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What Is the Impact of Dexamethasone and Prednisolone Glucocorticoids on the Structure of Meiobenthic Nematode Communities?

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Abstract: The toxic effects of two commonly used glucocorticoids, the dexamethasone and prednisolone, on meiobenthic nematodes were assessed in a laboratory experiment for 30 days. Nine treatments were employed, comprised of two single and mixed concentrations of dexamethasone and prednisolone (i.e., 0.14 and 1.4 $\mu\text{g}\cdot\text{L}^{-1}$). The exposure to both glucocorticoids showed significant effects on the abundance and taxonomic diversity of nematodes. Such changes were mainly induced by the decrease in the abundance of the species *Microlaimus honestus*, considered to be sensitive to prednisolone and by the increase in *Enoplolaimus longicaudatus* abundance, which can be considered tolerant. The exposure to these glucocorticoids also led to a decrease in 2A feeding groups, 2–4 mm body-size interval, and c-p3 life history type in most treatments, with type of life history and shape of amphids as the most relevant functional traits impacted by these two glucocorticoids. The results could also be explained by the potential antagonism between these two pharmaceuticals.

Keywords: glucocorticoids; meiobenthic nematodes; diversity; functional traits



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1. Introduction

The pharmaceuticals released in aquatic ecosystems are consumed by wildlife, bioaccumulated and further transferred through food webs [1–4]. This environmental issue associated with potential toxic effects on aquatic biota has a long history of research [5,6]. However, currently, little is known about the fate and toxicological effects of synthetic glucocorticoids on aquatic habitats and biota. These drugs are used in the treatment of various autoimmune and inflammatory diseases [7] but are frequently excreted in the environment without undergoing physiological transformations into inactive metabolites [8]. Moreover, most glucocorticoids reach the aquatic habitats through wastewater effluents [9–11].

The prednisolone is one the most frequently consumed glucocorticoids [12], reaching mean concentrations in wastewaters within the range of $\text{ng}\cdot\text{L}^{-1}$. However, concentrations of prednisolone in the range of $\mu\text{g}\cdot\text{L}^{-1}$ in wastewater effluents from the hospital and industrial areas were also recorded [11]. Previous studies showed that prednisolone had deleterious effects on the physiological processes during the ontogenetic development of zebra fish larvae (*Danio rerio*, see [13]) or on the number of leukocytes from the adults of *Pimephales promelas* even at low concentrations as 1 $\mu\text{g}\cdot\text{L}^{-1}$ [14].

The dexamethasone is another glucocorticoid used primarily as an anti-inflammatory drug [15]. Ref. [16] proved that the dexamethasone induced developmental abnormalities in zebra fish, but the toxic effects of exposure in the range of $\text{ng}\cdot\text{L}^{-1}$ on aquatic habitats and biota are practically unknown, requiring additional investigations [11].

The current study investigated the toxicological effects of glucocorticoids on meiobenthic taxa, a crucial component of the 'small food web' (i.e., protists, bacteria and meiofauna). Thus, a closed microcosm study focused on the response of free-living marine nematodes following contamination with prednisolone and dexamethasone was carried in laboratory. The free-living marine nematodes are routinely used as reliable bioindicators in ecotoxicology [17–20]. Their small size and short life cycle, associated with their ease of laboratory maintenance, makes them ideal organisms in toxicology experiments [21,22]. Outcomes from our bioassay will allow us to identify tolerant nematodes towards glucocorticoids. To reach this goal, many factors should be considered, namely (1) the buccal armature affecting directly the manner of catching and the type of preys consumed, but also (2) other factors such as the body size and shapes of the organs involved in the feeding process like chemodetection efficacy and locomotion easiness for searching food items. The identification of tolerant morpho-functional groups is necessary for further applications where these nematodes will be reused not for bioindication purposes but as bioremediators in sustainable development projects. This aims to neutralize the harmful effects of pharmaceutical pollution in marine areas and to avoid its transmission to future generations.

