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Fractal analysis reveals pernicious stress levels related to boat presence and type in the Indo–Pacific bottlenose dolphin, *Tursiops aduncus*

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ABSTRACT

The stress induced in the Indo–Pacific bottlenose dolphin, *Tursiops aduncus*, by boat presence and type was investigated in a highly urbanized coastal environment, the Port Adelaide River-Barker Inlet Estuary, South Australia. The level of stress experienced by bottlenose dolphins was inferred from the distribution patterns of their dive durations. Dive duration has previously been shown to increase under boat traffic conditions, and is considered as a typical avoidance behavior. Dive durations were opportunistically recorded from land-based stations between January 2008 and October 2010 in the absence of boat traffic, and in the presence of kayaks, inflatable motor boats, powerboats and fishing boats. Subsequent analyses were based on nearly 6000 behavioral observations. No significant differences in dive durations were found between control observations (i.e. absence of boats) and boat interferences, which could erroneously lead to conclude that boat traffic did not induce any stress in *T. aduncus*. In contrast, the scaling exponents of the cumulative probability distribution of dive durations obtained in the absence of boat traffic and under different conditions of boat interferences show (i) that the presence of boats affected the complexity of dive duration patterns and (ii) that stress levels were a function of boat type. Specifically, the complexity of dive duration patterns (estimated by the scaling exponent ϕ) did not significantly differ between control behavioral observations and behavioral observations conducted in the presence of kayaks. A significant increased in behavioral stress (i.e. decreasing values of ϕ) was, however, induced by the presence of fishing boats, motorized inflatable boats and powerboats. This demonstrates that traditional approaches based on the analysis of averaged behavioral metrics may not be sensitive enough to detect changes in the distribution pattern of behavioral sequences, hence underestimate the potential consequences of e.g. chronic exposure to low levels of stress. It is finally emphasized that fractal analyses of behavioral variables, and in particular the analysis of their cumulative probability distribution function, may provide a non-invasive, objective and quantitative framework that can be used to assess the changes in stress response, and subsequently evaluate the welfare status of organisms under various conditions of abiotic and/or biotic stress.

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1. Introduction

The increasing anthropogenic disturbances introduced in the oceans through shipping, military activities, oil and gas exploration, wind farms, and recreation may then interfere with cetaceans, and affect their physiology [1,2], behavior [3,4], and ultimately their fitness and population dynamics [5–7]; see also Refs. [8,9] for reviews. This issue is particularly relevant for cetaceans inhabiting urbanized coastal areas that are increasingly exposed to a variety of potential human disturbances [10]. A range of behavioral changes have hence been observed following exposure to anthropogenic disturbance, including alteration in speed and direction of swimming [11], alteration of behavioral budgets [12], changes in habitat usage [13], changes in vocalization frequency and regularity [14], and modification of breathing frequency [11]. More specifically, small motorized vessels have increased as a source of anthropogenic disturbance due to their rise in popularity [15,16]. This has led to concern over the potential impacts of boat traffic on dolphin behavior [17–19]. Besides physical injury and death [20] and changes in vocalizations presumably to enhance acoustic signal detectability [14,16], the response of dolphins to motorboats varies depending on the type of vessel, the duration of interaction and their relative distance, and dolphin sex [19,21–23]. For instance, slow-moving or immobile vessels do not cause immediate stress on the dolphin community, while fishing vessels are at times chased by dolphins [24,25], and fast moving boats disrupt behavior and social life [18,26,27]. Motorboats consistently elicit, however, a range of behavioral changes including increased swimming speed [28,29], decrease in resting behavior [30], directional changes [19,28,29], decreased inter-animal distance [29,31,32], increased breathing synchrony [17], and longer dive durations [18,21,29,33].

