley canyon at 400 m and 920 m (see Table 2) offer the opportunity of studying these nektobenthic movements in a coordinated fashion and their effect on ecosystem functioning at different depths on the continental margin (Huchet et al. 2010).

### *Indirect seasonal (i.e., photoperiodic) regulation of deep-sea ecosystems: characterisation of endogenous and exogenous signals for growth and reproduction*

The existence of a mechanism controlling behaviour on both diel and seasonal bases through detection of photophase or scotophase duration (i.e., photoperiodism) is currently under discussion for several vertebrate and invertebrate animal models (reviewed in Refinetti 2006). Such a common mechanism could explain reported rhythms related to the growth and reproduction of a number of vertebrate and invertebrate species. Photoperiodic responses in marine animals may involve a circadian clock that can determine the temporal difference between long and short days (i.e., photoperiodic time measurement), for example, measuring the reciprocal temporal distance at dusk and dawn behavioural peaks (reviewed by Aguzzi & Company 2010).

Deep continental margin species from the Western Mediterranean Sea appear to carry out bathymetric displacements according to seasonal variations in the length of the photoperiod, though these presently remain non-quantified (Aguzzi et al. 2009a). Seasonal behaviour modulation in species performing nektobenthic displacements may be the result of changes in interspecific prey-predator relationships in varying light conditions (Kornfeld-Schor & Dayan 2003). In this context, networks of bathymetrically linked video observation nodes may contribute to the characterisation of these poorly understood phenomena.

The bathymetric ranges of the distributions of animal species may also change on a seasonal basis according to synchronisation of moulting and reproduction cycles (Aguzzi & Company 2010). Modulation of the biological rhythms in benthic species may follow a photoperiodic-like pattern, even at depth ranges where solar light is completely absent and where the only available light source is bioluminescence. However, in the dark deep sea, although most species, particularly at abyssal depths seem to exhibit continuous or semi-continuous reproductive patterns (reviewed in Young 2003); studies performed in the last decade on deep continental margin areas have demonstrated the occurrence of seasonal reproductive cycles at aphotic depths (Company & Sardà 1998 2000, Company et al. 2003). This finding suggests that there is photoperiodic regulation of the physiology of animals in these deep strata (Aguzzi et al. 2007).

The nature of the environmental clues employed by deep-water continental-margin species to time their seasonal rhythms to the photoperiod are presently unknown. Seasonal phytodetritus concentration peaks within the upper water column may act as a temporal feeding synchroniser for benthos reproduction (Ecklebarger &

Walting 1995, Lisin et al. 1997). This synchronisation may allow these species to obtain a suitable food supply in their larval phase, thus increasing the survival rate of the dispersing offspring (Gage & Tyler 1992, Tyler et al. 1994). Again, continuous video monitoring of benthic deep-sea communities coupled with concurrent multiparametric collection of habitat data may prove critical for understanding the cause-effect relationships between species' behavioural rhythms and habitat fluctuations.

### *Faunal changes in a globally warming world*

The current and predicted impacts of global climate change on living organisms are a cause of major concern (Walther et al. 2002). The importance of monitoring such transformations in marine communities is absolutely clear within the marine science community (Occhipinti-Ambrogi 2007, Lepetz et al. 2009, Jennings & Brander 2010). Climate-driven variation affects aquatic organisms, which show complex behavioural responses in displacement and habitat utilisation correlated with temperature and salinity changes in continental margin areas (Hawkins et al. 2008). In coastal areas, poleward shifts of species distributions (Perry et al. 2005, Lenoir et al. 2011) and the increasing success of thermophilic taxa versus the retreat of cold water species (Bianchi & Morri 2003, Azzurro 2008) can be considered among the most obvious consequences of this phenomenon in marine communities. Other detectable effects are related to shifts in phenology, i.e., in the timing of life-history events that occur at specific times each year (Edwards & Richardson 2004.). To follow and understand these phenomena, generalising analyses from a local to a global scale should be conducted. These analyses will obviously require sampling to be greatly extended in both time and space, which usually implies that an extensive socio-economical effort will be required to sustain the required technology (Giske et al. 2001).

New approaches, new technologies and, most importantly, new ways to collect and share data are thus critical elements in exploring patterns that act at the largest geographical scale (Decker & O'Dor 2003). In this context, the role of modern ocean observation methods, such as marine cabled seafloor observatories, can be considered as promising and cost-effective, not only in deep water  but also in coastal areas (e.g., OBSEA; see Table 2). These devices, which collect samples remotely, can handle large volumes of data; however, they have not yet been employed to carry out long-term studies on faunal changes in association with modifications of selected habitat characteristics, such as temperature or light intensity/turbidity conditions in relation to eutrophication. Nevertheless, researchers around the world are working diligently towards finding new solutions to monitor marine communities over large space and time scales (Decker & O'Dor 2003, Lepetz et al. 2009). Cabled marine observatories are perfectly suited for this purpose, and research could immediately benefit from these powerful innovations, building the capacity for world-wide marine research. These remote video recorders can support continuous, efficient semi-quantitative sampling in a variety of different environmental conditions, from near shore to the deep sea. By using these devices, it will become possible for researchers to plan long-term studies for the real-time identification of changes in the geographic distribution of target species based on to an early detection system, as intended within the frame of the Ocean Tracking Network (OTN 2011).

One of the main advantages of the suggested approach is the potential to overcome the variability generated by many other climate-independent forcing factors that can influence species distributions. The coordination of as many observatories as possible throughout the seas of the world would allow the realisation of complex experimental designs suited to achieve a general picture of global responses to climatic changes on a continuous basis. This coordination would imply the need for an effort related to the development of standardised methodologies and coordination with a ‘network’ of different observations representing the sole feasible way of disentangling global signals from other sources of variability that act at regional and local scales (Briand 2008, Lepetz et al. 2009).

To limit and to better allocate effort, the acquisition of remotely video-collected data could be restricted to a small set of indicator species (i.e., key species) (Briand 2008), and these indicators should be chosen from sensitive organisms that are easy to track and identify. Among other potential biotic indicators, fishes exhibit excellent qualities for both ecosystem and climate monitoring, such as high dispersal potential, ecological differentiation, general non-resilience, and sensitivity to temperature (Stephens et al. 1988, Roessig et al. 2004). Moreover, their large size, ease of identification and the simplicity of their automated classification based on morphometric tools (Aguzzi et al. 2009c, 2011b) make them ideal candidates for sensing studies using remote devices, such as cabled observatories.

### *The connectivity of benthic populations as a result of rhythmic behaviour in cyclically changing seascapes*

In the marine environment, the rhythmic displacement of species occurs within seabed or water column corridors associated with recurrent oceanographic features (Roff & Evans 2002). Roff & Taylor (2000) characterised different strata of the water column and seabed as patches using parameters such as water temperature, depth/light, stratification/mixing regimes, substratum type, and exposure/slope. They termed the marine components identified by this classification as 'seascapes'. Marine seascapes are different from landscapes, as they are more dynamic, intermittent, and present a more complex geography (Longhurst 2007). The activities of animals occur within certain seascapes associated with entraining geophysical cycles (i.e., being constituted by a set of particular and recurrent habitat conditions fitting with species ecological niche requirements). Seascapes hence rhythmically modulate the rhythmic behaviour of animals, and seascape characteristics can be measured in a multiparametric fashion by seafloor cabled observatories.

Farina & Belgrano (2004) defined as the ‘ecofield’ the portion of the surrounding environmental space directly perceived by animals during their behavioural activities of energy collection, preservation, and transformation. Internal tidal currents, light intensity cycles, and variations in the length of photoperiods, represent signals that cyclically affect the ecofield of animals (reviewed by Aguzzi & Company 2010), modifying the predisposition of their populations to undergo rhythmic displacements within seascapes as corridors.

Rhythmic behavioural activation may contribute to the potential dispersion of benthos among geographically separated groups. In this sense, behavioural rhythms may be used to explain dispersals by adults (and not only of larvae) among distant populations. Characterisation of how animals displace in their associated medium when reacting to temporal changes in its condition is of importance for characterising these potential corridors (reviewed in Kritzer & Sale 2006). Cabled platforms allow the multiparametric measurement of marine processes through time by concurrently acquiring several different classes of oceanographic, geologic, and chemical data. Video observations can be efficiently used to portray the presence of animals within a multi-dimensionally measured habitat framework, which is also represented by multiparametric assets at a corresponding time. This multiparametric monitoring in association with bio-video observations could provide a coherent operative and experimentally testable view of species ecological niches: the presence of animals can be measured at any moment in relation to (and possibly as a result of) habitat condition and the multidimensional characterisation of this habitat condition depends on the available sensors.

Under this scenario, multiparametric video observatories within a network of distant nodes could provide important data on why certain deep-sea species exhibit certain geographic ranges of distribution (Levin et al. 2001), clarifying their level of connectivity based on the enhancement or suppression of the activities of adults at the onset of certain habitat conditions (i.e., seascape turnover in a certain observational area). For example, tidal pull may generate deep-sea currents of consistent speed. Benthic fishes may suppress their swimming activity upon water flow speed increases to maintain a certain location, or alternatively, they may exhibit increased activity leading to their entering the dispersing flow (Aguzzi et al. 2010b).

Cabled multiparametric video observatories endowed with moored arrays may be used to portray the behavioural reaction of benthic animals with different levels of motility (e.g., crawlers, walkers or swimmers) to internal tidal-driven flow variations. This research methodology may provide important data on behaviour and dispersion ranges in relation to stepping stone models that has been proposed for dispersal through favourable habitats such as the whale falls (reviewed by Smith & Baco 2003).

