Behavioral fractality in marine copepods: Endogenous rhythms versus exogenous stressors

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A B S T R A C T

The presence of endogenous rhythms in the swimming behavior of five common species of copepods (i.e. minute marine crustaceans) was investigated through comparisons of the scaling properties of their three-dimensional trajectories and cumulative probability distribution functions of move lengths recorded during the day and at night. Beside clear inter-specific differences in their behavioral scaling properties, the five species exhibited an increase in path tortuosity at night, consistent with an increase in food foraging activity. Given the absence of food under all experimental conditions, this suggests the presence of an endogenous swimming rhythm consistent with the widely reported pattern of ascent at dusk resulting in copepods entering the food-rich surface layer at night. The impact of the stress exerted on swimming behavior by changes in the light regime (i.e. light and dark conditions respectively experienced at night and during the day) and the related copepod behavioral adaptivity was also investigated. The low and high fractal dimensions respectively observed during daytime in the dark and during night-time under conditions of simulated daytime indicate that these organisms have the ability to adjust the complexity of their swimming path depending on exogenous factors, independent of their actual endogenous rhythms. The scaling exponents of the cumulative probability distribution function of move lengths exhibit a significant decrease during daylight hours under simulated night-time conditions and during the night under simulated daytime conditions, suggesting an increase in the stress levels experienced by the five species considered. It is finally shown that the stress exerted on endogenous behavioral diel variability by exogenous cues has a species-specific effect on copepods.

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1. Introduction

Behavioral time series, though they often appear erratic, reveal 1/f like spectra; they are fractal-like because they display self-similar fluctuations over a wide range of time scales [1,2]. Long-range correlation in biological systems is adaptive because it serves as an organizing principle for highly complex, nonlinear processes and it avoids restricting the functional response of an organism to highly periodic behavior [3]. For example, 1/f temporal fluctuations are found in the heart rate of healthy individuals [4], respiratory intervals in animals [5] and neuronal discharges during sleep [6]. Long-range correlations have been also observed in the stride interval of human gait [7,8]. Fractal analysis of behavioral sequences has also been shown to be an effective tool for the non-invasive assessment of the general health of wild animals [2,9–11].

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In response to stress, animals typically increase their metabolic rate and energy intake [12–14]. They also perform compensatory responses that involve their endocrine system and behavior to improve the probability of survival [15]. As a consequence, a stressed animal will reduce the complexity of behavioral display [16–19]. The subsequent change in pattern distribution of behavior sequences is likely to be related to the underlying dynamics of physiological components and can be used to detect the level of stress under different environmental conditions. For example, the time series of interbeat intervals in healthy subjects have more complex fluctuations than patients with severe cardiac disease [3,20]. Similarly, the long-range correlations related to behavioral sequences of wild chimpanzees were characterized by higher fractal dimensions for healthy individuals [9].

Copepods are millimeter-to-centimeter scale crustaceans that are among the most abundant animals on earth and are at the base of the food chain, transferring energy from primary producers to higher trophic levels. As such they play an important role in the functioning of marine ecosystems and in biogeochemical cycles through their sensitivity and response to climate change [21–23]. More specifically, the swimming behavior of copepods, widely investigated in relation to a range of abiotic and biotic cues [24–28], present circadian rhythms such as diel vertical migration (i.e. the pattern of ascent at dusk and descent at dawn is performed, resulting in the copepods entering the food-rich surface layer at night; [29,30]) and the related increased foraging and grazing activity at night. Migration behaviors have also been observed under conditions of constant darkness [31,32], suggesting the presence of endogenous diel swimming rhythms.