Chronic exposure to even low levels of stress can induce long-term consequences at the population level [5,22]. The understanding and mitigation of the potential impacts posed by boat traffic, hence the identification of potential long-term ramifications, requires an objective qualitative assessment of the behavioral stress of dolphins inhabiting anthropogenically impacted coastal areas. This is critical for dolphin welfare as the habituation to boat traffic reported for bottlenose dolphins [23] did not imply the absence of stress, hence may be thought as a pernicious threat. In addition, behavioral studies in general, and in marine mammal ecology in particular, still heavily rely on standard behavioral metrics such as time allocated to different behavioral sequences, and the related statistical comparisons of mean duration or frequency. This is particularly problematic as those traditional metrics seem much less sensitive to changes in behavioral complexity than fractal analysis [34–37]. Fractal analysis of behavioral sequences is an effective tool for the non-invasive assessment of the general health of wild animals, both invertebrates [34–36] and vertebrates [37–43]; this hence demonstrates the generality of fractal analysis in animal stress and welfare assessments. The related long-range correlations indicate that behavioral sequences do not occur at random (i.e. as a white noise) and result from short-term correlations, but instead result from highly organized complex processes that may generate long-term autocorrelation [36].

In this note, we investigate the complexity pattern of the diving duration in the Indo–Pacific bottlenose dolphin (*Tursiops aduncus*) inhabiting a highly urbanized South Australian estuary, under various conditions of boat traffic, i.e. kayak, motorized inflatable boat, powerboat and fishing boat. It is demonstrated that behavioral complexity consistently decreases in the presence of boats, despite the non-significant changes observed between mean diving durations. The implications of our findings for animal welfare and conservation are briefly discussed.

2. The Indo–Pacific bottlenose dolphin (*Tursiops aduncus*)

The Indo–Pacific bottlenose dolphin (*Tursiops aduncus*; Fig. 1) is a species of bottlenose dolphin (i.e. *Tursiops* sp.), a genus well-known through its appearance in aquarium shows around the globe and the popular television show *Flipper* created in the 1960s. The bottlenose dolphin is also one of the most well known and studied dolphin species [44]. *Tursiops aduncus* inhabits both tropical and temperate marine waters throughout the Indo–Pacific region, occurring around oceanic islands and along continental coastlines [45]. In Australia, this species is distributed around the whole coastline, where it commonly frequents shallow estuarine and coastal waters [46]. Specifically, *T. aduncus* are found in South Australian coastal waters and Gulfs [47], in particular the Port Adelaide River–Barker Inlet estuary, which supports a population of resident individuals [48].

In comparison to other dolphin species the bottlenose dolphin in general is robust, and easily recognized by it relatively short rostrum, hooked dorsal fin and coloration pattern from slate gray to charcoal with a lighter ventral pigmentation [44]. Maximum length of adults can reach up to 2.6 m; however throughout its distributional range considerable size variability between individuals has been noted [49,50]. Bottlenose dolphins are commonly long-lived animals with longevity of up to fifty years [51]. Female Indo–Pacific dolphins are thought to reach sexual maturity between the ages of 12–15 years, with estimates of males reaching sexual maturity between 8–13 years [44]. They are a highly social and complex species, living in a fission–fusion society. Individuals associate in small groups, ranging from approximately five to ten animals [45]. However, group composition regularly fluctuates with associations changing on a hourly to daily basis, or in some cases lasting years [52]. Indo–Pacific bottlenose dolphins generally associate with members of the same sex, with males forming strong alliances and females associating with other females of varying age and kinship [53,54]. Little is known about the Indo–Pacific dolphin's specific dietary habits; however they have been known to feed on a range of prey including various fish species and cephalopods [55].

Fig. 1. An adult Indo–Pacific bottlenose dolphin, *Tursiops aduncus*, about to reach the surface; note the bubbles coming out from the blowhole and indicating exhalation.

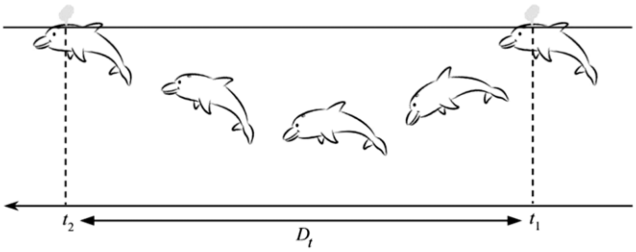

Fig. 2. Schematic representation of the diving duration D_t, defined as the time interval $(t_2 - t_1)$ between two surface blows.