### *Community-based responses to seismic events: towards a new biologically based alert system*

Grant & Halliday (2010) reviewed a number of reports on the putative anticipated perception of seismic events by animal communities. Some species may disappear from a certain area a few hours prior to these events, without resuming their presence and behavioural activities for several days. In this context, the study of responses of single species and communities to earthquakes could be used to predict these events if automated methods of animal monitoring could be developed with sufficient precision.

Cabled multiparametric seafloor stations could be used to implement early warning systems based on sudden drops in video-counted individuals for a certain abundant species. With the increased automation efficiency of video image analysis, the number of individuals of key species in certain geologically active areas could be used to predict an incoming seismic event based on anticipated population disappearances from the FOV of cameras. In the case of Japan, this technological possibility could be explored using existing functioning platforms, such as that deployed at a depth of 1100 m in Sagami Bay (Central Japan; see Table 2). Long-lasting automated visual counting of zoarcid eelpout fishes has already been successfully carried out using this platform (Aguzzi et al. 2010b). A decrease in the population of zoarcid fishes occurred in 1996 after a major seismic event in the area (Figure 10). Unfortunately the frame sampling rate was too low (one picture each 12-h) to demonstrate whether this decrease truly anticipated the event. Drops in individual counts prior to a seismic event could be resolved using a higher rate of frame acquisition (e.g., a frame per 10 min). The disappearance of animals below a certain threshold level (defined by an average computed from observational counts of previous days) may be indicative of the anticipatory perception and reaction of zoarcids related to the incoming seismic event.

If this dynamic could be proven, then it could be incorporated into an automated alert system. However, the Sagami population of zoarcids show rhythmic changes in local counts correlated with internal tidal motions (Aguzzi et al. 2010b), which greatly complicates such an operation. Other non-swimming and more benthic species could be then studied in relation to this application because their behaviour may be less dependent upon tidal flow changes.

### *Time series responses to stochastic pulses of nutrients in deep-sea ecosystems*

A potent method for understanding the dynamics of complex systems in engineering (e.g., electronics, mechanical structures, and acoustics) and economics is to test the impulse response of the system. A single impulse typically results in a deflection and a series of oscillations of decreasing amplitude, depending on the damping and resonant frequency of the system (e.g., ringing of a bell struck by a hammer). Similar principles can be employed to study the reaction of deep-sea ecosystems to stochastic pulses of nutrients. Deep-sea areas host food-depleted environments (reviewed by Childress 1995). The enrichment of the deep seafloor with an artificial pulse of particulate organic matter can be studied by video monitoring of the response of fauna and micro-bacteria (Witte et al. 2003a,b, Gillibrand et al. 2006). Deployment of cetacean carcasses mimics natural carrion falls exploited by a succession of organisms (Smith & Baco 2003). Kemp et al. (2008) used DOBO (see Section on Automated video-imaging from cabled observatories for the study of behavioural rhythms) to perform time-series observations of porpoise (*Phocoena phocoena*) carcasses for up to 6 months at bathyal depths and recorded rhythmic alternations between the numbers of squat lobsters (*Munidopsis crassa*) and octopus arrivals (*Benthoctopus* spp.). Glover et al. (2010b) used real-time video from a cabled observatory to record the time series of events at whale bones deployed on the seafloor and showed that such systems have great potential for elucidating the response characteristics of marine ecosystems.

## Conclusions

The use of cabled observatories for the assessment of population/stock size, biodiversity, species dynamics, ecosystem functioning, and the responses of communities to deterministic or stochastic changes in environmental conditions requires an international effort regarding methods standardisation for comparative studies. Future actions should promote a coordinated strategy for the use of permanent observatories, and standardised imaging census protocols should be adopted to permit large-scale analyses. These simple actions will supply a ‘missing tool’ in the exploration of global environmental changes and allow targeting which are the greatest challenges at different spatial and temporal scales. The methods here proposed are straightforward, and the development of automation in video and habitat data recording, processing and analysis could certainly capitalise on existing cabled stations. To realise these goals, the cooperation of a global network of interested scientists, companies and governments is certainly desirable.

The study of behavioural rhythms of marine animals by means of cabled permanent video observatories at all depths of continental margin areas could be successfully performed by adherence to of prerequisites related to image acquisition, processing, handling and interpretation of the results. Suggestions for the efficient and reliable implementation of video studies of rhythmic behaviour using permanent observatories are listed below:

1. Using video-imaging at permanent cabled video stations to portray local population sizes and biodiversity should be addressed with caution. Spatial limitations of these observational points may impede species detection in a given and larger area. It should be also noted that animal tracking without implementation of trajectory computing does not allow previously counted individuals to be discarded in consecutive frames. Video observations can only be used to disclose the biasing effect of behavioural rhythms on population/stock and biodiversity assessments obtained from trawling when the timing of sampling is not considered in relation to local day-night (depth-dependent) or internal tidal and inertial (geographic-dependent) entraining cycles. Space biases could be reduced, hopefully in the near future, by expanding video acquisition locations as a part of observatory networks.

2. Organism classification procedures must be implemented to facilitate the extraction of biological information from video sources following the combined use of morphometric tools and multivariate statistics (e.g., KNN and PLSDA). Supervised classification of organisms within frame subsamples including the creation of training sets (libraries of images), increases classification efficiency (i.e., a machine-learning approach). That process must be differentially tuned for each deployed cabled observatory, being the local environmental context, video and light equipment and residing species often different.

3. Animal counts obtained from the automated processing of videos must be placed within the larger context of synchronously collected habitat data (i.e., gathered at the same time frequency). This requires implementation of multiparameter data banks in which biological, geological, and physical oceanographic data can be processed together for comparison. For such processing, the following steps already in use in the field of chronobiology are proposed in order to allow i) identification of the periodicity and phase of the fluctuations; and ii) establishment of temporal linkage in their phases (i.e., maxima timings), thus studying potential cause-effect relationships between geophysical fluctuations and population behavioural responses.

4. Among the diverse sensors available, light measuring devices are surprisingly still missing from platforms located within benthic areas of the twilight zone range. The diurnality and nocturnality of species represent fundamental characteristics of their ecological niche. Diel changes in light act cyclically at a very basal level of central nervous system functioning in animals (i.e., on biological clocks), hence potentially setting the timing of consequent behavioural activities.

5. The implementation of automated video image analysis for species tracking and classification, as well as the development of efficient and user-friendly multiparametric information treatment tools should result in the creation of a new

generation of embedded technology coupling software for image processing and statistical data treatment.

6. Multiparametric cabled observatories can overcome present limitations in gathering biological (i.e., video-imaging), geological, chemical, and finally physical oceanographic information, which in turn favour the understanding of ecosystem functioning. In this context, there is for the first time the opportunity to place any biological observation (i.e., video-counted animals) acquired at a high constant frequency, within a multidimensional space of habitat parameters also measured at a corresponding moments. A species niche is a hyperspace made by vectors representing the variation of habitat variables constraining its survival (i.e., the Hutchinson definition; Whittaker et al. 1973). Multiparametric cabled observatories within a network measuring biologic, geological, chemical, and oceanographic parameters represent the first operative tool to study species niches over space and time.

7. Multiparametric monitoring represents the necessary basis for determination of presence or absence of a given species and, hence definition of population rhythmic behaviour within certain configurations of habitat variables. Although the behaviour of species and communities can also be depicted over time using acoustic technologies, only video-imaging can ultimately discriminate individuals of given species without the need for verification by other means e.g., pelagic/benthic trawling). Therefore, video cameras can be placed at the highest rank of a multisensor scale of complexity in which oceanographic (physical) and chemical (geologically- or life-derived) variables are at the base (Figure 11). This ranking of sensors is related to the character of the obtained information, with individual, population or species detection being the result of surrounding physical, chemical, and geologic conditioning (i.e., the ecological niche measured in a multiparametric fashion).

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**Table 1** Main studies on video image analysis of moving biological objects

| Method | Theme | Citation |
|---|---|---|
| Coarse-coded channel for classification | Automated taxonomy of dinoflagellates for microscopy | Culverhouse et al. 2003 |
| Segmentation and neural network-based classification | Automatic counter to identify and measure phytoplankton | Embleton et al. 2003 |
| Wavelet-based method for identification based on profile | Cell motion detection | Liu et al. 2003 |
| Movement tracking | Bacterial rotation filming | Shotton et al. 2000 |
| Image subtraction and segmentation for identification | Remotely Operated Vehicle (ROV) observations for pelagic community monitoring | Edgington et al. 2003 |
| Saliency-based system with Kalman Filters for tracking | | Edgington et al. 2004 |
| Saliency-based system with Kalman Filters for detection and tracking | | Edgington et al. 2006 |
| Bayesian classifier for detection and tracking | | Edgington et al. 2007 |
| Image subtraction for detection and tracking | | Walther et al. 2004 |
| Saliency-based algorithm for detection | Permanent cameras for benthic community monitoring | Cline et al. 2007, Aguzzi et al. 2009c, 2010b |
| Artificial neural networks and discriminant analysis for semi-automated sizing and counting | Dual (stereo) camera system for cultured fish | Simmonds et al. 1996, Williams et al. 2006, Costa et al. 2006, 2009c, Nucci et al. 2010 |
| Image texture and colour analysis for classification | Reef monitoring | Spampinato et al. 2008 |

