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Activity rhythms in the deep-sea: a chronobiological approach

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1. ABSTRACT

Ocean waters deeper than 200 m cover 70% of the Earth's surface. Light intensity gets progressively weaker with increasing depth and internal tides or inertial currents may be the only remaining zeitgebers regulating biorhythms in deep-sea decapods. Benthopelagic coupling, exemplified by vertically moving shrimps within the water column, may also act as a source of indirect synchronisation to the day-night cycle for species living in permanently dark areas. At the same time, seasonal rhythms in growth and reproduction may be an exogenous response to spring-summer changes in upper layer productivity (*via* phytoplankton) or, alternatively, may be provoked by the synchronisation mediated by an endogenous controlling mechanism (*via* melatonin). In our review, we will focus on the behavioural rhythms of crustacean decapods inhabiting depths where the sun light is absent. Potential scenarios for future research on deep-sea decapod behaviour are suggested by new *in situ* observation technologies. Permanent video observatories are, to date, one of the most important tools for marine chronobiology in terms of species recognition and animals' movement tracking.

2. MARINE CHRONOBIOLOGY AND THE DEEP-WATER ECOSYSTEM

The evolution of life occurs in deterministically cycling environments. The rotation of the earth and its movement in relation to the sun and the moon produces predictable cycles in most habitat parameters. Geophysical cycles impose a patterning in the biological activity of species. Ultimately, species fitness is determined by the capacity to anticipate the onset of favourable or unfavourable environmental conditions associated with these cycles (reviewed by 1).

Chronobiology is the scientific study of biological clocks and their rhythms, which occur at virtually all levels of organisation from cells to ecosystem communities (2). The development of molecular, physiological, ethological and ecological chronobiology is an ongoing process for terrestrial vertebrates and invertebrates, specifically mammals and insects (3-4). Biological clocks and their rhythmic outputs have been described at the behavioural, physiological and molecular levels, particularly with reference to the day / night cycle (circadian clock) and in relation to seasonal changes in day

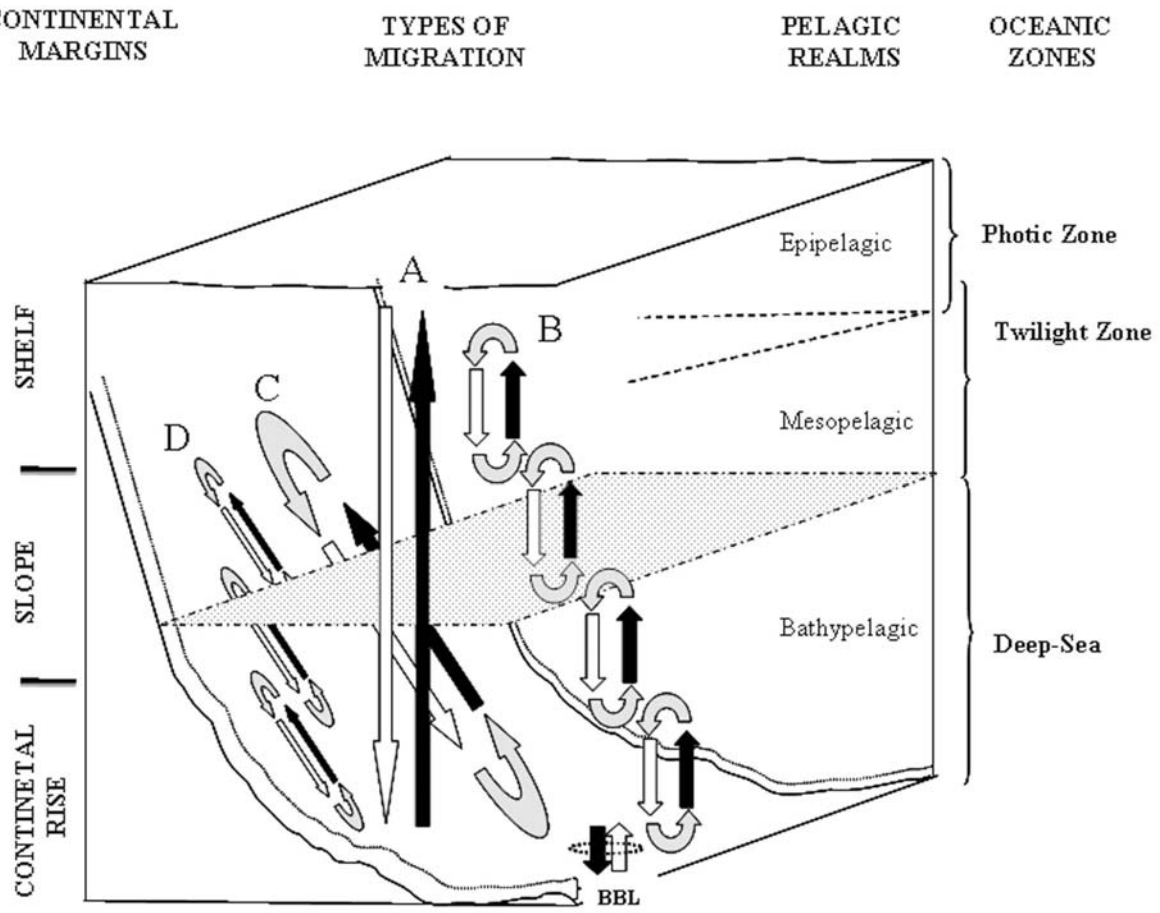

Figure 1. The description of different types of behavioural rhythms and mechanism of benthopelagic coupling in relation to the benthic and the pelagic environment (black arrows-nocturnal movement; white arrows-diurnal movement). Vertical migrators move in the pelagic domain. Nektobenthic species move along the seabed within the benthic boundary layer (BBL). Endobenthic species present rhythmic activity in and out of the substrate. The benthopelagic coupling may indirectly convey information about the status of the day-night cycle within the twilight zone to depth areas below it as based on pelagic or nektobenthic long-range (A, C) or staggered (B, D) migrations. The deeper limit of the twilight zone is represented by the horizontal dashed rectangle.

length (photoperiodism). The molecular architecture of the circadian clock has shown autoregulatory negative feedback loops for gene expression and their protein products (5-6) whereas physiological research has highlighted the role of hormones in the regulation of activity on a diel and seasonal basis, increasing our understanding of seasonal rhythms in growth, reproduction, hibernation or moult (7).

The study of biological rhythms in marine organisms is comparatively less developed. Light represents one of the most important environmental parameters for marine circadian biology. With this in mind, the marine *milieu* should be conceived as three-dimensional with depth as the major axis of light variation. Light intensity is attenuated by water itself, but it is also weakened by the amount and kinds of dissolved and suspended materials in the water (reviewed by 8). Field measurements of underwater irradiance show a negative exponential decay with depth. Two processes are important for this progressive extinction: absorption and scattering. Absorption removes photons, acting on overall intensity whereas scattering changes the direction of their propagation. Scattering does not directly remove light but rather increases the length of the path that photons must travel, hence increasing the chance that they will be absorbed by water or other dissolved particles. As a consequence of these processes, light is usually detected down to approximately 1000 m depth, depending on the local turbidity conditions in the water column (Figure 1; 9-10). Blue light at 480 nm is the only wavelength that is invariantly present across the twilight zone (i.e., 0-1000 m depth).

In the context of marine chronobiology, biological rhythms are phenomena chiefly described for coastal species (11). The identification of molecular and physiological markers that aid in the determination of the

nocturnal or diurnal character of fish and crustacean decapod behaviour has consistently improved over the past few years (e.g., 12-13). In the case of decapods, the most extensively studied group, complex rhythms in behaviour and physiology have been found in association with conflicting geophysical modulations produced by tidal forces and light intensity fluctuations (14). Similar data are scant for species that inhabit the deep-water habitats of the continental margin shelves and slopes and are especially lacking for deep-sea species.