3. Environment, diving duration and stress assessment

3.1. Study site and behavioral observations

The stress potentially induced by boat traffic was assessed as a function of vessel type in a highly urbanized environment, the Port Adelaide River–Barker Inlet Estuary. This estuary, located on the north-eastern side of Gulf St. Vincent, is a sheltered, marine dominated estuary [56] and is considered to have unique conservation significance and commercial value [57]. It is, however, subjective to a range of anthropogenically-driven pollution and disturbances, including the increase in urban development and recreational boating [56]. The related need to develop and implement conservation strategies led this area to be proclaimed the Adelaide Dolphin Sanctuary in June 2005, seeking to protect both the resident dolphins and their habitat. Baseline information on how bottlenose dolphins interact with both the space-time properties of their habitat and anthropogenic activities are still critically lacking [48].

In this context, the present work investigates the influence of vessel type (i.e. kayaks, motorized inflatable boats, powerboats and fishing boats) on the distribution patterns of dive duration in the Indo–Pacific bottlenose dolphin *Tursiops aduncus*. Dive duration has previously been shown to increase under boat traffic conditions, and it is considered as a typical avoidance behavior [18,21,29,33]. Here, we specifically investigate the dive durations D_t as the time intervals between two successive surface exhalations (Fig. 2), while *T. aduncus* individuals were traveling, i.e. moving in a persistent, directional way [58]. Dolphin behavior was observed using binoculars, and dive durations were recorded using a hand held chronometer and internally stored until analysis. Behavioral observations were limited to solitary individuals; control observations were conducted in the absence of any boat on the water, and the potential for boat interactions was investigated when a vessel was within 100 m from a traveling individual. Direct signs of boat avoidance or attraction were never observed. Observations were opportunistically conducted from land-based sites located in the Port Adelaide River–Barker Inlet Estuary from January 2008 to October 2010. All behavioral observations were conducted in areas with a 4 knot speed restrictions to limit the potential bias due to strong discrepancies in boat speed. Note that *T. aduncus* could not be confused with another species of bottlenose dolphins as they are the only species occurring in the Port Adelaide River–Barker Inlet Estuary area [48]. Dive durations were recorded in the absence of boat traffic ($N = 17$), and in the presence of (i) kayaks ($N = 12$), (ii) inflatable motor boats ($N = 5$), (iii) powerboats ($N = 4$) and (iv) fishing boats ($N = 8$). This resulted in a database comprising 1275 behavioral observations conducted in the absence of boat traffic, and 1848, 1172, 801 and 804 data points for behavioral observations respectively conducted in the presence of kayaks, inflatable motor boats, powerboats and fishing boats.

3.2. Dive duration and stress assessment

Previous applications of fractal analysis to behavioral patterns [34–43] have found reduced behavioral complexity under conditions that challenge the animal. Behavioral complexity hence consistently decreases under stressful conditions; this

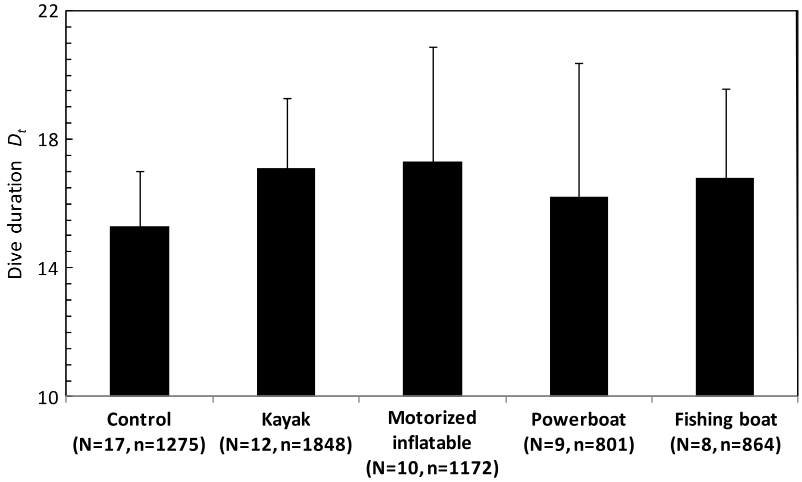

Fig. 3. *Tursiops aduncus* dive duration D_t (seconds) shown for control observations conducted in the absence of boat traffic, and in the presence of different boat types. N is the number of individual *Tursiops aduncus* observed under each condition, and n the total number of dive durations recorded. The error bars are the standard deviations to the mean.

has been observed in the motion behavior of a range of marine invertebrates under short-term exposure to hydrocarbon contamination [34], unnatural light conditions [35], and temperature and salinity stress [37], but also in the locomotor activity of rats intoxicated with tetrachloroethylene [37], the head lifting behavior and feeding vs. non-feeding activity sequences in pregnant and parasitized female Spanish ibex [38,39], the reproductive behavior of fathead minnows exposed to lead [40], the behavior of diseased chimpanzees [41], the activity of food limited chickens [42], the vigilance of juvenile laying hens stressed by mechanical constraints and blood withdrawal [43] and the behavioral activities of pigs under chronic stress treatments [43].