**Table 1** Continues

| Method | Theme | Citation |
|---|---|---|
| Neural networks and discriminant analysis on shapes | Fish species classification by computer vision | Storbeck & Daan 2001 |
| Discriminant analysis of shapes | Fish classification from photograph banks | Strachan & Nesvabda 1990 |
| Frame subtraction and image filtering for tracking | Measurements of rhythmic movements in individual lobsters in laboratory conditions | Aguzzi et al. 2009d |
| Frame subtraction, image filtering, and shape matching for tag identification and tracking | Measurements of rhythmic movements in a group of lobsters in laboratory conditions | Menesatti et al. 2009a,<br>Aguzzi et al. 2011c |
| Landmark based geometric morphometry | Morphologic discrimination of scleractinian reef corals | Budd et al. 1994 |
| Multi-target initiation, clutter modelling, event analysis and multiple hypothesis filtering for tracking | Permanent infrared cameras for bat monitoring | Betke et al. 2007 |
| Mirroring systems for three-dimensional trajectories tracking | Tracking of swimming-flying species | Chraskova et al. 1999 |
| Frames subtraction, segmentation and pattern matching for tracking | Tracking of flying insects filmed under natural conditions | Voss & Zeil 1995 |

**Table 2**. Marine multiparametric cabled seafloor observatories bearing video cameras and equipped for the long-term and real-time monitoring of continental margin ecosystems. Information on geographic location and water depth (m) is provided along with the web page of reference (URL, as active or inactive at last access the 07/06/2011) and numeral code as referenced for Figure 8B.

| Code | Name/Acronym | Location | Depth | URL |
|---|---|---|---|---|
| 1 | Monterey Accelerated Research System (MARS) | Monterrey (California, USA) | 891 | www.mbari.org/mars<br>ACTIVE |
| 2 | Victoria Experimental Network Under the Sea (VENUS) | i) Saanich Inlet (British Columbia, Canada)<br>ii) Bonne Bay Observatory (Strait of Georgia, New Foundland, Canada) | 100<br>300 | www.venus.uvic.ca<br>ACTIVE<br>http://bbo.physics.mun.ca/background<br>INACTIVE |
| 2 | North East Pacific Time Series Undersea Network Experiment (NEPTUNE) | i) Barkley Canyon (Juan de Fuca plate, Canada)<br>ii) Hydrothermal vent site (Juan de Fuca plate, Canada) | 400<br>920<br>3200 | www.neptunecanada.ca<br>ACTIVE |
| 3 | Deep-ocean Environmental Long-term Observatory System (DELOS) | Angola | 1500 | www.delos-project.org<br>(not presently cabled being retrieved yearly for data downloading)<br>ACTIVE |
| 4 | JAMSTEC Off- Hatsushima (DONET) | Sagami Bay (Japan) | 1175 | www.jamstec.go.jp/scdc/top_e.html<br>ACTIVE |
| 5 | JAMSTEC off-Cape Muroto (DONET) | Nankai Trough (Japan) | 3572 | www.jamstec.go.jp/scdc/top_e.html<br>ACTIVE |
| 6 | Life-Streamed Underwater Video Observatory (ESONET) | Koster Fjord (Sweden) | 30 | www.sams.ac.uk/sams-news/Media%20releases/world-first-scientific-video-underwater-observatory<br>ACTIVE |
| 7 | Expandable Seafloor Observatory (OBSEA-ESONET) | Western Mediterranean (Vilanova i la Geltru, Spain) | 20 | http://sites.upc.edu/~www-sarti/OBSEA/info/intro/intro_eng.html<br>ACTIVE |

## FIGURE LEGENDS

**Figure 1** Variation in species composition as reported from 24-h trawl hauls conducted continuously over consecutive days at different depths (i.e., shelf: 100 m; slope: 400 m) in the western Mediterranean in autumn-winter (October 1998). The temporal variation in the species composition within different samples is shown by similarity index-based cluster analysis (A), which discriminates day from night hauls (bold black in grey boxes) (adapted by Bahamon et al. 2009). Variations in catches are the product of changing species abundances (B) according to day-night cycles (grey vertical bars indicate night), as can be seen for some ecologically and economically important crustacean, fish and decapod species (all values are surface density estimates (no. ind. km$^{-2}$) standardised as a percentage of the maximum for comparison). Fish catch patterns were obtained from NERIT project unpublished data. Decapod catch plots were adapted from different studies, all reviewed in Aguzzi et al. (2009a).

**Figure 2** Seasonal differences in population sampling on the shelf (100 m) and on the slope (400 m) for species in the example described in Figure 1B. Total sampled animals are reported for two different seasons for comparison: autumn-winter (October; 32 and 34 catches on the shelf and the slope, respectively) and spring-summer (June; 32 catches on the shelf and the slope). Differences in population catchability were considered as significant (*) when the total number of animals sampled in October and June showed variation equal to or greater than twofold. Data for fishes were obtained from NERIT project unpublished results. Decapod estimates were drawn from Aguzzi et al. (2009a).

**Figure 3** Simulated temperature sections drawn in the x, y, and z directions in a water parcel sampled at different periods (*x* axis, time 1 to time 4) in two locations (*y* axis; locations m and n) and at three depths (*z* axis, surface, middle waters, and bottom). The volume array characterises the conditions for a water parcel, with temperature slightly decreasing with time but strongly decreasing with depth from 21 to 13°C. This simulation was performed according to the deployment configuration of the Operational Observatory of the Catalan Sea (OOCS; http://www.ceab.csic.es/~oceans/) maintained by the Centre for Advanced Studies in Blanes (CEAB-CSIC) in the NW Mediterranean Sea (41° 39.7' N; 2° 54.6' E; operating depths between 50 and 200 m). The simulation was performed considering the environmental conditions found in late summer in the area. OOCS employs an oceanographic buoy moored at the sampling station between the continental shelf and the slope at the head of the Blanes submarine canyon. Apart from the meteorological conditions provided by the buoy's instrumentation, the upper 100 m are monitored with multi-parametric submarine instruments measuring the temperature, salinity, current velocity and direction, visible light, turbidity, dissolved oxygen and chlorophyll fluorescence conditions every 30 m (Bahamon et al. 2011).

**Figure 4** Protocol for the discrimination of clam shells between two species, *Tapes decussatus* and *T. philippinarum*, as carried out by Elliptic Fourier Analysis (EFA) as an example of automated video image classification on a specimen profile (adapted from Costa et al. 2008b, 2010). Dorsal digital images (A) are acquired in colour and subsequently transformed to grey-scale (B). Image binarisation (C) is required to detect the shell outline. A series of points are placed equidistantly (each 2º; D) in a Euclidean space starting from an arbitrary origin; a set of *x,y* coordinates is then computed for each point. The points are joined, and an outline is obtained (E; profile acquisition). The resulting harmonic function is fitted with an increasing number of ellipses and a matrix (F) comprising all individuals (in columns) with all fitted ellipses (in rows, from A to Z, each with its own four coefficients: a, b, c, and d) in the rows is obtained. Supervised or non-supervised multivariate analyses can be performed to screen the sample for any clustering of shapes. A mean shape outline (G) can be then obtained for each clustering to represent the within cluster morphological variation.

**Figure 5** Example of automated processing for identification of the galatheid squat lobster, *Munida quadrispina*, in Saanich Inlet using the VENUS platform video camera based on Red-Green-Blue (RGB) colour coordinates values, Fourier Descriptors (FDs) and Scale invariant Feature Transform (SFIT), as adapted from Aguzzi et al. (2011a). For the colour content analysis, the original RGB image (A) is filtered and corrected for the background by means of a top-hat filter (B). The next step is segmentation using a threshold value corresponding to the 95th percentile of Euclidean distances (C). Then, the Euclidean distances between the Red and Green channel are calculated for each pixel of the filtered TopHat image (D); the FDs of the animal shape are then classified by PLSDA modelling, where red circles show the correctly classified objects, and the green profile shows the object outline (E). For SIFT analysis, the Red channel of the original RGB image is used at filtering (F). Then, features extraction with the SIFT algorithm is performed, and all of the extracted features are shown in green, while all of the features recognised as belonging to *Munida* are shown in red (G).

**Figure 6** Flowchart describing the processing of animal tracking and classification in automated video image analysis of digital videos obtained from the permanent deep-sea station of Sagami bay (1100 m depth, Central Japan) (adapted from Aguzzi et al. 2009c). The identification of moving animals (within a circle: an eelpout; a red crab, *Paralomis multispina*; and finally a snail, *Buccinum soyomaruae*) occurs at frame subtraction (A-B) and after image binarisation and area filtering (C; the removal of fouling allows identification of the animals' shapes). At profile extraction (D), shapes and RGB content coordinates can be automatically processed for classification in relation to morphological models of reference (i.e. from a library of images as training set) by means of supervised multivariate statistics.