3. INTERNAL TIDES AND INERTIAL CURRENTS AS POTENTIAL NON-PHOTIC ZEITGEBERS FOR DEEP-SEA DEMERSAL SPECIES

The deep-sea represents a novel context for biological rhythms research. Accordingly, a characterization of potential zeitgeber is of extreme importance. In the deep-sea below the depth where light is present, the only available photons interacting with organism are the product of biological activity (i.e., bioluminescence). From an ecological point of view, the deep sea seems to be an extreme, although constant, environment (reviewed by 15). Pressure is high, with an increase of one atmosphere for every 10 m depth. Generally, temperature decreases gradually with depth being of around 2ºC on abyssal plains.

The deep-sea environment is of interest for marine chronobiology because inhabiting species may show a diel regulation of their biorhythms with geophysical cycles other than the light intensity fluctuation. In the ocean, internal waves moving water masses from the surface down to the seafloor occur at different frequencies. Energy propagates itself as tidally-driven waves generated by the sloshing of the barotropic tide over the seabed. Energy can also take the form of inertial waves generated by superficial wind regimes. The vertical movement of the ocean, typically on the order of a few meters for the tidal rise and fall at the surface, is accompanied by barotropic currents that move water under the surface (reviewed by 16-17). Inertial currents can also be episodically generated by storms. Accordingly, near-inertial energy passage can occur in finite bursts.

Internal tides and inertial currents propagate horizontally and vertically within the water column (18). In the water column, the vertical propagation is almost uniform from the surface to the sea bottom. Internal tides can travel thousands of kilometres from their source. Inertial waves can also propagate over long ranges where the intensity of either wave decreases with distance from its origin. In this context, the interaction between internal tides and inertial waves generates mixed frequency patterns over variable geographic scales. This interaction is also of interest in relation to deep-sea chronobiology; when internal waves occur in a deterministic fashion, tides and inertial currents may acquire the status of geophysical cycles for the regulation of behavioural rhythms.

The friction generated by internal waves produces a turbulent mixing over bathyal and abyssal seabed areas (19). This turbulence releases fundamental elements such as carbon and nitrogen trapped within the sediment and introduces them into the water column. These waves re-suspending and transporting sediment potentially represents an important cyclic entraining chemical signal for the behavioural regulation of demersal deep-sea communities (see Section 6).

4. ACTIVITY RHYTHMS IN THE THREE-DIMENSIONAL MARINE SCENARIO

In marine decapods, the vast majority of behavioural rhythms take the form of swimming or locomotor activities. We can classify different types of displacement depending on which segment of the water column/sea bed is crossed during the 24 h (Figure 1). Animals can perform epi-, meso- or bathypelagic diel vertical displacements depending on which depth ranges are crossed within the water column (reviewed by 20-21). Epi- and mesopelagic movers perform migrations of a few hundred meters within upper-intermediate or lower layers of the pelagic zone. Conversely, bathypelagic species often reside in deeper pelagic realms and show diel migrations toward superior and more photic zones hence, encompassing the whole twilight zone. Swimming activity has been reported for decapods in the epi-, meso-, and bathypelagic categories. Crustaceans use pleopod movement to ascend in the water column and they suspend that motion in order to passively sink (22).

Some species perform vertical displacements in which they touch the seabed, entering the benthic boundary layer (i.e., the depth stratum of water column-seabed interface) at least once every 24 hours. Such migrations acquire a benthopelagic character which can extend to migrations of several hundred meters (23-24). Benthopelagic movers show the same swimming motion of epi-, mesopelagic, and bathypelagic species in the water column, but certain levels of locomotor activity can occur when animals move onto the seabed.

Nektobenthic decapods undergo another type of displacement generally along the seabed within the benthic boundary layer in relation to precise depth gradients that encompass shelves and slopes (25). These animals move alone or in schools (26), performing large displacements that can encompass distances ranging from several hundred meters to a few kilometres (27). This displacement is characterised by both walking and swimming along or near the sea bed (reviewed by 28).

Finally, endobenthic decapods represent a pool of species that reside within the seabed sediment during periods of behavioural inactivity (reviewed by 29). When they emerge from the substratum, these burrow constructors display active locomotion around the entrances to their burrows, often showing site fidelity and strong territoriality (reviewed by 30). These species possess the ability to return repeatedly to the same refuge site after foraging excursions. On the other hand, buriers (i.e., those that simply cover themselves with the sediment) can emerge and perform mixed swimming and walking

activities in a fashion similar to nektobenthic species but with no bathymetric-oriented direction (31-32). Since their active displacement does not occur along a depth gradient, the level of site fidelity for areas within a certain bathymetry is presently unknown (33).

5. BENTHOPELAGIC COUPLING AS A MECHANISM OF INDIRECT ENTRAINMENT TO DAY-NIGHT CYCLES IN THE APHOTIC DEEP-SEA

Benthopelagic coupling is the transferral of organic or inorganic matter between the pelagic and the benthic compartment of the oceans (reviewed by 34-35). Passive transport occurs when the organic or inorganic matter sinks or follows the flow of water (reviewed by 36). Conversely active transport is the transfer of matter mediated by animal movement. Diel migrants feed in the upper layers of the water column at a certain time of day (reviewed by 33) and when animals retire toward the deeper benthic realms, they bring the ingested organic and inorganic matter with them. The rate of transfer of that matter is faster than passive particle sinking (37). In a chronobiological context, benthopelagic coupling is of interest because it can indirectly synchronise deep-sea communities to photic signals in the upper layers of the water column.

Predator-prey relationships are possibly important for indirect synchronisation of behavioural rhythms in demersal deep-sea species. Benthopelagic natantian decapods (i.e., prawns and shrimps) are active in benthopelagic coupling (38-39) and are strong vertical migrators that, in some cases, cross both the twilight zone and the aphotic depth strata below it (see Section 4) (reviewed by 23-24, 40). Because decapods are generally located at intermediate levels of marine food webs (41-43), their rhythmic presence on the seabed may influence predators and prey and their behavioural activities.

Time-series trawl sampling in the Mediterranean Sea shows diel variations in the quantity of hauled prawns and shrimps at a depth range between 700 m and 1500 m (44). This indicates the presence of a day-night modulation in their behaviour both above and below the twilight zone. Deep-sea pelagic shrimp of the genera *Acanthephyra*, *Systellaspis*, *Pasiphea* and *Sergestes* undertake extensive vertical migrations and are often captured by trawling during the daytime in the aphotic depth strata (e.g., below 1000 depth in the western Mediterranean). Species of this category probably cross the inferior border of the twilight zone in a rhythmic fashion, conveying information about the time of day (by retiring to darker depths during the day) to resident deep-sea communities. A similar dynamic may be encountered in nektobenthic species such as the red shrimp *Aristeus antennatus* or pandalid shrimps of the genus *Plesionika*.

The indirect synchronisation of behavioural rhythms in deep-sea fauna via benthopelagic coupling may be modelled according to a rhythmic displacement of benthopelagic and nektobenthic types (Figure 1). Benthopelagic species can perform large vertical displacements within the water column. Long-range movements may similarly occur in the nektobenthos (e.g., 27). Individuals may rhythmically invade the benthic boundary layer of deep-sea areas, potentially altering the behaviour of resident demersal species in a temporally predictable fashion as transitory members of these communities.

The indirect synchronisation of behavioural rhythms may also occur by means of short, vertical, staggered, partially overlapping migrations (Figure 1). These migrations have been extensively studied and modelled for species in the pelagic realm (reviewed by 45), but are still poorly characterised in the nektobenthic realm (33). Staggered movements occur when pools of animals (of the same species or of different species) undergo vertical diel migrations through distinct but partially overlapping strata. Staggered patterns are usually separated by 10-30 min (46). In that manner, the information about the day-night cycle is indirectly transferred to the aphotic deep-sea through the steps in the vertical chain of species.

One mechanism for indirect entrainment similar to that of benthopelagic coupling in the deep sea has been proposed for cave-dwelling communities. Cave communities show a cline in the distribution of species sorted by their degree of adaptation to light, temperature, and humidity conditions. Troglobitic (i.e., cave restricted) species are relegated to the deeper realms of caves because they are less tolerant of fluctuations in these habitat parameters (47-48). Species distributed close to the cave entrance may show migrations toward and away from it. For other troglobitic species, any synchronicity with the external light cycle may therefore be dependent upon their proximity to cave-entrance species as based on a staggered migration principle. The linkage of that indirect entrainment is the behavioural response to predictable variations experienced in interspecific interactions.