By analogy with self-organized critical systems that build-up and release stress in intermittent pulses [59–61] the level of stress arising in individual dolphins from an exposure to different vessel types was described by the scaling properties of the cumulative probability distribution function of dive duration D_t greater than a determined duration t [34]:

$$P(t \leq D_t) = kt^{-\phi} \tag{1}$$

where k is a constant and ϕ the scaling exponent describing the distribution. The exponent ϕ is estimated as the slope of $P(t \leq D_t)$ versus t in log–log plots, and is expected to decline under stress [34,35,39].

4. Boat types and stress levels in the bottlenose dolphin *Tursiops aduncus*

4.1. Boat types do not influence dive durations

Dive durations were significantly non-normally distributed both in the absence and the presence of boats (Kolmogorov–Smirnov test, $p < 0.01$), and were consistently positively skewed. This indicates the presence of long dives, more often than in the Gaussian case. Dive durations (D_t; Fig. 3) were 15.3 ± 1.7 s($\bar{x} \pm sd$) for control observations ($D_t \in [8.1-93.2]$), and 17.1 ± 2.2 s, 17.3 ± 3.6 s, 16.2 ± 4.2 s and 16.8 ± 2.8 s in the presence of kayaks ($D_t \in [7.3-89.7]$), motorized inflatable boats ($D_t \in [9.2-96.3]$), powerboats ($D_t \in [9.1-97.7]$) and fishing boats ($D_t \in [9.5-92.6]$), respectively (Fig. 3). No significant differences in dive durations were observed between control observations and boat interferences (Kruskal–Wallis test, $p > 0.05$; [62]). For each observational conditions, no significant differences in dive duration were observed between individuals (Kruskal–Wallis test, $p > 0.05$), indicating the absence of any bias related to inter-individual variability in *T. aduncus* behavior. This sharply contrasts with the elevated inter-individual variability observed in invertebrate motion behavior in the absence of any biotic and biotic cues [63], and might suggest the existence of fundamental differences between vertebrate and invertebrate innate behavioral properties. The resolution of this issue is, however, far beyond the scope of the present study, and further work is needed to critically and quantitatively infer the intrinsic differences between innate and acquired behavioral properties of invertebrates and vertebrates.

The increase in dive duration variability from control observations ($sd = 1.7$ s) to observations conducted in the presence of boats (Fig. 3), and the clear increase in variability from kayaks ($sd = 2.2$ s), motorized inflatable boats ($sd = 3.6$ s) and powerboats ($sd = 4.2$ s), with fishing boats having a milder effect ($sd = 2.8$ s) both suggest an increase in inter-individual variability under stressful conditions. However, in the absence of further analysis (as classically observed in the marine mammal literature, e.g. Refs. [18,21,29,33]), the non-significant differences induced by boat presence and type in *T. aduncus* dive durations would erroneously indicate the absence of behavioral impact of boat traffic. While this could be questioned

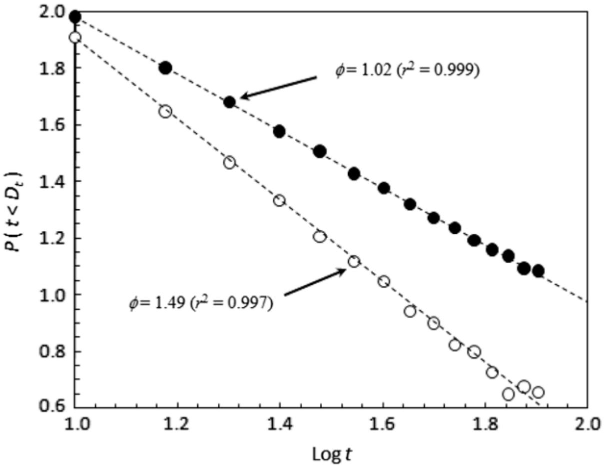

Fig. 4. Log–log plot of the cumulative probability distribution function $P(t \leq D_t)$ of dive duration D_t greater than a determined duration t for bottlenose dolphins *Tursiops aduncus* observed in the absence of boat traffic (open dots) and in the presence of powerboats (black dots).

by the observed increase in dive duration variability (Fig. 3), we will demonstrate hereafter that the patterns of the scaling exponents ϕ that characterize the scaling properties of the cumulative probability distribution function of dive duration D_t provide valuable information about the pernicious effects of boats on *T. aduncus* behavior.