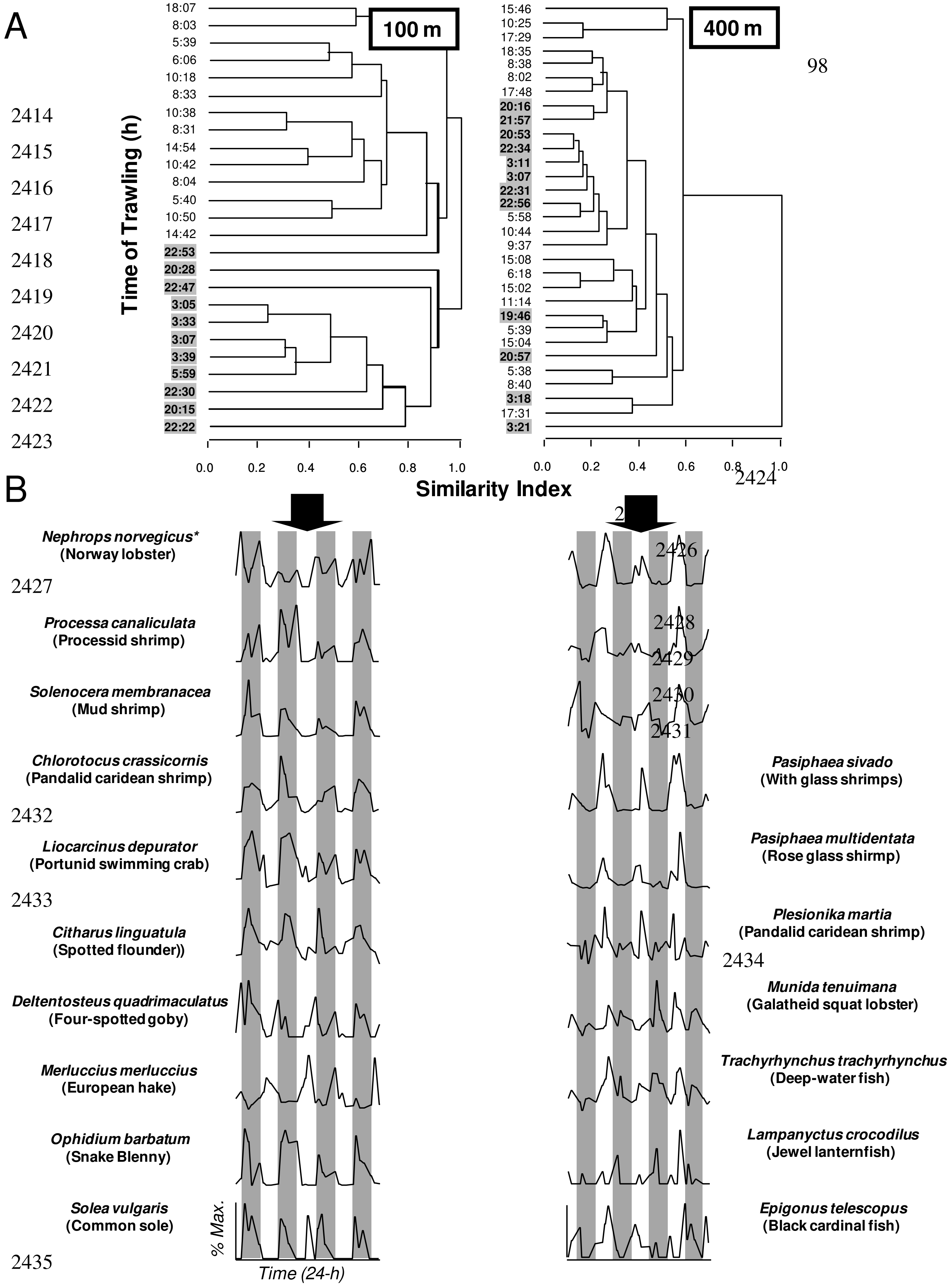
**Figure 7** Proposed conceptual scheme for automated and integrated time-series analysis of different biological (black) and habitat (grey) datasets obtained from permanent marine multiparametric stations. Arrows indicate the sequence of different steps involved in the data processing (A, data acquisition; B, data filtering; C, periodogram screening; D, data sub-set partitioning; E, waveform computing, and active phase duration assessment by the establishment of a daily threshold mean, i.e., Midline Estimating Statistic of Rhythm-MESOR; and finally, F, integrated waveform analysis). In the ultimate stage (F), the continuous thick horizontal line depicts the temporal duration of significant increases (i.e., the peak) in the fluctuation of biological (black) and habitat (grey) parameters. The dashed vertical rectangle in this analysis indicates the sensible time lapse (ΔT) when several habitat indicators (grey) significantly increase their state of fluctuations prior to the consequent increase in the biological response (black) as a marker of a cause-effect relationship.

**Figure 8** World distribution maps depicting oceanic zones with different sunlight exposure where field studies detected the occurrence of variations in sampled species based on behavioural diel rhythms (A), along with tidal forces (B). Computed global mean sea surface photosynthetically available radiation (PAR, measured as $E_i m^{-2} dy^{-1}$) was obtained by Aqua MODIS satellite measurements for the period between 4 July 2002 and 28 February 2011 (digital data provided by NASA). The approximate locations of most recent trawl studies at depths equal to or greater than 40 m where diel changes in benthic or demersal fishes were reported in this PAR global scenario. A depth threshold of 40 m was chosen because this is the average depth of worldwide light extinction (Lorenzen 1972). The present authors selected only studies on diel catches for fishes because these are usually more prone to automated tracking and classification given their size compared with other species, such as crustaceans (Aguzzi et al. 2011b). For zones where multiple studies were conducted, only the most recent one has been considered. The approximate locations of presently existing cabled seafloor observatories (see Table 2 for codes) is presented in relation in relation to internal tidal forces (amplitude of the principal lunar semidiurnal constituent of tides, M2 at a period of 12.42 hours) and propagation (adapted from: http://www.aviso.oceanobs.com, by Legos and CLS-Space Oceanography Division, distributed by Aviso/CNES). Cotidal lines depict the phase every 30 degrees originating at amphidromic points, where the tidal range is zero.

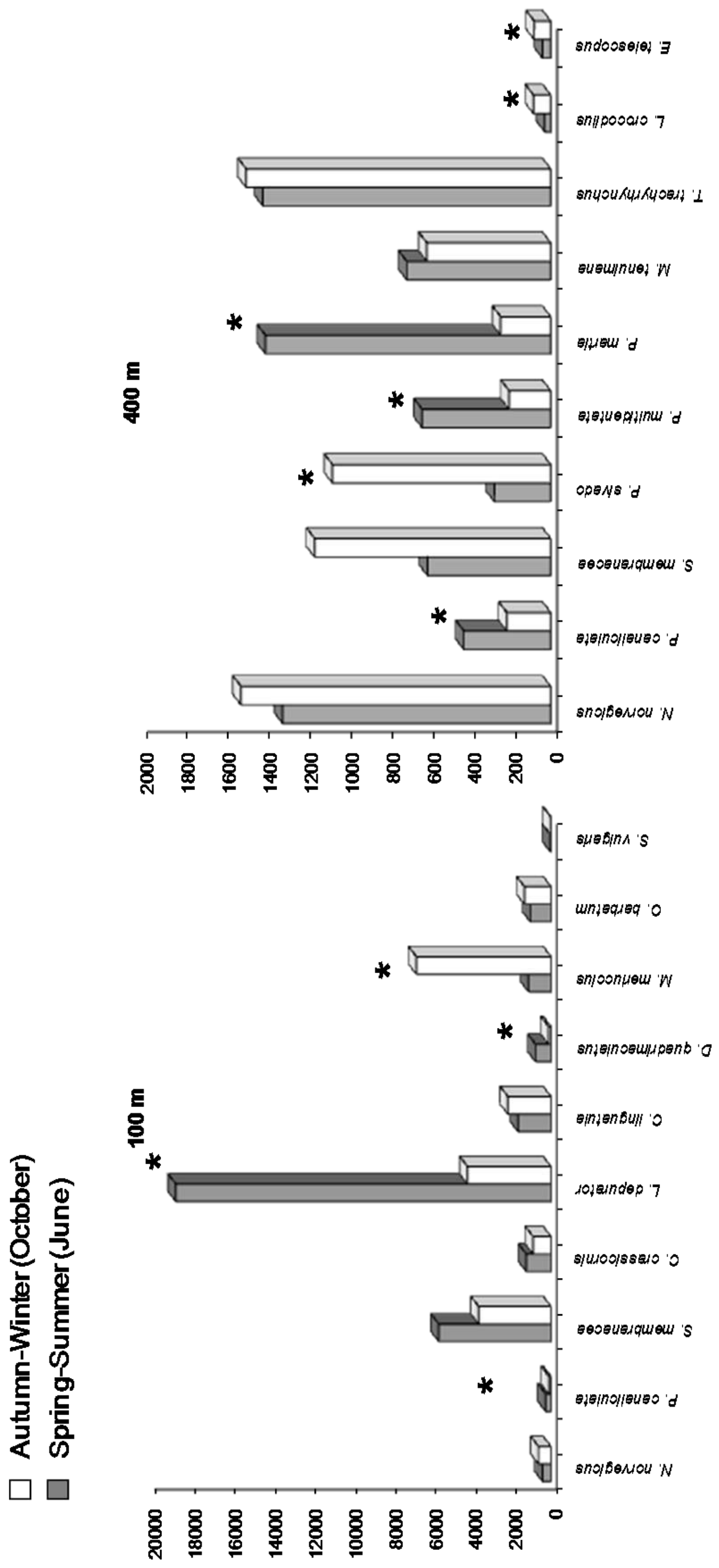
**Figure 9** Proposed bathymetric network of permanent multiparametric video stations (A) on the shelf and slope (indicated as 1 and 2, respectively) and modelled visual count patterns over 24-h (B) for diel nektobenthic horizontal migrations (DHMs) and diel vertical benthopelagic migrations (DVMs). A bathymetric integrated network of video cameras is required to portray the role of both types of migrations in the temporal functioning of deep water and deep-sea ecosystems. DHM and DVM can be distinguished by comparing the numbers of tracked animals at equivalent times at stations 1 and 2. Arrows depict the diurnal (white) and nocturnal (black) direction of population displacement.