6. THE BASES OF THE ENTRAINMENT IN THE DEEP-SEA

Any analysis of behavioural rhythms entrainment based on internal tides and inertial currents should consider the reduced motility of deep-sea animals as provoked by the decrease in visual-predation pressure for the complete absence of light. In the dark deep-sea realms, visual predation is unfeasible and a general reduction in locomotor-swimming capability is an evolutionary trend observed in several phylogenetically distant groups such as decapods, fishes and cephalopods (49).

Behavioural observations of synchronised substratum emergence, swimming or locomotion based on tidally controlled flow changes were reported in several coastal decapods both at adult and larval stages (reviewed by 50). This tidally controlled activity occurs in relation to differential habitat uses such as sheltering, colonisation and feeding. For deep-sea species, similar data are scant due to the technological limitations of sampling repetition by trawling and direct observation by permanent video stations (see Section 10).

In this context, we wish to put forward two hypotheses explaining the potential entrainment in deep-sea decapods to internal tides and inertial currents: 1) a mechanic entrainment may occur for the response of animals to speed changes in the water flow component parallel to the seabed and 2); a chemical entrainment may occur for the response of animals to changes in surrounding food odours according to the hydrodynamic variation (51). This latter may be of importance for deep-sea organisms since they inhabit a nutrient-poor environment.

The animal entrainment may occur at predictable water flow increases in order to facilitate the dispersal in organisms with low motility rates (52-53). Adults may enter water flumes with a frequency that corresponds to their phases of behavioural activation. In this sense, hydrodynamic behavioural synchronisation may possibly be of evolutionary value for dispersal and colonisation in the deep-sea. In bottom areas where tidal currents are strong, population distribution and the genetic structuring of animals are influenced by the hydrodynamic conditions (reviewed by 54). That observation suggests the occurrence of a long-rage dispersal of both larval and adult individuals within tidal water flow corridors (55).

The input pathway for this type of entrainment may be specialised sensors that can respond to slight changes (nanometre scale) in hydrostatic pressure. These enable decapods to synchronise their behaviour with minimum variations in the flow regime (56-57). Many penaeid prawns show strong tidally-based substratum emergence behaviour and swimming patterns (reviewed by 58-59). The pelagic squat lobster *Munida gregaria* swims towards the surface when displaced to deeper waters by internal waves (60). Deep water pandalid shrimps counteract the effect of tidal drift by active swimming since sensitive to small hydrostatic changes (49, 61). Similar receptor systems could be also present in deep-sea demersal decapods, acting as sensors for changes in the speed of water currents at the onset of internal tides or inertial pulls.

Food-entrained oscillators are a fairly novel finding of chronobiology (reviewed by 3-4). These non-photic oscillators can be entrained by a cycling of food administration. Decapods rely on chemical signals to extract key environmental information about predator and prey locations (62) whilst benthic crayfish use turbulence generated by hydrodynamic variations to localize food sources (reviewed by 63). In the deep-sea, predictable variations in chemical stimulation at moments of tidal- or inertial-driven seabed turbulence may be of fundamental importance to alter the behaviour of decapods in a synchronous manner favouring entrainment. Demersal deep-sea necrophagous amphipods emerge from the substratum in relation to water speed variations (64). This behavioural response is used by poorly motile decapods to move to a new location as well to approach carcasses (65).

The indiscrete temporal nature of chemical stimuli given by tidally-driven water flow can cause a synchronous flicking in decapods antennules (66). This flicking enhances the receptor's ability to detect changes in stimulus concentration whilst some species of lobster also compensate for changes in water flow by changing their rate of movement while sampling surrounding odours (67). In the deep-sea, the response of decapods to near-bottom currents occurs when these currents contain important directional cues for detecting food (68).

Benthic decapods may respond to variations in current flow for hydrodynamic and chemical stimulation, but behavioural alterations can be also evoked by the presence of predators or prey (reviewed by 69). Entraining to the day-night vertical migrations of pelagic and nektobenthic predators or prey (i.e., benthopelagic coupling) is a definite possibility (see Section 5). Any entrainment of demersal deep-sea communities to species periodically present at their depth can conflict with local geophysical internal tide- or inertial-driven fluctuations in hydrodynamic conditions (see Section 3).

Conflictive behavioural rhythms related to tidal and day-night co-occurring cycles, have been observed in coastal crabs (reviewed by 70) and the technical and conceptual difficulties in studying their rhythms are recurring in deep-sea species. In coastal waters where tides are present, animals experience both diel light variations and changes in local hydrodynamics, temperature, and salinity. The behavioural response to conflicting environmental cycles is probably responsible for noisy time series patterns. In the deep-sea, internal tides and inertial currents create temporally and highly geographically variable hydrodynamic patterns. In the case of species with wide geographic ranges, different populations may experience weak or strong tide-associated selective pressures depending on local topography (70). Within the photic zone, the divergence in behavioural rhythms leading to the establishment of tidal or day-night based regulation may depend on the reciprocal connectivity (i.e., gene flow) between communities.

7. PHOTOPERIODIC RESPONSES IN THE APHOTIC DEEP-SEA

Over time, demersal communities along deep-water continental margins and in deep-sea areas show marked increases and decreases in biomass. These changes suggest the existence of seasonal regulation in the growth and reproduction of these species (71). That synchronisation is associated with seasonal variations in the length of the photoperiod, suggesting the occurrence of a photoperiodic response whose endogenous basis is currently unknown in deep-sea species.

There is an exogenous component to the response of deep-water and deep-sea demersal communities to seasonal variations in primary production in the photic layers of the pelagic zone. Primary production has solar, climatic, and oceanographic controls, resulting in deterministic seasonal variations in sinking inorganic and organic matter (see Section 5) (72). During algal blooms, the reported primary production doubles. These seasonal increases in sinking phytodetritus may trigger reproduction in oceanic decapods deeper than 1000 m (reviewed by 73).