Light has long been recognized to have a significant impact on the behavior of copepods, with much research emphasizing the effects of light on vertical migration; see [33] for a review. Most copepods possess only a simple eye consisting of paired dorsal ocelli and a single ventral ocellus [34]. While these simple photoreceptors are not capable of image formation, photoreception is extremely sensitive in copepods, as behavioral responses such as positive or negative phototaxis have been observed for light intensities as low as 0.1 µM photons m⁻² s⁻¹ [35]. Photoreception hence plays a direct role in predator avoidance by copepods through photophobic responses to rapid increases or decreases in light intensity [36], but also indirectly helps copepods to evade attacks by visual predators by remaining in low light environments during daylight hours [37]. Due to intrinsic positive phototaxis involved in vertical migration patterns of copepods and their behavioral sensitivity to light [33,35–37] and the advance of infrared lighting and imaging systems, most behavioral observations are now conducted in the dark [27,28,38–41]. This has, however, not always been the case, e.g. [42–46]. From both methodological and ecological perspectives, this generally leads to question the impact of experimental conditions (light versus dark, day versus night) on copepod swimming behavior, and more specifically the subsequent relevance of comparing results obtained under different conditions. The present work explores the ability for fractal analysis to detect the stress potentially induced by experimental conditions in the swimming behavior of five common species of marine copepods. This was tested through investigations of the fractal properties of high-resolution three-dimensional swimming paths and the cumulative probability distribution function of move lengths.

2. Methods

2.1. Study organisms and behavioral observations

The five species of copepods investigated (Acartia clausi, Centropages typicus, Paracalanus parvus, Pseudocalanus elongatus and Temora longicornis) were collected in the coastal waters of the eastern English Channel on June 14, 2006. Immediately after collection adult females were sorted by pipette under a dissecting microscope and acclimated for 2 h in a 2 l beaker containing fresh in situ seawater prior to the behavioral experiments. All subsequent handling of animals was done at 18 °C in a temperature controlled room.

For each species, experiments were conducted with adult females in a temperature controlled dark room (18 °C) to assess their behavioral properties (i) during daylight hours under simulated daylight conditions (daylight experiment, DE), (ii) during the night in the dark (night experiment, NE), (iii) during daylight hours in the dark (simulated night experiment, SNE), and (iv) during the night under simulated daylight conditions (simulated daylight experiment, SDE). Prior to each experiment, ten females were selected from the female stock, transferred in the experimental filming setup (a cubical 3.375 l Plexiglas container, 15 × 15 × 15 cm) filled up with fresh in situ seawater vacuum filtered through Whatman GF/F glass–fiber filters (porosity 0.45 µm) and allowed to acclimatize for 15 min [27,28]. The three-dimensional trajectories of the copepod were recorded at a rate of 25 frame s⁻¹ using two orthogonal, synchronized infrared digital cameras (DVSony DCR-PC120E) facing the experimental container. The various components of the setup were adjusted so that the copepods were adequately resolved and in focus.

During the daylight experiment and the simulated daylight experiment, the test chamber was illuminated with two diffuse cold lights placed 0.5 m above and below the chamber, to ensure homogeneity of the light source and avoid phototropism. This resulted in an illumination of ca. 12 µE m⁻² s⁻¹ in the vessel, approximately equal to the full daylight conditions experienced by the organisms in their natural environment [47,48]. During the simulated night experiment and the night experiment, the only light source was provided by six arrays of 72 infrared light emitting diodes (LEDs), each mounted on a printed circuit board about the size of a business card (i.e. 9.3 cm long and 4.9 cm wide) connected to a 12 volts DC power supply. To account for possible endogenous swimming rhythms, copepods were filmed around midday for the daylight experiment and the simulated night experiment, and around midnight the same day for the night experiment and the simulated daylight experiment and at night the same day for the night-time experiments.
For each experimental condition, ten individual females were recorded swimming for 30 min, after which valid video clips were identified for analysis. Each experimental condition was triplicated. Valid video clips consisted of pathways in which the animals were swimming freely, at least two body lengths away from any chamber’s walls or the surface of the water [27,28]. Selected video clips were captured (DVgate Plus, 25 frames s⁻¹) as MPEG movies and converted into QuickTime TM movies (QuickTime Pro), after which the x, y and z coordinates of swimming pathways were automatically extracted and subsequently combined into a 3D picture using LabTrack software (DiMedia, Kvästgård, Denmark). The time step was always 0.04 s, and output sequences of (x, y, z) coordinates were subsequently used to characterize the motility. Duration of individual observations varied, and swimming paths ranged between 15 s and 4.5 min. However, to ensure the statistical relevance of our analysis paths of the same duration (i.e. 2 min) were selected, and the same number (N = 66) of swimming paths was considered for the behavioral analysis of each species and each treatment. Each behavioral analysis was then based on 198,000 data points.