**Figure 10** Time series (between 1 April and 20 May 2006) of visually counted eelpouts (zoarcid fishes) prior and after to two major seismic events (on 20-21 and 30 April and 2 May) as reported by the imaging system of the Sagami Bay multiparametric cabled observatory (1100 m depth; Hatsushima, Central Japan). Digital frames acquired at midday and midnight (the rhomb series), depict a consistent drop in the number of counted individuals prior to and after the first of these seismic events. This pattern was detected by adding a two-step moving average (thick black line). Seismic data were collected from the USGS-NEIC Earthquake Database (http://earthquake.usgs.gov/earthquakes/eqarchives/epic/epic_rect.php; Last access: 19/04/2011).

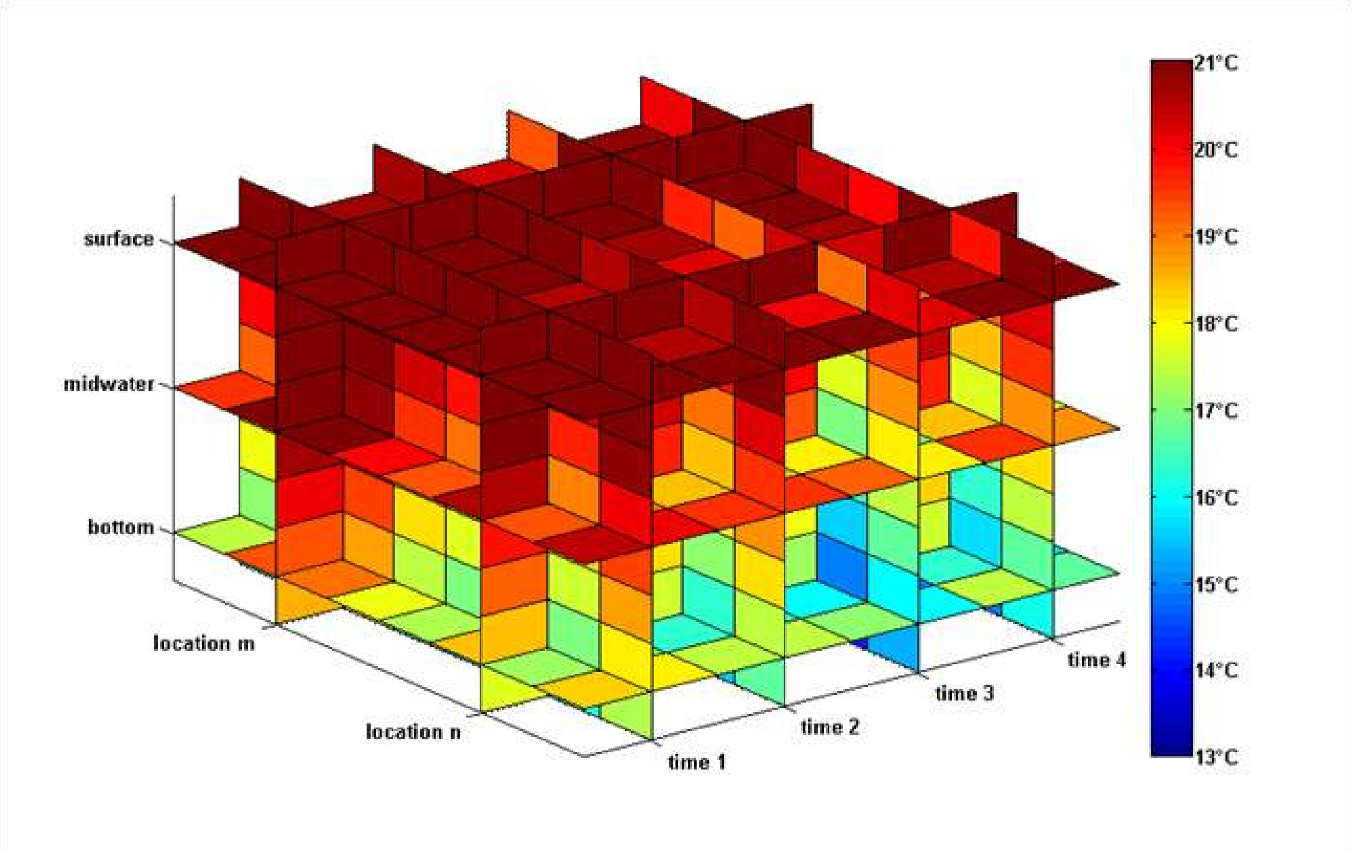
**Figure 11** Organisation scheme for measuring capability at different levels of ecosystem complexity using sensors installed in multiparametric cabled observatories. Sensors for physical measurements (i.e., chemical, geological, and oceanographic) portray ecosystem dynamism at a very deterministic baseline level, accounting indirectly for events affecting life performances. At intermediate levels of environmental complexity, there are sensors that measure biological performance only in an indirect manner (e.g., chlorophyll sensors). A maximum level of environmental complexity is obtained through direct biological process measurements, which are taken by video-imaging sensors that directly allow species recognition and the counting of individuals within local populations (by automated video image analysis).



**Aguzzi et al., Fig. 1**



**Aguzzi et al., Fig. 2**



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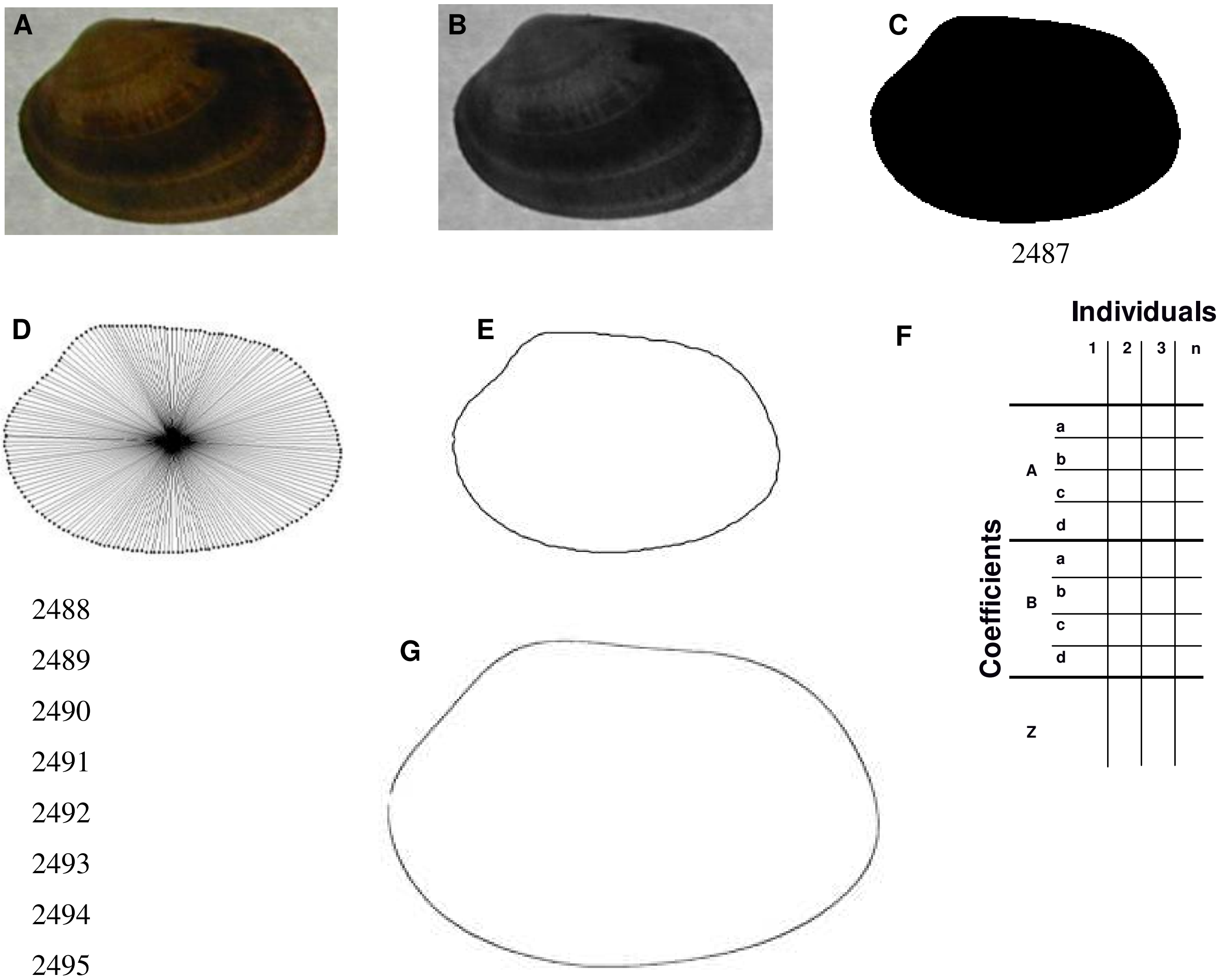
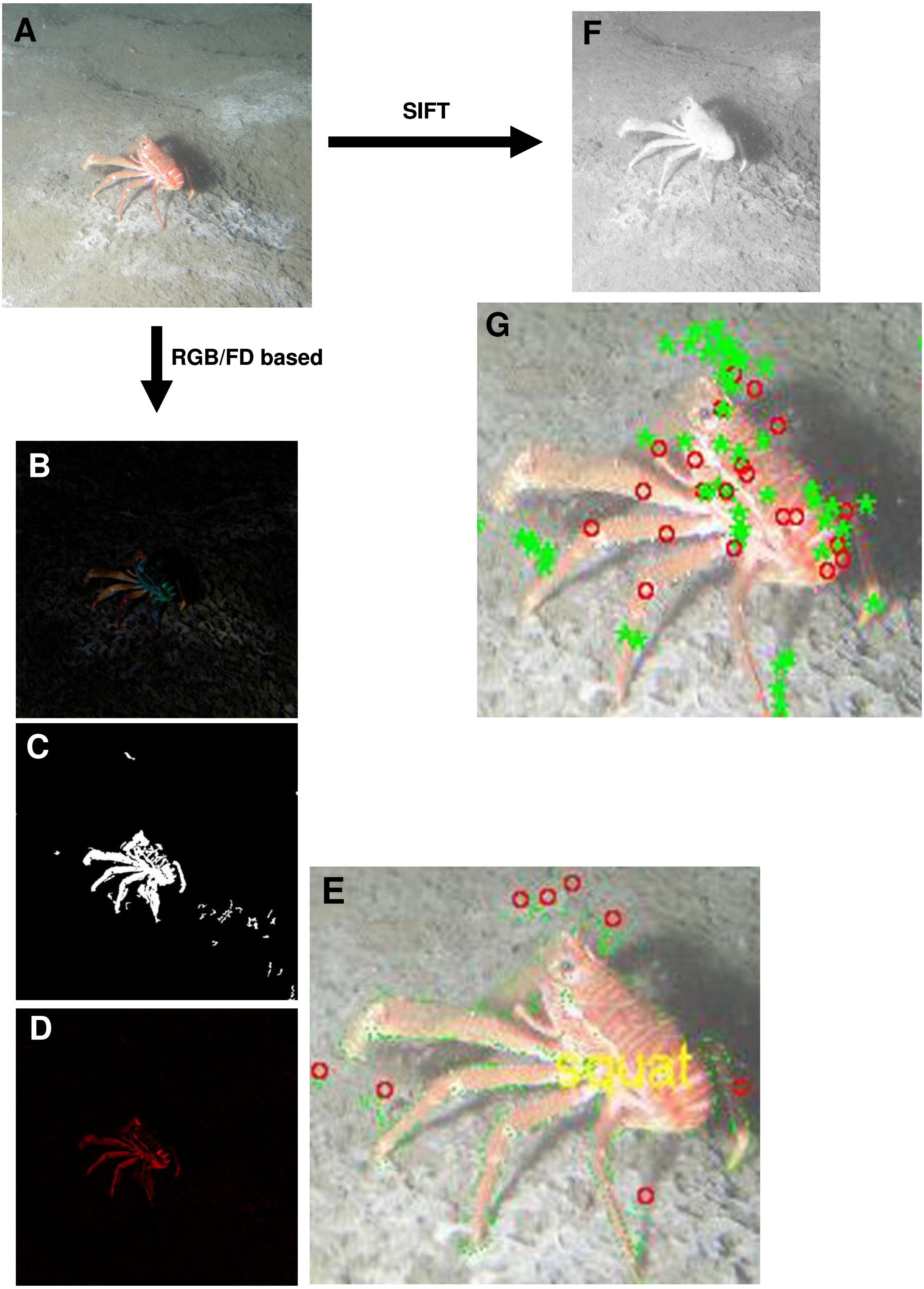