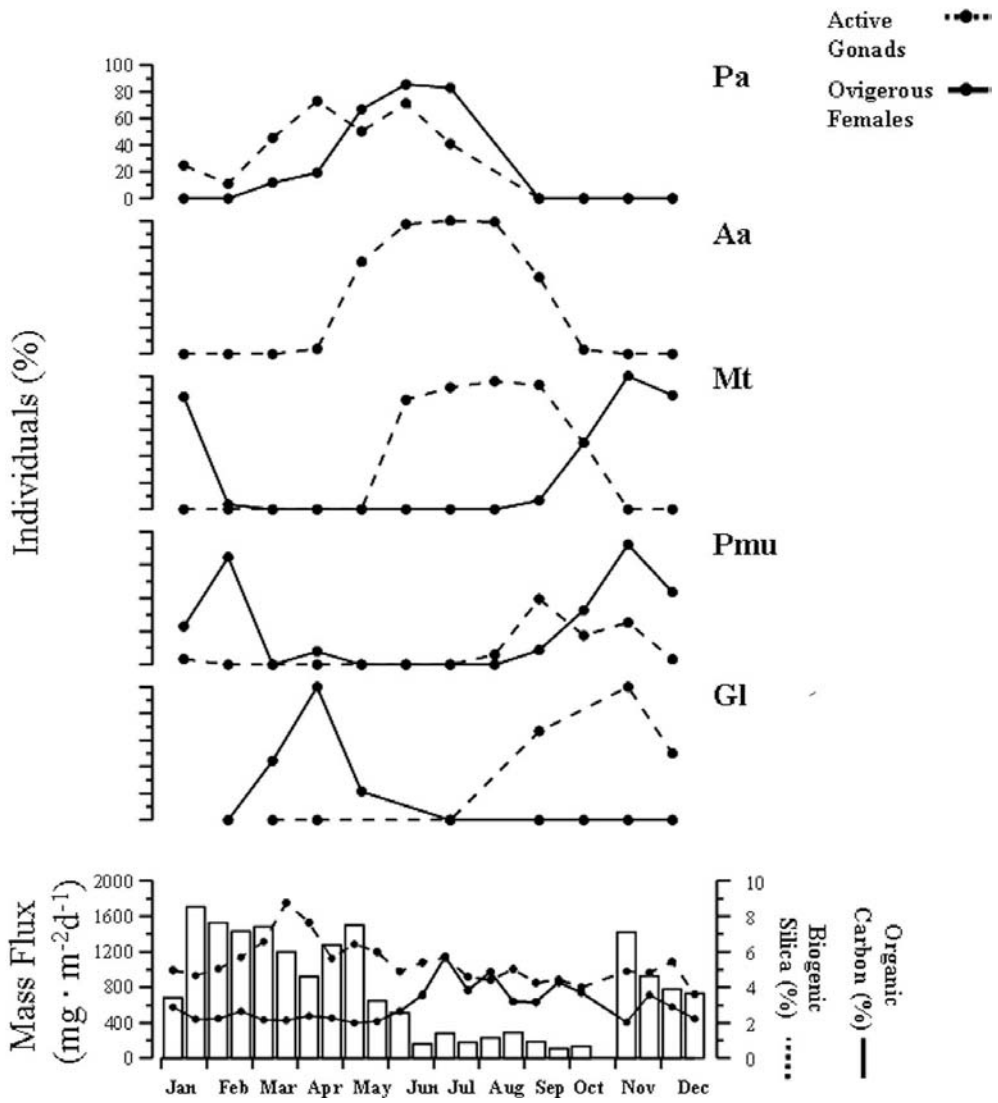

Figure 2. The relationship between specific season of maximal reproductive activity (i.e., recrudescent gonads) and the quantity of sinking particulate organic (carbonate) and inorganic (silicate) matter is evidenced in deep water decapods by looking at species in progressively deeper waters (adapted from 76). Species are ordered by increasing depth. Pa-the Pandalid shrimp *Plesionika acanthonotus*; Aa-the red prawn *Aristeus antennatus*; Mt-the squat lobster *Munida tenuimana*; Pmu-the glass shrimps *Pasiphaea multidentata*; Gl-the geryonid crab *Geryon longipes*

Seasonal increases in primary production provide enough organic input to efficiently sustain gametogenesis in deep-sea species (74). This production also provides food for the larval phase, which increases the survival rate of the dispersing offspring (73-75).

Middle-slope-dwelling species show maximum reproductive activity at different times of the year (Figure 2; 76). For several demersal decapods in the Mediterranean, seasonality of the reproductive period becomes more apparent with an increase in depth. This temporization in reproduction seems to be related to the seasonal decreases in organic and inorganic input. These decapods exhibit marked complex, exogenous, environmental-modulated control of reproductive timing. When seasonal data on the bathymetric shifts in the timing of reproduction are compared with variations in matter input, deeper species seem to lack any sort of synchronisation. This result suggests that the differential availability of energy does not affect the reproductive processes of all species equally. More work is required to examine a possible endogenous photoperiodic response.

The presence of a seasonal oscillator contributing to the regulation of growth and reproduction of demersal deep-sea decapods should not be disregarded *a priori*. Such an endogenous mechanism has already been proposed for the cold-seep shrimp *Alvinocvaris stactophila* living 600-700 m below the surface (77). Laboratory tests under light-darkness simulating a day-night alternation, showed

marked peaks during the scotophase. Because the experiments were not performed under constant darkness due to technical constraints, the endogenous nature of the rhythm could not be proved. However, the observation that light can modulate behaviour in a deep-water species suggests that other biorhythms can be under photic control.

Alvinocvaris seasonally regulates its reproduction and growth (78). Other species close to the inferior border of the twilight zone (close to 1000 m depth) can show growth and reproduction cycles as well as marked diel behavioural rhythms (see Section 5). The diel control of activity may represent the basis for photoperiodism in species within the twilight depth range.

The presence of a mechanism controlling behaviour on a diel and a seasonal basis, through the measurement of the photophase or scotophase duration, has been poorly studied in marine invertebrate species (79). Several coastal decapods demonstrate crepuscular regulation of their activity rhythm (reviewed by 33) similar to what has been described for the fruit fly *Drosophila melanogaster* (80-81). For coastal ditch shrimps of the genus *Palaemonetes*, the reciprocal distance between crepuscular activity peaks may be the mechanism controlling their seasonal reproduction (82). In coastal crabs, crepuscular peaks seem to be controlled by coupled morning and evening oscillators (83-84). In deep water continental margins where photons are still present, a similar variability in the relationship between morning and evening oscillators according to the length of the scotophase may be the mechanism by which animals synchronise their biology with the seasons.

In the deep-sea, data on the locomotor activity rhythms of resident decapods are almost absent. The presence of an endogenous photoperiodic mechanism regulating growth and reproduction cannot be distinguished from any reactive response (i.e., masking) to seasonal fluctuations in organic and inorganic matter associated with the primary production variations in the upper photic layers. Long laboratory assays under constant photoperiods or tests with Nada-hammer protocols (i.e. skeleton photoperiods) are a feasible method for establishing the endogenous nature of such regulation without transient manifestations (79). Unfortunately, the unfeasibility of keeping deep-sea animals in laboratory facilities makes experimental tests very difficult (11).

8. MELATONIN IN DEEP-SEA DECAPODS

Melatonin has been detected in bacteria, eukaryotic unicells, macroalgae, plants, fungi and various animals (e.g., 85-86). The key enzyme regulating its biosynthetic pathway, N-acetyltransferase (NAT), has been found in mammals and in several invertebrates including decapods (e.g., 4, 87-90). Its ubiquitous presence in multicellur organisms and day/night cycling suggests that it has an evolutionarily conserved role in conveying information about the photic status of the ecosystem to the internal physiology of the organism (86).

Melatonin rhythms were measured in the hemolymph, eyestalks (91-94), and optic lobes (92) of decapods, and they showed marked phase variability in melatonin production peaks. This finding calls into question the physiological function of melatonin in relation to the ecology of different species (95). The use of different tissues and different units to report concentration data along with the use of different experimental methodologies (i.e., radioimmunoassay, ELISA, and immunohystochemistry), makes any interspecific comparison of melatonin rhythms difficult (96).

In decapods, the role of melatonin is associated with the circadian regulation of behaviour. Eyestalk melatonin conveys environmental timing cues (i.e., light intensity) to locomotor centres. Increases in melatonin are associated with locomotor peaks and with increases in glucose and lactate concentrations (92-94). Diel variations in melatonin concentration were measured in the eyestalks of the fiddler crab, *Uca pugilator*, that were exposed to 12 hour light-dark cycles in the laboratory. Concentrations peaks were reported for the photophase as too in the freshwater prawns of the genus *Macrobrachium* (91). However for the red swamp crayfish *Procambarus clarkii*, data on melatonin levels in relation to photophase and scotophase are contradictory (96-97).

Uca crabs can also show tidally driven hemolymph melatonin rhythms (94). This observation suggests that the tidal or inertial frequency regulation of activity rhythms in deep-sea decapods can be modulated via melatonin. A tidally driven rhythm in the blood melatonin has been characterised in deep-sea fishes (98). Functional melatonin receptors were found in the brain of bathypelagic species (99-100). The role of this hormone at the level of their central nervous system physiology seems to not be related to the perception of diel variations in light (101). In bathypelagic fish, melatonin may be involved in the monitoring of ambient light as a part of the physiological mechanism that controls the bathymetric distribution (99). Pineal organs in deep-sea demersal fish develop when larvae inhabit photic strata of the water column. At adulthood, animals migrate towards the aphotic seabed, but melatonin synthesis is still active. Melatonin may shift from acting as a photosensor to hydrodynamically synchronising various organs to alternative temporal cues such as rhythmic changes in water speed (102).