2.2. Quantitative analysis of swimming behavior

The geometric complexity of swimming trajectories was quantified using fractal analysis. The fractal dimension D was estimated by measuring the length L of a path at various scale values λ. The procedure is analogous to moving a set of dividers of fixed length λ along the path. The estimated length of the path is simply the product of N (number of dividers required to “cover” the path) and the scale factor λ. The number of dividers necessary to cover the object then increases with decreasing measurement scale, giving rise to the power law relationship:

\[ L(\lambda) \propto \lambda^{1-D} \]  

(1)

where L is the measurement scale and \( L(\lambda) \) is the measured length of the path, \( L(\lambda) = N\lambda \). The fractal dimension D is estimated from the slope of the log–log plot of \( L(\lambda) \) versus \( \lambda \).

By analogy with a self-organized critical system that builds up stress and then releases the stress in intermittent pulses, the level of stress arising from each experimental condition was described by a power law, which states that the cumulative probability distribution function of move length L greater than a determined length l follows [49]

\[ N(l \leq L) = kl^{-\phi} \]  

(2)

where the move lengths L correspond to the distances travelled by a copepod every 0.04 s, k is a constant and \( \phi \) the scaling exponent describing the distribution. The exponent \( \phi \) is estimated as the slope of \( N(l \leq L) \) versus \( l \) in log–log plots, and is expected to decline under stress [2,10,11]. Note that Eq. (2) differ from the power law relationship used previously used to identify Lévy flights in a range of marines including microzooplankton [50] and gastropods [51]:

\[ P(l_d = l) = kl^{-\mu} \]  

(3)

where \( l_d \) is the displacement length, l a threshold value, k a constant and \( \mu \) (1 < \( \mu \) ≤ 3) characterize the power law behavior of the tail of the distribution. Note that the move lengths L (Eq. (2)) differ from the flight path lengths \( l_d \) (Eq. (3)) which are defined as sequences of straight-line movements between the points at which significant changes in direction occurred; a significant change in flight direction is deemed to have arisen when the direction of the current flight segment (joining two successive recorded positions) and that of the flight segment immediately following the last deemed change in direction, is more than 90° [52,53].

The distribution of both the fractal dimensions D and scaling exponents \( \phi \) were significantly non-Normal (Kolmogorov–Smirnov test, \( p < 0.01 \)). Non-parametric statistics were then used throughout this work. Multiple comparisons between experimental conditions were conducted using the Kruskal–Wallis test (KW test hereafter), and a subsequent multiple comparison procedure based on the Tukey test was used to identify distinct groups of measurements (Tukey test hereafter).

3. Results and discussion

3.1. Behavioral fractality reveals endogenous rhythms and adaptivity to exogenous cues

The fractal dimension D significantly differed between experimental conditions for the five studied species (KW test, \( p < 0.05 \)). The daytime dimensions (\( D_{\text{DE}} \)) were significantly lower (Tukey test, \( p < 0.05 \)) than those estimated from the night (\( D_{\text{NE}} \)) and simulated night (\( D_{\text{SNE}} \)) experiments (Fig. 1). The fractal dimensions estimated under simulated night conditions did not significantly differ from those estimated under night conditions (\( p > 0.05 \); Fig. 1), hence they were pooled for each species and referred to as \( D_{\text{N}} \) hereafter. Similarly, the fractal dimensions estimated under simulated daytime conditions (\( D_{\text{DSE}} \)) did not significantly differ from those estimated under daytime conditions (\( p > 0.05 \); Fig. 1), hence they were pooled for each species and referred to as \( D_{\text{D}} \) hereafter. More specifically, the fractal dimensions \( D_{\text{D}} \) were statistically separated into two groups under daytime conditions as (i) \( T. longicornis (D_{\text{D}} = 1.10 \pm 0.04), A. clausi (D_{\text{D}} = 1.11 \pm 0.05) \) and \( P. elongatus (D_{\text{D}} = 1.12 \pm 0.04) \), and (ii) \( C. typicus (D_{\text{D}} = 1.21 \pm 0.05) \) and \( P. parvus (D_{\text{D}} = 1.29 \pm 0.05) \). The fractal dimensions \( D_{\text{N}} \) were similarly segregated into two groups as: (i) \( A. clausi (D_{\text{N}} = 1.24 \pm 0.07), T. longicornis (D_{\text{N}} = 1.25 \pm 0.07) \) and \( P. elongatus (D_{\text{N}} = 1.26 \pm 0.06) \), and (ii) \( C. typicus (D_{\text{N}} = 1.38 \pm 0.07) \) and \( P. parvus (D_{\text{N}} = 1.43 \pm 0.07) \).
1.8
1.6
1.4
1.2
1.0 Fractal dimension $D$