**Aguzzi et al., Fig. 4**



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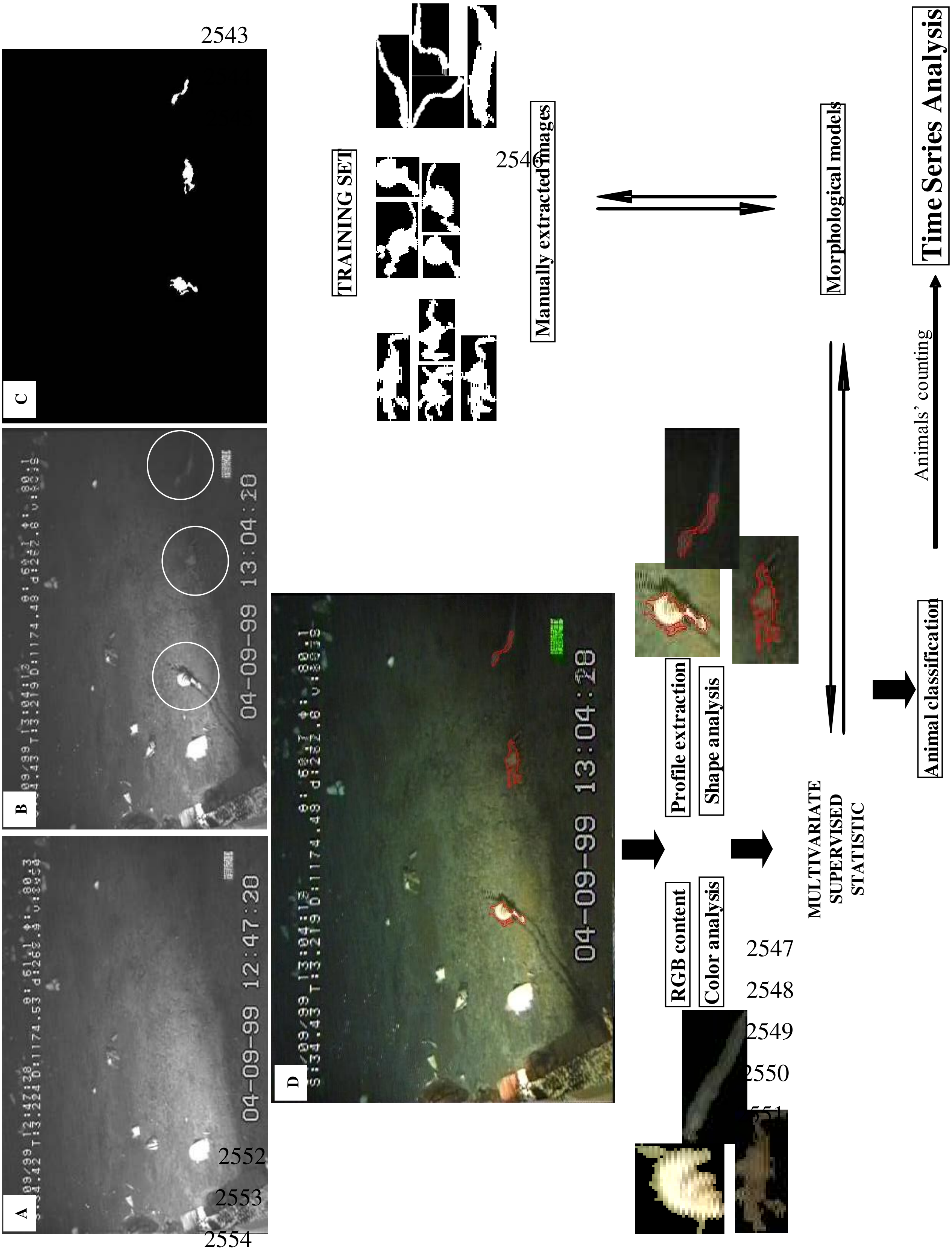