The association of melatonin and locomotor rhythms has only been extensively studied for decapods of inland or marine shallow coastal waters (e.g., reviewed by 86-87, 103). Unfortunately, to our knowledge, no data exist on melatonin presence in deep-sea decapods. The closest exception is the Norway lobster, *Nephrops norvegicus*, which is not a deep-sea decapod and possesses a depth-dependent weak diel melatonin rhythm. This species shows a wide bathymetric distribution encompassing upper shelves and upper and middle slopes down to approximately 700-800 m. Recently Aguzzi *et al*. (95) measured a dampening of hemolymph melatonin concentrations in *Nephrops* under different light intensities

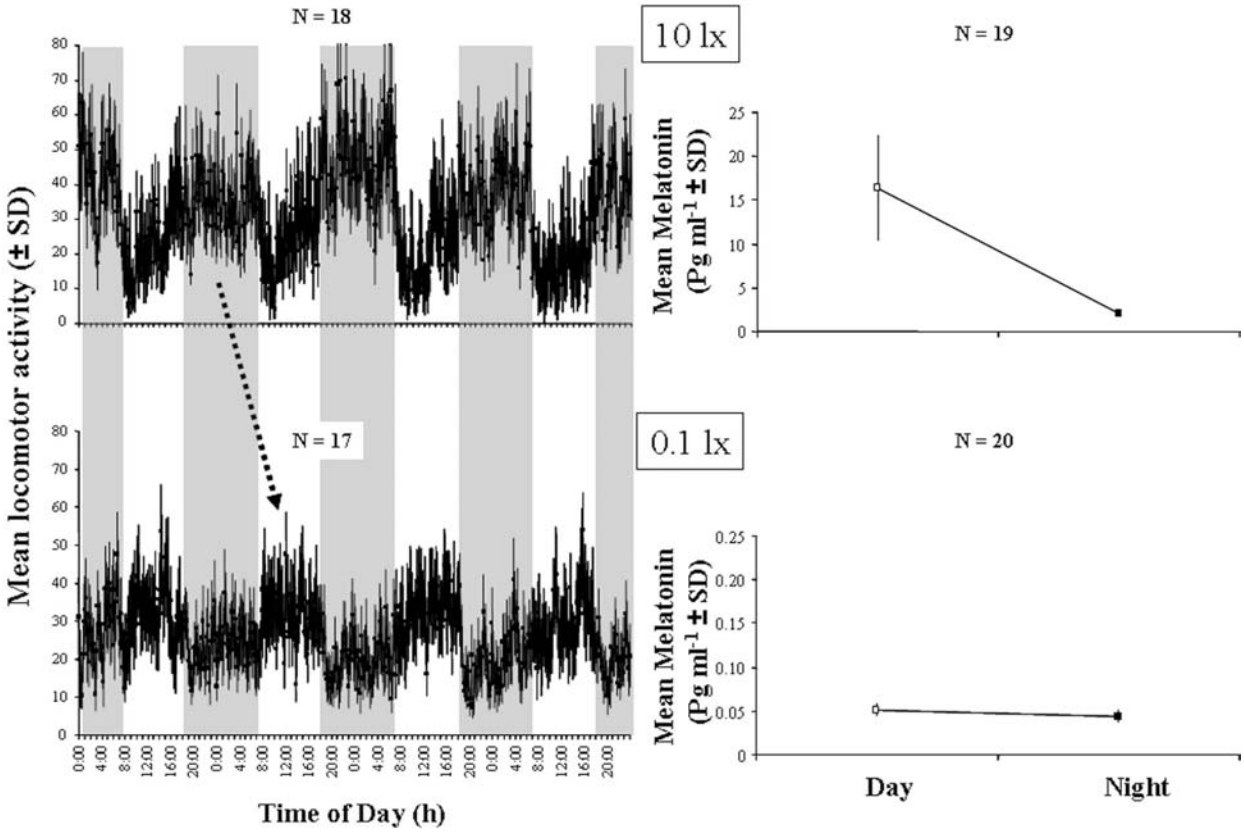

Figure 3. Reported shift in timing of peaks of locomotor activity (on the left) and associated hemolymph melatonin patterns (on the right) in *Nephrops norvegicus* exposed to different light intensity conditions (i.e., 10 lx and 0.1 lx; 95). Maxima in locomotor rates are displayed at night under 10 lx and shift their timing toward daytime under 0.1 lx. Under the same circumstances, hemolymph melatonin markedly reduces its concentration diminishing the diel variation between photo- and scotophase (the potential presence of crepuscular peaks still remains untested given the absence of sampling at that time). Grey vertical bars represent the night duration; the oblique dashed black arrow indicates the timing shift in the maxima of locomotor activity.

from 10 to 0.1 lx, which simulated depths from the lower shelf to the upper slope (Figure 3). A non-significant increase in daytime melatonin was found under 10 lx treatments and not under 0.1 lx treatments. The animal's locomotor rhythm also changed from nocturnal to diurnal within that light intensity range (see Section 9). On the one hand, these data mean that melatonin is not involved in the control of locomotor activity rhythm. On the other hand, they indicate that the metabolism of that hormone is still related to the light cycle.

A putative role for melatonin in growth, moulting, and reproduction was proposed for shallow water decapods. In the prawn *Macrobrachium rosenbergii*, a sexual dimorphism was found in the optic lobes for NAT activity and melatonin concentration (90). Although the duration of the light phase directly increases NAT and melatonin concentration in the optic lobes, no direct effect on gonadal growth has been demonstrated as of yet (91).

9. THE NORWAY LOBSTER: A CHRONOBIOLOGICAL MODEL FOR THE DEEP-SEA

Decapods have proven to be an excellent model for studying the behavioural rhythms in the deep-sea. Crustaceans have an open circulatory system composed of a series of sinuses and they don't have gas bladders (104-105). These features allow them to survive the stress of capture, which includes pressure changes associated with bringing them to the surface (106) making them more suitable to lab studies than other deep-sea (in)vertebrates which often require collection by submersibles or remotely operated vehicles (ROVs) with high-pressure trap chamber facilities (107).

Deep-water tunnel makers or shallow-water shelterers usually have predictable activity patterns when a burrowing media is available (108-111). In the case of nephropid lobsters, adults of the genus *Homarus* (e.g., American lobster, *Homarus americanus*) usually inhabit shelters in rocky areas. These lobsters present a clear nocturnal behavioural pattern with or without the presence of rock shelters (112-113). A clear behavioural rhythm is also seen with the use of running-wheels, a typical instrument for mammalian chronobiological tests (114). *Nephrops* that dig tunnels in muddy bottoms also display good behavioural rhythms with or without the use of structures simulating their burrows (115-117). In all these species, the activity cycle is defined by two markedly different, but well characterized nocturnal phases (117):

activities inside the refuge including maintenance operations, and locomotor-sustained emergence activities (118).

We suggest that activity rhythms should focus on endobenthic decapods with a wide bathymetric distribution since populations at different depths are exposed to widely variable local photic levels, hydrodynamic tidal and / or inertial cycles. One good experimental model is to compare phylogenetically related species which also show similar ecology, *Nephrops* and *Homarus americanus*. Populations of the former are present in deeper waters. Differently, *Nephrops* inhabit continental shelves and slopes, relying on the presence of a suitable silt and clay seabed substratum (reviewed by 119). As they move from shelves to slopes, they are subjected to light regimes of markedly different intensities and spectral qualities (reviewed by 116).

The behaviour of *Nephrops* in the field can be studied by repeatedly trawling in a temporally scheduled fashion (reviewed by 116). The number of individuals captured is proportional to the number of animals undertaking burrow emergence activities that are sustained by an increase in the locomotor rate. Field studies with trawling repeated over consecutive days in the Mediterranean showed dramatic emergence rhythms in populations from depths of both the continental shelf (approx. 100 m) and slope (approx. 400 m) (10). Peaks in catches across this range shifted from crepuscular hours to midday (reviewed by 116). These data fit with data from fishery reports from the Atlantic where peaks in catches are shift from night time to crepuscular hours moving from depths of 10-30 m down to 100-150 m (e.g., 120-123).