A. clausi C. typicus P. elongatus P. parvus T. longicornis

Fig. 1. The fractal dimensions $D$ measured on adult female copepods swimming freely under simulated daylight conditions during daylight hours (white bars) and at night (light grey bars), and under dark conditions at night (black bars) and dark conditions during daylight hours (dark grey bars). The error bars are the standard deviation.

3.2. Endogenous and exogenous rhythms induce stress in copepod behavior

Log–log plots of $N(l \leq L)$ versus $l$ (Eq. (2)) exhibited very strong linear behaviors over the whole range of available move length $l$ (Fig. 2) with coefficient of determination $r^2$ consistently greater than 0.99. The resulting scaling exponent $\phi$ significantly varied between the experimental conditions (KW test, $p < 0.05$). The values of $\phi$ obtained for the daytime...
and night-time experiments were never significantly different ($p > 0.05$; Fig. 3), and were then pooled for each species and referred to as $\phi_{DN}$ hereafter. In contrast, they were both consistently significantly higher than the indistinguishable $\phi$ values estimated under conditions of simulated night during daylight hours and conditions of simulated daytime during the night ($\phi_{SDN}$ hereafter, Fig. 3). The values of $\phi_{ON}$ and $\phi_{SDN}$ significantly differed between the five species studied ($p < 0.05$). More specifically, $\phi_{ON}$ were statistically separated in three groups of decreasing values estimated for (i) $A.\ clausi$ ($\phi_{ON} = 1.81 \pm 0.05$), (ii) $P.\ parvus$ ($\phi_{ON} = 1.74 \pm 0.04$), $C.\ typicus$ ($\phi_{ON} = 1.71 \pm 0.04$), and $T.\ longicornis$ ($\phi_{ON} = 1.69 \pm 0.05$), and (iii) $P.\ elongatus$ ($\phi_{ON} = 1.61 \pm 0.04$). $\phi_{SDN}$ were also separated in three groups of decreasing values which were respectively estimated for (i) $P.\ parvus$ ($\phi_{SDN} = 1.47 \pm 0.04$), $A.\ clausi$ ($\phi_{SDN} = 1.44 \pm 0.05$) and $P.\ elongatus$ ($\phi_{SDN} = 1.43 \pm 0.05$), (ii) $C.\ typicus$ ($\phi_{SDN} = 1.33 \pm 0.03$) and (iii) $T.\ longicornis$ ($\phi_{SDN} = 1.22 \pm 0.04$).