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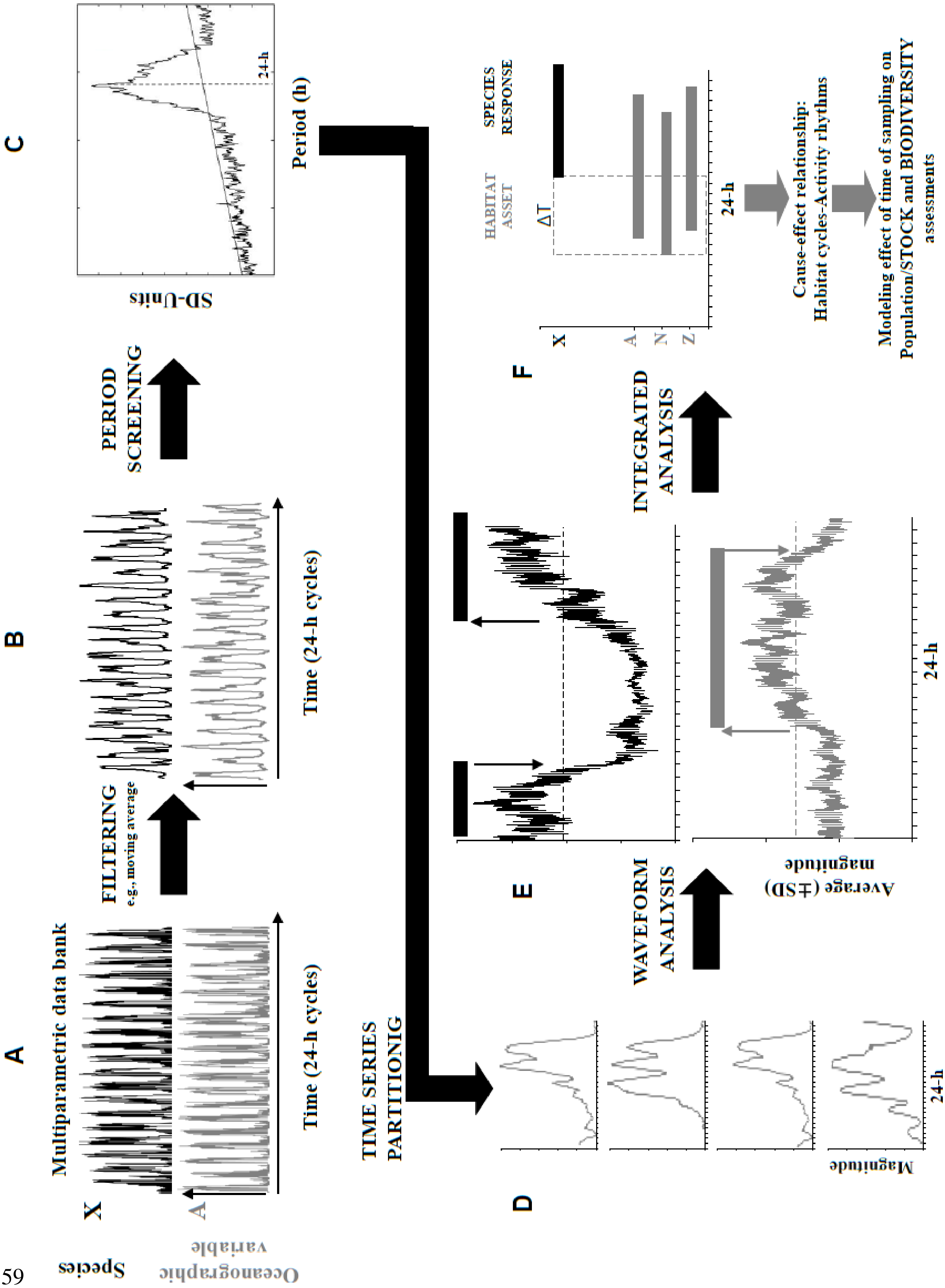
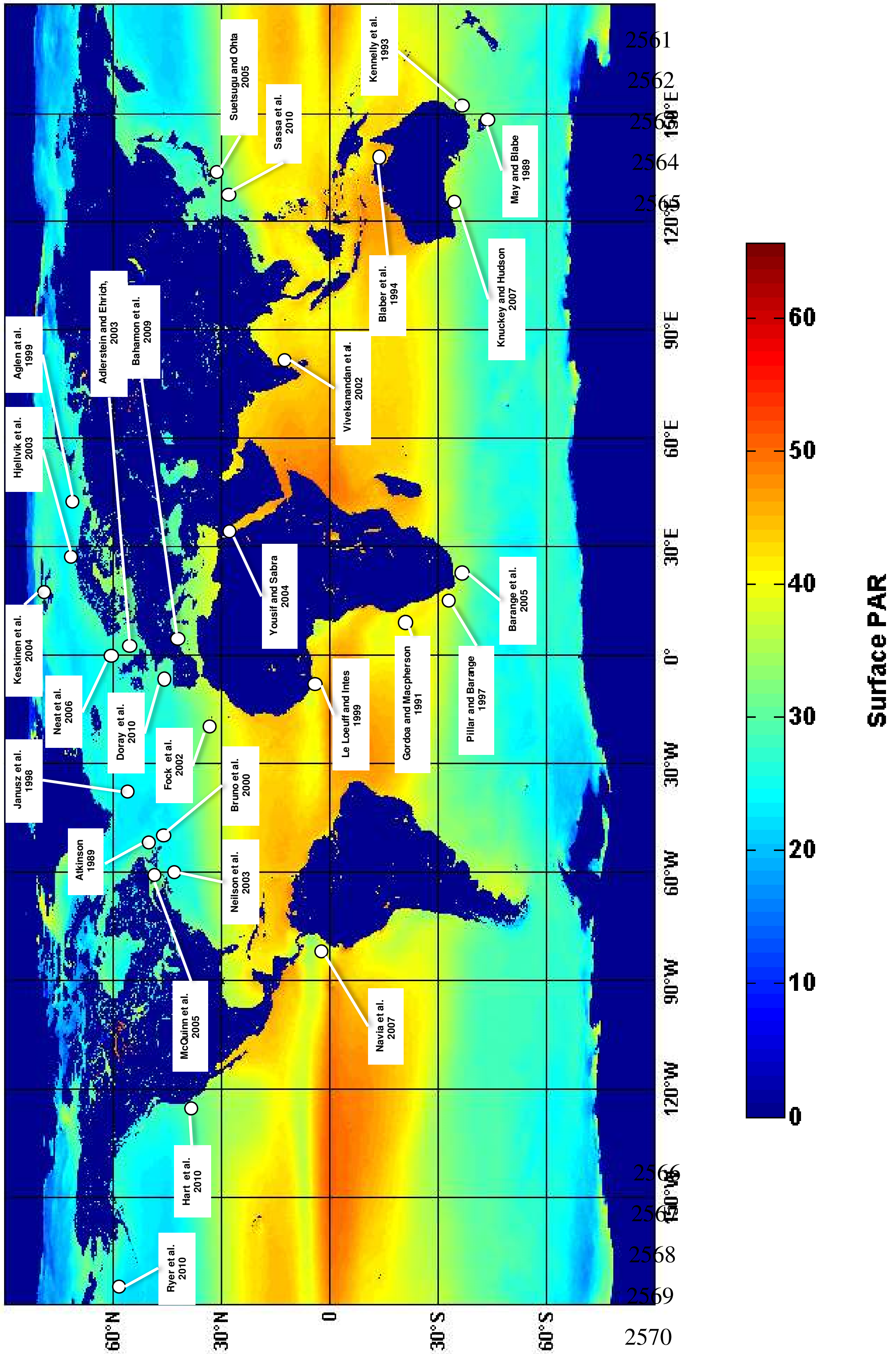
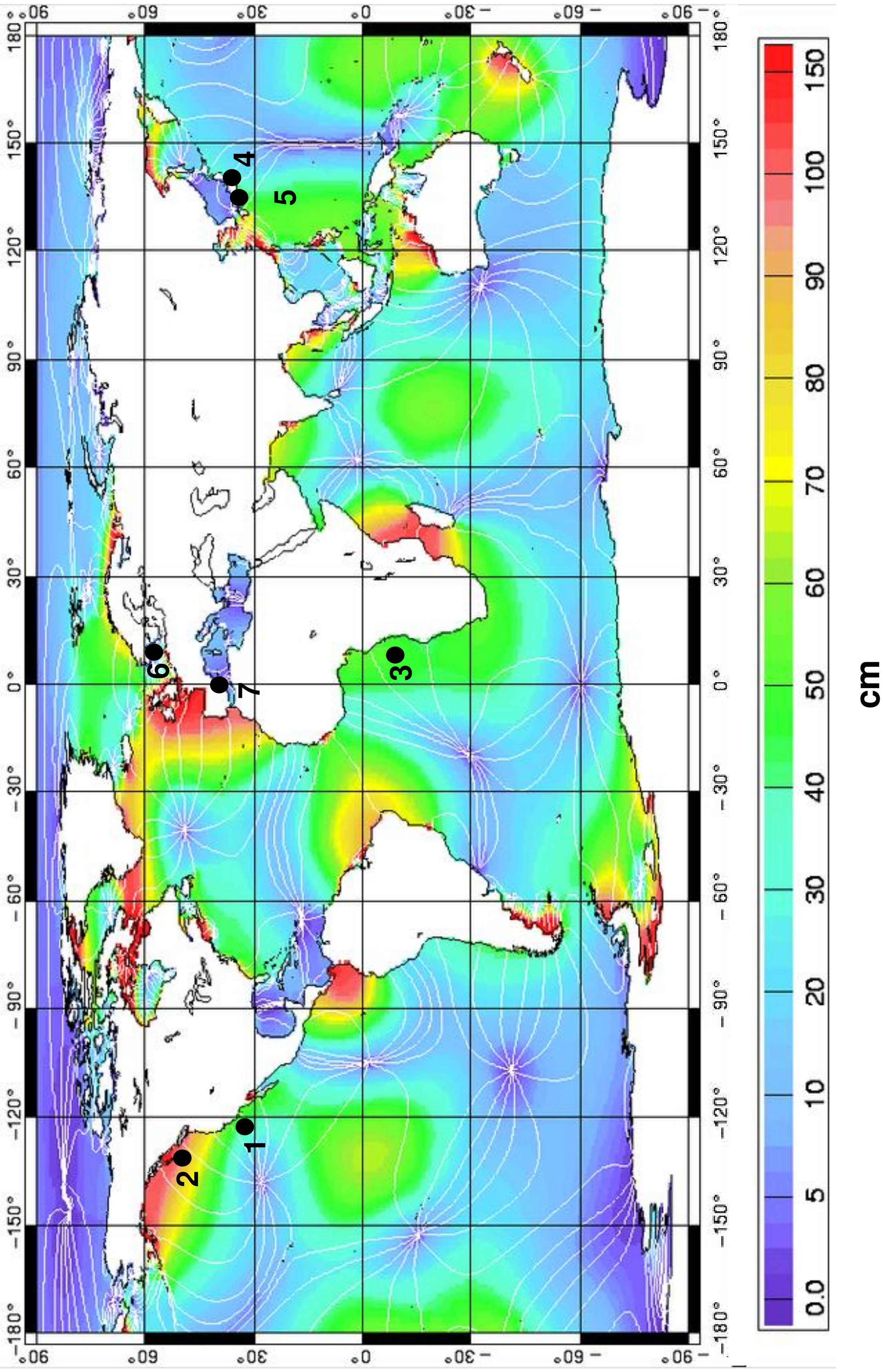