In the laboratory *Nephrops* show diel rhythms in burrow emergence related to the photoperiod regimes of monochromatic blue (i.e., 480 nm) light (117, 124). These rhythms are expressed under a range of different intensities simulating an increase in depth. Aguzzi *et al*. (95) provoked a shift in the timing of burrow emergence (from night to day) with the use of decreasing light intensity regimes (i.e., from 10 lx to 0.1 lx) simulating photic conditions at different depths. In the shallow water areas of the upper shelf, *Nephrops* is a nocturnal species, showing high activity rates at full moon phases when illumination is high (reviewed by 16, 33). With an increase in depth, the species experiences a shift in the timing of its burrow emergence from the crepuscular hours to the daytime. This timing varies with the variation in the intensity of monochromatic blue light. During behavioural tests with different blue-light regimes, Aguzzi *et al*. (117) observed a free-running periodicity in a minority of animals. This phenomenon is currently under investigation, but it may be that with an increase in depth and a concomitant reduction in the experienced light intensity, the entrainment of *Nephrops* is no longer possible. At these depths, light is so reduced that other Zeitgebers may come into play i.e. the inertial currents of the Mediterranean where tides are absent (see Section) (125).

Different activity rhythms have been reported for *Nephrops* depending on whether they were studied in the field or in the lab. In the field, locomotor rhythms (i.e., catch patterns) show a 24-h periodicity on the shallow shelf (nocturnal) and upper slope (diurnal) with an intermediate depth where these rhythms are crepuscular (upper shelf) (10). Similar periodicities can also be recreated in the lab (96). Other laboratory tests in constant darkness point to multiple periodicities at 24 h, 12 h and 18 h (115). In particular, the 18 h periodicity has not readily understandable ecological meaning. It was firstly observed in animals freshly collected from waters deeper than 450 m (125). The coping of that data with local seabed flow regimes allowed Aguzzi *et al.* (125) to hypothesize that this periodicity was created by an entrainment to factors others than the light intensity cycle, such as inertial frequency currents (an 18-h periodicity at 40 degrees latitude).

Comparing constant darkness data with field observations, Aguzzi and Chiesa (126) proposed a model of the *Nephrops* clock that could adapt to different light intensities and depth conditions, within the tidal-free context of the Mediterranean. This model consists of a population of independent but coupled neural circadian oscillators organised into four groups. The state of their coupling in relation to the intensity of blue light (used in the clock input pathway) may explain the nocturnal or diurnal rhythms seen in the laboratory as well as the catch patterns observed in the field (Figure 4) (127). Under bright light cycles simulating shallow shelf depths areas, it is proposed that behavioural rhythms are controlled by the four groups working in a coupled fashion. The overall modulator output generates therefore a diel nocturnal rhytmicity of circadian character when animals are transferred to constant darkness conditions. A nocturnal rhythm is recorded in the laboratory light.-darkness cycles similar to that reported by temporally scheduled trawling of the upper-middle shelf. With a reduction in light intensity, a 12 h periodicity occurs because the four groups of circadian oscillators split their phases of functioning. That splitting drives the observed 12-h rhythm as a sub-multiple of the 24-h period observed in bright light laboratory tests. This coupling justifies the crepuscular catch patterns reported on the lower shelf. With a further reduction in the light intensity (as depth increases moving down to the upper slope), oscillators groups completely uncouple from each other. During the daytime, only one group out of four is functioning and the others are dampened.

Ultimately, animals may experience the overwhelming influence of inertial currents with increased depth (i.e., on the middle slope). In constant darkness experiments in the lab, animals show 18-h rhythms that can be only explained by a complete uncoupling in the phases of the 4 groups of motorneurons. A further 6 h phase uncoupling lead to an overall 18 h period. This is true not only if a constant phase relationship is preserved, but also if damping decreases the amplitude of fluctuation of different oscillators over time.

The decoupling of clock neurons has been used to interpret multiple rhythms observed in the activity of different invertebrates such as the Antarctic krill *Euphausia superba* (22). Such an interpretation is derived from the

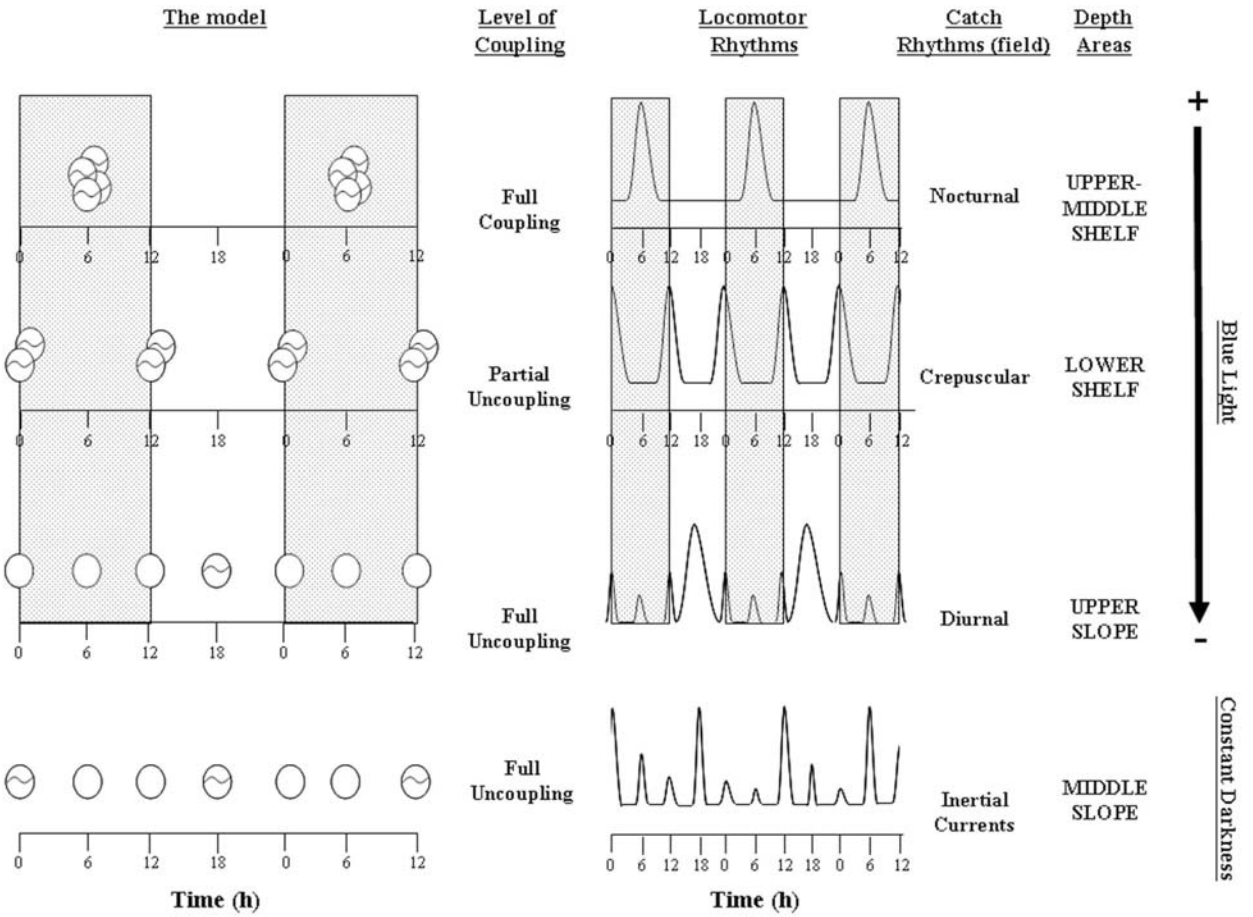

Figure 4. The model accounting for the presence of circadian and ultradian 18 h and 12 h rhythms in the behaviour of *Nephrops norvegicus* as recorded in the laboratory under varying blue-light conditions simulating depth as well as in the field sampled by temporally scheduled trawling. The existence of four groups of pacemakers (circles) is postulated (circles with the internal wave represent the functional oscillator, being those empty a representation of non working oscillator). The degree of phase splitting varies in the laboratory and in the field: a 24-h rhythmicity of nocturnal or diurnal type (on the upper-middle shelf and upper slope, respectively); an overall 12 h crepuscular periodicity (on the lower shelf); overt 18 h rhythmicity when an ulterior splitting occurs in association with variable damping (i.e., on the middle slope). The scheme depicting the state of oscillators coupling (on the right) is depicted during two and a half days along time series profiles a day and a half long.

behavioural model of *Drosophila* (reviewed by 3). Behavioural arrhythmia in populations because of incoherence in phase among rhythmic individuals is compared to arrhythmia in a group of autonomous oscillators resulting from a desynchronisation in their phase of functioning. *Drosophila* possesses groups of autonomous oscillators that act as morning and evening oscillators (reviewed by 81). These oscillators can consist of multiple pacemakers with their phase of functioning coupled together by light intensity. Under normal conditions, both groups are coupled to create a bimodal activity band. Without that coupling, the clock falls apart and ultradian rhythms appear with the splitting in phase functioning for the two oscillator groups.