4.2. Boat types do influence dive duration pattern

The highly significant linear behavior of the log–log plots of $P(t \leq D_t)$ versus t (Eq. (1); Fig. 4) indicates that the cumulative frequency distribution of dive duration is compatible with a power-law behavior, hence an underlying fractal structure. Note that after randomization of data, the distributions were better fitted by an exponential distribution than by a power-law ($p < 0.01$). The power-law behaviors shown in Fig. 4 imply that the diving pattern of bottlenose dolphins is far from Gaussian. As a consequence, comparisons of experiments with different durations using mean values of behavioral metrics (e.g. dive duration, distance traveled) that have a fractal structure are unlikely to be meaningful, because those mean values intrinsically depend on the duration of the experiment.

In contrast to dive durations, the scaling exponent ϕ exhibits a clear pattern as a function of observational conditions (Fig. 5). An analysis of covariance [62] indicates the presence of significant differences between the exponents ϕ ($p < 0.01$). A subsequent multiple comparison procedure based on the Tukey test [62] to determine which ϕ differ from the others showed that (i) the exponent ϕ obtained in the absence of boats ($\phi = 1.49 \pm 0.06$) and in the presence of kayaks ($\phi = 1.44 \pm 0.07$) could not be distinguished and were significantly higher than those obtained for the other observational conditions. The exponent ϕ obtained in the presence of fishing boats ($\phi = 1.36 \pm 0.05$), motorized inflatable boats ($\phi = 1.21 \pm 0.05$) and powerboats ($\phi = 1.02 \pm 0.03$) all significantly differ from each other ($p < 0.01$). This indicates that the presence of kayaks did not modify the distribution pattern of diving duration in *T. aduncus*, hence the related stress is likely to be minimal. In contrast, the presence of motorized inflatable boats, powerboats and fishing boats consistently significantly decrease the exponent ϕ of the cumulative probability distribution function of dive duration D_t greater than a determined duration t, i.e. $P(t \leq D_t) = kt^{-\phi}$. More specifically, the significant gradual decrease in the exponent ϕ observed in the presence of fishing boats, motorized inflatable boats and powerboats indicates an increase in the related stress experienced by bottlenose dolphins.

The results are consistent with previous observations showing a decrease in the complexity of animal behavioral display under stressful conditions, e.g. when individuals are diseased or parasitized, or in a polluted environment [34–43]. More specifically, the values of the exponent ϕ found in the present work are substantially higher than those found for non-parasitized (i.e. not stressed; $\phi_{NS} = 0.892 \pm 0.04$) and parasitized (i.e. stressed; $\phi_S = 0.701 \pm 0.05$) Spanish ibex females [39]. However, the relative differences between the exponents found for not stressed and stressed animals, expressed as the stress ratio r_{stress} ($r_{stress} = (\phi_{NS} - \phi_S)/\phi_{NS}$) were in the same range of values for Spanish ibex ($r_{stress} = 0.27$) and bottlenose dolphin *T. truncatus* ($r_{stress} \in [0.14 - 0.46]$). Similar conclusions can be drawn from a recent work assessing the stress levels induced by light conditions on five species of marine copepods (i.e. millimeter-scale free-swimming crustaceans) motion behavior [35]. The exponents ϕ characterizing stressed copepods ($\phi_S \in [1.22 - 1.47]$) and unstressed copepods ($\phi_{NS} \in [1.61 - 1.81]$) also differ from values found for stressed ($\phi_S \in [1.02 - 1.31]$) and unstressed ($\phi_{NS} \in [1.44 - 1.49]$) dolphins. However, the stress ratio found for copepods ($r_{stress} \in [0.11 - 0.28]$) are consistent with our findings, i.e. $r_{stress} \in [0.14 - 0.46]$. While further work would be needed to confirm this hypothesis, this suggests that (i) the absolute values of the exponent ϕ may be specific to the type of organisms under consideration, and (ii) the relative differences in the exponent ϕ between stressed and unstressed organisms might exhibit some sort of universality.