The scaling exponent $\phi$ did not significantly differ for the behavior recorded under daytime and night-time conditions (Fig. 3). This indicates similar levels of stress in copepods under those distinct conditions, and may be considered as reference stress values for each species under experimental conditions matching their endogenous rhythm. In contrast, the clear decrease in $\phi$ observed during daylight hours under simulated night-time conditions (Figs. 2 and 3) and during the night under simulated daytime conditions (Fig. 3) suggests an increase in the stress levels experienced by $A.\ clausi$, $C.\ typicus$, $P.\ elongatus$, $P.\ parvus$ and $T.\ longicornis$. Specifically, the relative differences between $\phi_{ON}$ and $\phi_{SDN}$ were much higher for $T.\ longicornis$ (27.9%), $C.\ typicus$ (22.5%) and $A.\ clausi$ (21.6%) than for $P.\ parvus$ (15.5%) and $P.\ elongatus$ (10.9%). This suggests that the disturbance observations conducted in the dark during daylight hours and in the light at night exerts an endogenous behavioral diel variability is species-specific in copepods. In particular, the present work suggests that $C.\ typicus$, $T.\ longicornis$ and $A.\ clausi$ were the more stressed species.

### 3.3. Methodological considerations

Both the observed increase in the inter-individual variability of the fractal dimensions $D$ and the decrease in the scaling exponents $\phi$ obtained under simulated night-time conditions and simulated daytime conditions compared to those returned by daylight and night experiments question the relevance of behavioral experiments conducted in the dark using IR imagery during daylight hours, or more generally any behavioral study conducted in conditions that hamper the endogenous rhythms discussed above. A direct consequence of the observed decay in the behavioral complexity of copepods observed in the dark during daylight hours is a likely decrease in their foraging efficiency, hence their encounter rates with both preys and sexual partners. In this context, it is strongly advised that future behavioral studies should be conducted under experimental conditions that are representative of the endogenous diel rhythm of the test organisms (i.e. light and observations during daylight hours and night-time) to avoid potential biased results and conclusions.

Note that the exponents $\phi$ returned by the scaling properties of the cumulative probability distribution function of move lengths $L$ (estimated every 0.04 s) greater than a determined length $l$ (Eq. (2)) fundamentally differ from the power law relationship (Eq. (3)) used previously used to identify Lévy flights in the trajectories of e.g. marine invertebrates [50,51]. The flight paths lengths $L_f$ used in Eq. (3) are defined as sequences of straight-line movements between the points at which significant changes in direction occurred; a significant change in flight direction is deemed to have arisen when the direction of the current flight segment (joining two successive recorded positions) and that of the flight segment immediately following the last deemed change in direction, is more than 90° [52,53]. As such, they are used to quantify the space-filling properties of a given trajectory; which can then be used as a way to quantify the foraging efficiency of the related foraging strategy. In contrast, Eq. (2) (and the related exponents $\phi$) quantifies the complexity of the actual successive displacements that occurred along that trajectory, hence carries no information about the efficiency of a search pattern. Instead, Eq. (2) provides information about the internal stochastic structure of the walk performed along a foraging path that may (or may not) be a Lévy flight.
4. Conclusion

The fractal organization of motion behavior optimizes the cost–benefit ratio, as demonstrated by comparing random and fractal search efficiency [69]. It also explains why locomotor activity does not occur randomly in time, but is rather fractal-like [70]. As such, the behavioral approach introduced here to assess zooplankton stress from the scaling properties of the cumulative probability distribution function of move length \( l(N(l \leq L) = k^{-\phi} \) could conveniently be used to assess relative changes in the behavioral complexity of marine invertebrates in a wide range of ecologically relevant situation related to e.g. the quality and the quantity or food, and the presence of mates, predators or contaminants. In particular, as previously demonstrated for several fractal and multifractal measures of behavioral [19,27] and environmental [19,71,72] complexity, the differences between the values of the exponents \( \phi \) observed for a given species or environment under stressful and non-stressful conditions might be more informative on the related behavioral changes rather than the absolute values of \( \phi \). This is consistent with the information related to the relative decrease observed between the \( \phi_{DN} \) and \( \phi_{SDN} \) (see Fig. 3). A similar approach, based on the structure of sequential behavior patterns (e.g. moving versus non-moving) has previously been successfully applied to assess stress in a variety of terrestrial and aquatic vertebrates [1,2,9,10,16–18], and in the copepod Centropages hamatus under conditions of naphthalene contamination [11]. While further work is needed to generalize the use of fractal-based approach to stress assessment in marine invertebrates, the present work opens new perspectives in the understanding of the behavioral ecology of marine invertebrates.

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