10. THE TECHNOLOGY FOR STUDYING ACTIVITY RHYTHMS IN THE DEEP-SEA

The study of behavioural rhythms in decapods inhabiting deep-sea areas is often performed by temporally scheduled trawl haul surveys (see Section 9). There are economic constraints to sampling repeatability as well as technical limitations to deep-sea fishing that often make sample results not statistically relevant from the chronobiological point of view (84). Fluctuations in catches can be an indirect indication of species' activity rhythms because catch size is determined by the movement of individuals within limited sampling windows (reviewed by 33). Other sampling systems may be more suited such as remote sensing technology, which provides continuous and real-time recording of biological and habitat parameters. Among the wide arrays of recording devices usable in the marine environment, video-image analysis is the most promising for the characterisation of behavioural rhythms not only in decapods, but also in other groups such as fish and cephalopods (128).

Automated video-image analysis is useful for populations located in depth zones where internal tides and inertial currents exert strong effects. Deep-sea decapods

may show clear variations in their behavioural activity related to strong fluctuations in the hydrodynamics they experience. As water speed increases, animals alter their behavioural activity in order to cope with water drift effects (128). An animal's passage across the video camera's field of view may vary accordingly. This behavioural response should be synchronous for the local population, making it easier for the automated video-image analysis to detect overall patterns in behaviour.

Over the past two decades, interest in deep-sea exploration has increased. Because of this interest, permanent submarine monitoring stations measuring biological and physicochemical parameters have been built all across the world (27). In several cases, these permanent stations have video cameras. The ability to observe species within their local communities, estimate their biomasses and study their behavioural rhythms is also increasing. Unfortunately, the use of video cameras has been severely limited by the general lack in automation of analysis necessary to extract quantitative data from the available footage (reviewed by 128). With recent developments in the automation of footage processing, different biological parameters can now be measured. For the first time, chronobiological research can now be done in the deep-sea.

Detection, tracking and classification are elements of motion video analysis that will make counting animals over time much easier. An implementation of these steps will allow performing reliable remote chronobiological studies in the deep-sea. Detection is the recognition of the same object (an individual of a species) in different frames (129-130). Tracking takes place when that object is followed over several consecutive frames and is the first step in the analysis of digital videos. Classification is the grouping and categorisation of objects within a library of known objects through certain common properties (colour, shape, etc.) (135-137). It involves classifying displaced animals by species.

As a result of detection, tracking and classification, analytical protocols for the automated analysis of a great deal of footage should be able to identify different species and count the number of animals observed per species category over any arbitrary unit of time (Figure 5). Outputs of video-image analysis should be in the form of a time series of visual count data. One limitation of automated video-image analysis is revealed at the data analysis stage, because visual counting reports variations in a pool of sampled but not distinguishable animals. Negative results, as in the case of arrhythmia, may be reported because of the activity phase of different individuals. These results are a typical bottleneck for population studies dealing with the behavioural rhythms of different individuals at a single sampling site.

The development of protocols for automated behavioural tracking has been developed both in the laboratory and in the field. In the laboratory, video-image analysis is useful because other activity-measuring devices are less efficient for monitoring marine species than they are for terrestrial species (138-139). Laboratory telemetry, such as the active telemetry already in use in rodents (140-141), is problematic because salt water distorts the transponder emission (142). Acoustic and radio-acoustic telemetry requires elevated operational spaces for the hardware (i.e., receivers in a dry environment), and they can only detect decapods as they pass close to a listening source (i.e., the antenna) (143-144). Infrared actography (Figure 6), although very useful, requires delicate hardware such as cabling and LEDs that require careful handling. Their integrity is constantly threatened by the elevated humidity and marine condensation within refrigerated chambers, when often epoxy resins applications can not be applied (124).

Video-image analysis has the potential to replace IR actography in laboratory tests of behavioural rhythms. Under laboratory conditions, different kind of behavioural tests can then be carried out. Behavioural rhythms can be tracked in isolated individuals in order to study the functioning of the biological clock under different photic conditions, such as playing with monochromatic blue 480 nm light at different intensities to simulate different depths (see Section 8). Other behavioural tests can be carried out with groups of animals in order to observe how social interactions affect the circadian regulation (130). In behavioural tests with isolated individuals, the subtraction of consecutive frames for animal shape centroid identification is the fastest method (117, 145). Conversely, when animals are grouped and undistinguished, a tag technology has to be implemented (130). Plastic tags are a suitable method for distinguishing between different decapod specimens within a group. Tags can easily be recognised using different methods such as shape matching or geometric morphometric approaches (e.g., Fourier Descriptors). Frame subtraction for sensible object identification (i.e., tags that have moved) is always the first step. Then with shape matching, the program screens each frame seeking out tags that match a pre-memorised shape. With morphometric approaches, tag shapes can be identified by evaluating their outline using a Complex Fourier coefficients analysis where a pre-established function is fit onto the tag outline and fitting coefficient are calculated.

Quantitative motion video analysis for the monitoring of rhythmic behaviour is not often used in the marine environment. Published works utilising this technique chiefly refer to two main fields: microscopic analysis and underwater monitoring. The main goal of these studies in relation to organisms or cells is related to species identification, individual counting and measuring or motion tracking (146-147). An example of this research comes from the Monterey Bay Aquarium Research Institute (MBARI, California) where this technology is often devoted to the study of deep-sea pelagic organisms using remotely operated vehicles (ROV) that take videos (148-149). In recent years, researchers from that institute have