More generally, in response to stress, the organism typically performs a series of compensatory responses to improve the probability of survival, involving the endocrine system and behavior. For instance, decreasing fractal dimensions have

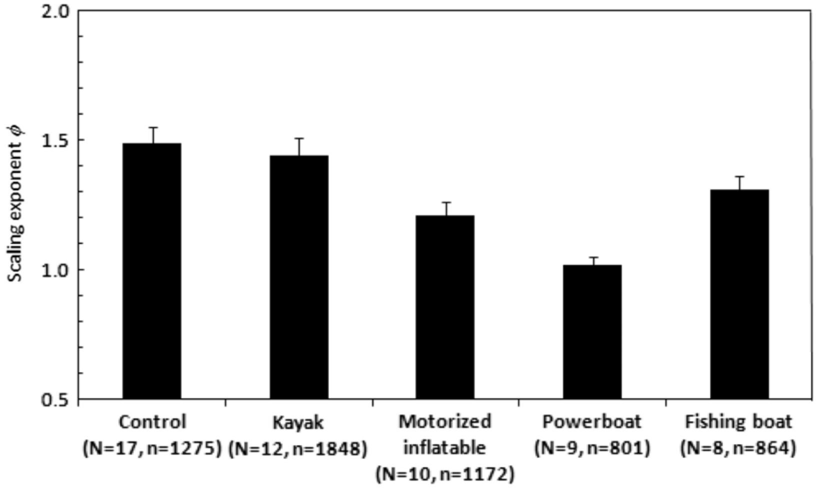

Fig. 5. The scaling exponent ϕ estimated from the dive durations of *Tursiops aduncus* recorded under control conditions in the absence of boat traffic, and in the presence of different boat types. N is the number of individual *Tursiops aduncus* observed under each condition, and n the total number of dive durations recorded. The error bars are the standard deviations to the mean.

been found in cattle's physiological responses to thermal stress [64]. The related loss of complexity observed here in the distribution pattern of dive duration under condition of boat traffic then results from a reduction in the energy balance of dolphins that is similar to a diseased animal [41] or human [65]. This is consistent with a loss of complexity appearing to be a general characteristic of stress in invertebrates [34–36], a range of vertebrates [36–43,66], and humans [60,67–69]. In summary, the change in pattern distribution of behavior sequences is related to the underlying dynamics of physiological components and can confidently be used to objectively detect and quantify the level of stress experienced by animals under different environmental conditions.

5. Conclusion

The use of fractal analysis in human health has a relatively long lasting history [65]. For instance, fractal analysis has been suggested to aid the assessment of cardiac risk and help forecast sudden cardiac death in humans, but also in the assessment of other regulatory systems, such as human gait control in health and disease [68]. The use of fractal analyses as a tool to measure the complexity of animal behavior is, however, still emerging [34–36,41–43,70]. Here, we show how the analysis of the dive duration distribution pattern in the Indo–Pacific bottlenose dolphin *Tursiops aduncus* provide valuable information that would not be available using conventional behavioral analyses. Namely, a standard behavioral approach based on a comparison of mean diving durations (classically used in the marine mammal literature, e.g. Refs. [18,21,29,33]) would have erroneously concluded to a lack of impact of boat presence and boat type. However, the consideration of the properties of the cumulative probability distribution function of dive duration D_t greater than a determined duration t, i.e. $P(t \leq D_t) = kt^{-\phi}$ demonstrate (i) that dolphin behavior is influenced by the presence of vessels, and (ii) that dolphin stress levels are a function of boat type.

The approach presented here has potential ramifications in both animal welfare and the related development and implementation of conservation strategies. This is particularly relevant for marine mammals living in coastal environments increasingly impacted by anthropogenic activities as regular and prolonged exposure to even very low stress levels may have long-term implications [5,13,22,70]. In this context, fractal analysis in general, and the analysis of the cumulative probability distribution function of a behavioral property may provide a non-invasive, reliable, sensitive, objective and quantitative framework that can be used to assess the changes in stress response, and subsequently evaluate the welfare status of organisms under various conditions of abiotic and/or biotic stress.

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