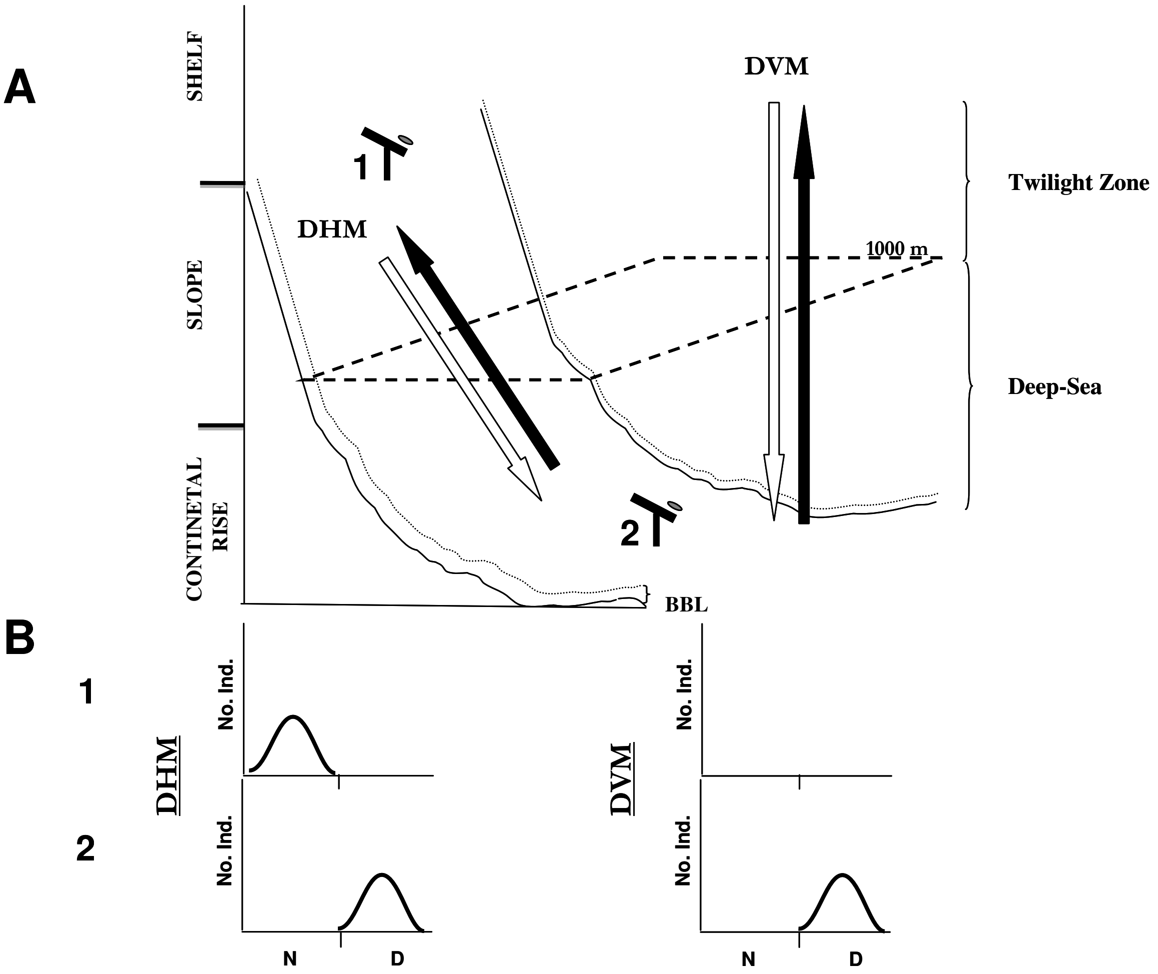
**Aguzzi et al., Fig. 7**



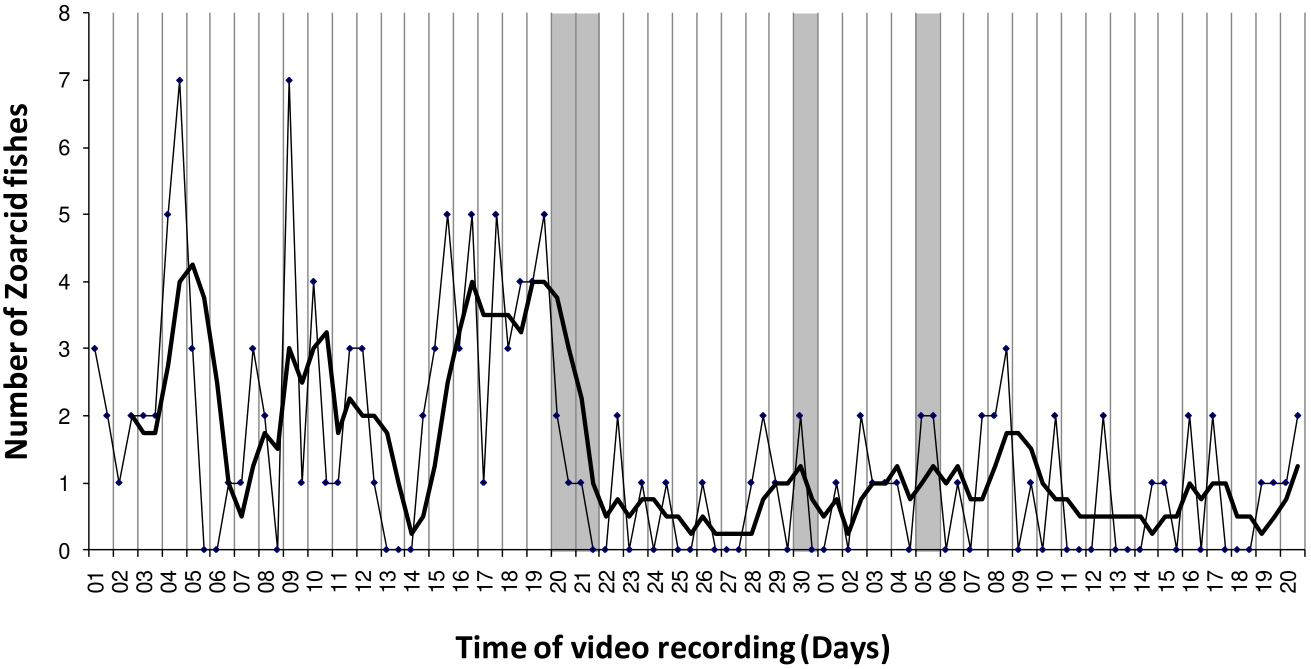
Aguzzi et al., Fig. 8A



**Aguzzi et al., Fig. 8B**



**Aguzzi et al., Fig. 9**



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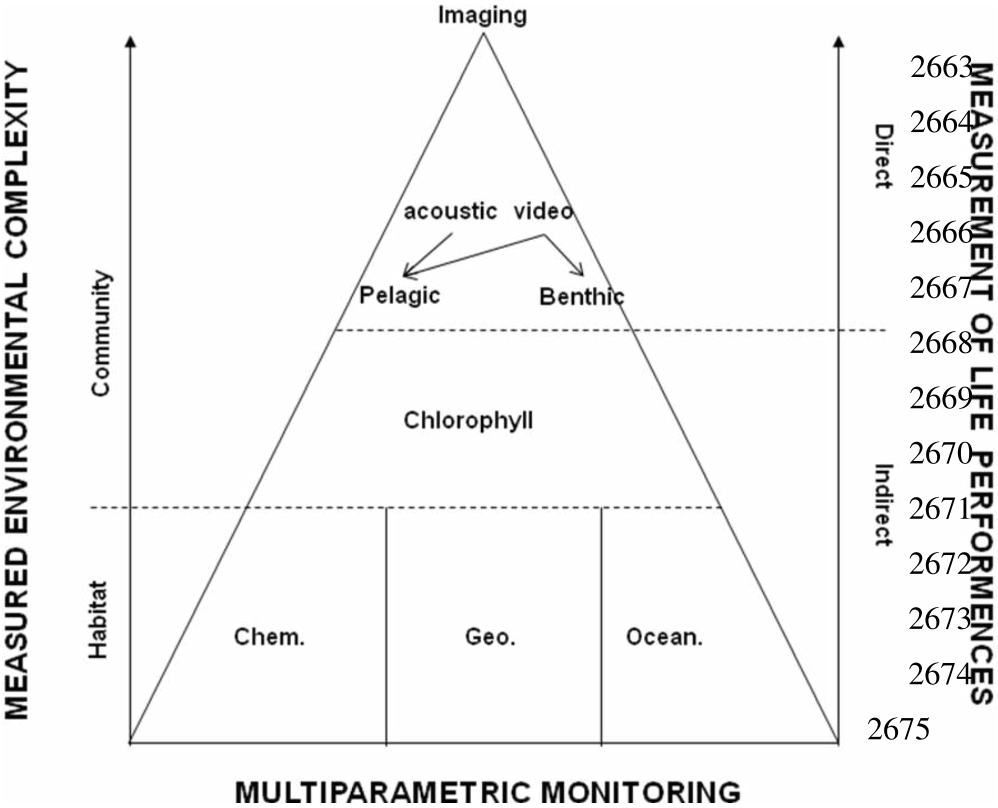

**Aguzzi et al., Fig. 11**