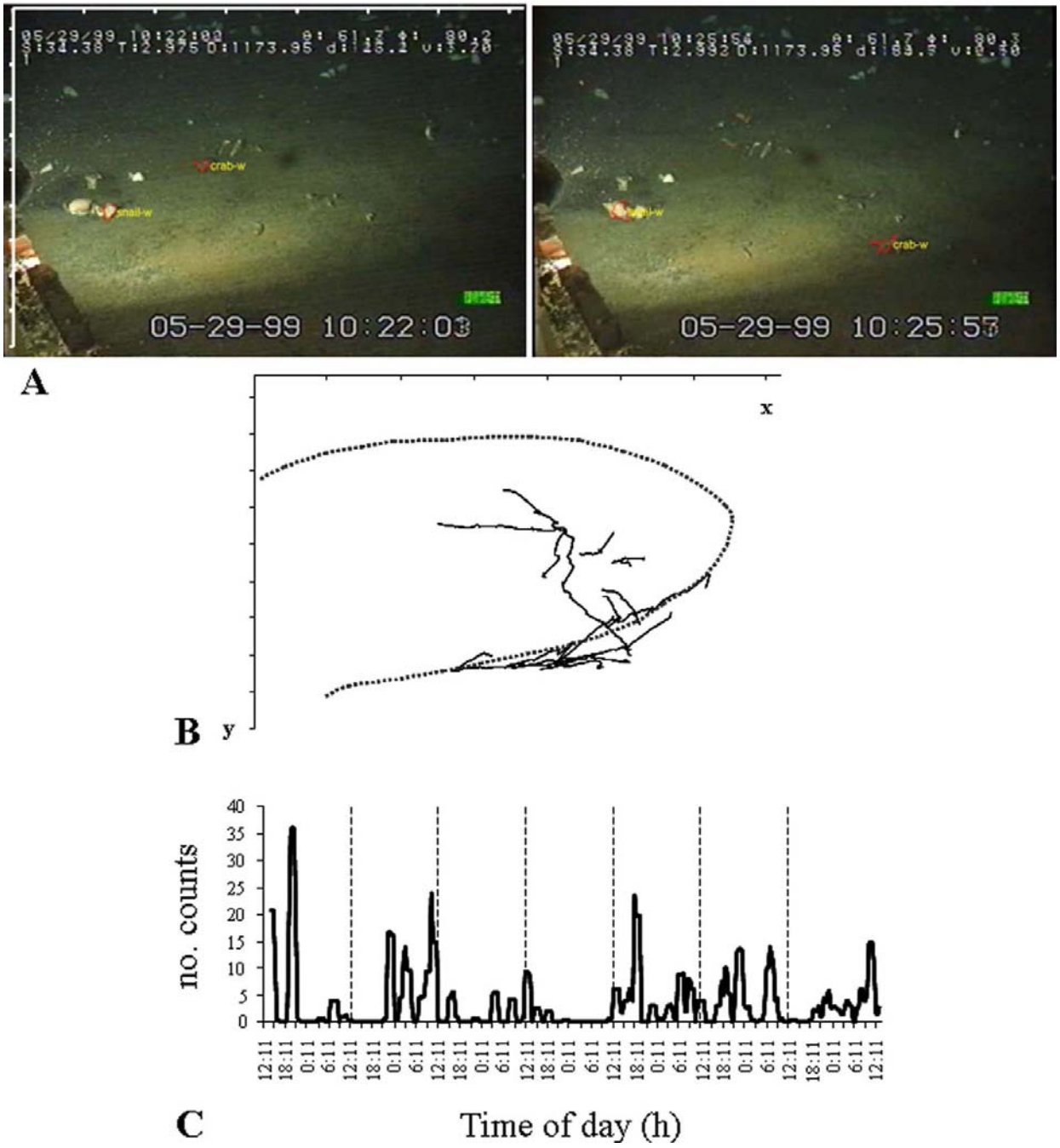

Figure 5. Schematisation of the automated video-image analysis processing of footage proceeding from the Real-Time Deep-Sea Floor Permanent Observatory of Sagami Bay (1100 m, Central Japan; adapted from 128). Two consecutive frames (A) prorating the displacement of the red crab *Paralomis multispina* are presented as an example of automated species identification (animals profiles were encircled after automated detection). Average trajectories of the animals in relation to video camera white lamps (B) are reported as an example of potential behavioural alterations at the moment of measurement (the enlightened seabed field is represented within the region of interest by the dashed line). The fact that red crabs cross the enlightened field shows how this species poorly senses video filming illumination (although no eventual damage to its optic apparatus has been yet studied). The results of the automated video image analysis are a time series of visual counts of animals per arbitrary unit of time (C). With that input, chronobiological analysis is possible for deep-sea decapods species in relation to tides.

created the Automated Visual Event Detection (AVED) software that processes digital images coming from the deep-sea permanent underwater observatory for the Monterey Accelerated Research System (MARS) (150-

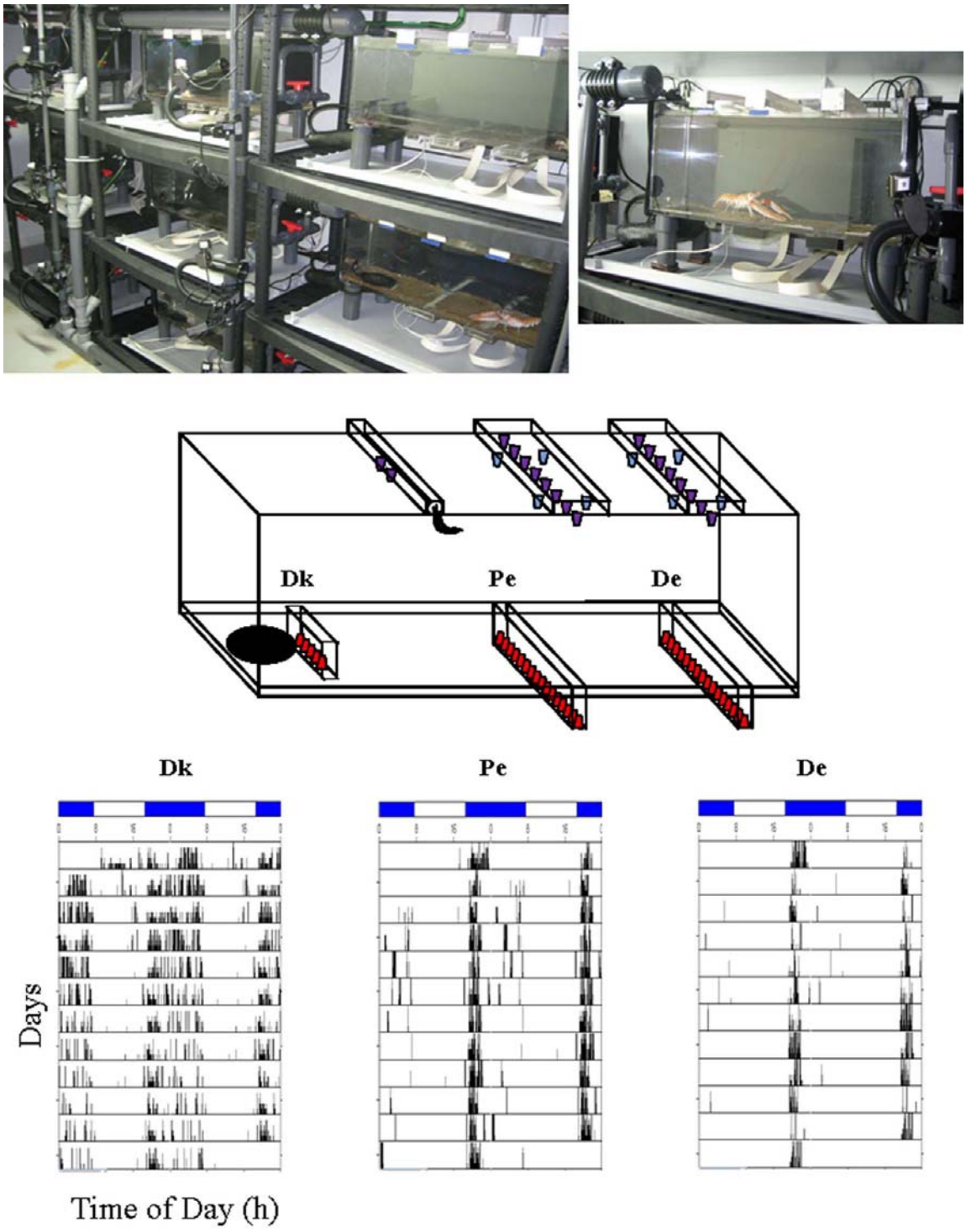

Figure 6. Actographic system used for the detection of the different components characterising the burrowing rhythm of *Nephrops norvegicus*. Photographs for the whole system of aquaria as well as a photograph for a single aquarium are presented along with a scheme depicting the positioning of emitting and detecting IR barriers and light cycle generators (see 124 for technological details). Different infrared barriers were placed at increasing distance from burrow openings in order to understand the behaviour of this species in relation to emergence location and duration (Dk, door-keeping; Pe, proximal emergence; De, distal emergence). Resulting time series of data are also presented in the form of double-plotted actograms as an example of outputs obtained in a behavioural study where animals were exposed over consecutive days to 12-h light-darkness cycles of blue monochromatic light (480 nm, light intensity 10 lx).

151). Other studies with marine organisms used automated-video digital image analysis with shallow-water species for fish recognition and measurement (152-153), tracking (154) and behaviour (155) as well as for aquaculture purposes (156-159).

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