2. Material and Methods

2.1. Collecting Site and Sediment Manipulation

Sediments were collected on the 14 August 2019 (7 a.m.) from a subtidal pristine beach in Bizerte Bay, Tunisia ($37^{\circ}15'07.34''$ N, $9^{\circ}56'26.75''$ E). The sediment was collected at 50 cm below water surface with several hand cores (surface of 10 cm^2 , inner diameter 3.6 cm); just the first 5 cm of sediment was sampled. The sediment collection was restricted to the upper 5 cm layer because more than 90% of the meiobenthos live in this microhabitat [21]. The collected sediments were stored in dark at constant temperature ($29\text{ }^{\circ}\text{C}$) for three days for acclimatization. The ambient temperature was inferred from meteorological data (<http://www.infoclimat.fr>; last accessed on 14 August 2019) of the previous month (14 July to 14 August 2019).

2.2. Sediment Contamination and Experimental Set-Up

Stock solutions of prednisolone and dexamethasone purchased from Sigma-Aldrich (Saint-Louis, MI, USA) were prepared by dissolution in filtered seawater ($0.7\text{ }\mu\text{m}$ pore-size Glas Microfibre GF/F (Dutscher, Issy-les-Moulineaux, France). The glucocorticoids thus dissolved were gently mixed into the sediment with a food mixer during the first day of the experimental period with a pace of 10 min at the beginning of each hour [21]. The concentrations used in the current experiment (i.e., 0.14 and $1.4\text{ }\mu\text{g}\cdot\text{L}^{-1}$) were derived from the minimum $\text{LC}_{50\text{s}}/24\text{ h}$ of the rotifer *Brachiorus calyciflorus* (i.e., 20.82 and $41.37\text{ mg}\cdot\text{L}^{-1}$ for prednisolone and dexamethasone, respectively, see [23]). These concentrations were first divided by 30, which is the number of days for the current experiment, leading to 0.694 and 1.379 (~ 1.4) $\text{mg}\cdot\text{L}^{-1}$, respectively. Given the higher toxicity of prednisolone compared to dexamethasone for *B. calyciflorus* [23], a preliminary experiment was set by using four concentrations: 0.14 , 1.4 , 14 , 140 , and $1400\text{ }\mu\text{g}\cdot\text{L}^{-1}$, respectively. Given that the sediment contaminated with 0.14 , 1.4 and $14\text{ }\mu\text{g}\cdot\text{L}^{-1}$ with prednisolone comprised meiofauna, the preliminary investigations were repeated, this time by spiking the sediment populated by a similar meiobenthic community with prednisolone and dexamethasone. This way, two final concentrations of 1.4 and $0.14\text{ }\mu\text{g}\cdot\text{L}^{-1}$ for both glucocorticoids were obtained, representing realistic values that are to be found in nature. [24] detected prednisolone and dexamethasone at concentrations of 1918 and $90\text{ ng}\cdot\text{L}^{-1}$, respectively, supporting the realistic nature of the chosen concentrations for the current experiment.

Overall, 27 microcosms were used ($n = 3$ per each type of treatment), as follows: one control set, two sets contaminated with prednisolone (hereafter P1 and P2) and two others with dexamethasone (hereafter D1 and D2). Finally, four sets were contaminated with mixtures of both employed concentrations of prednisolone and dexamethasone (hereafter P1D1, P1D2, P2D1, and P2D2).

The experimental microcosms were comprised of glass bottles (2 L) filled with 300 g of homogenized natural sediment and one liter of 40 μm pre-filtered (29 PSU) water, contaminated or not. Throughout the experiment, each microcosm was constantly aerated with an aquarium pump.

2.3. Structural and Functional Traits of Nematode Communities

Two stacked sieves of 1 mm and 40 μm mesh size, respectively [25], were used for collecting nematodes from sediment, followed by their fixation in 4% formalin solution [26] and staining with Rose-Bengal (0.2 $\text{g}\cdot\text{L}^{-1}$) [27]. Then, 100 individuals were randomly picked from each treatment under a dissecting microscope, transferred in 21% glycerol and mounted on microscope slides for taxonomic identification based on morphological features [28]. For taxonomic identification to genus and species levels, the keys of Platt and Warwick [29,30] and Warwick et al. [31] and the Nemys database developed and updated by nematologists at Ghent University [32], respectively, were used.

The appurtenance to trophic groups, tail and amphidial fovea shapes, as well as to type of life strategy, was established for each genus. The classification of amphids was based on the shape of fovea: circular (cr), spiral (sp), pocket (pk) or indistinct (id) (see [33]). The shape of tails was classified as conical (co), clavate/conico-cylindrical (cla), short/round (s/r) or elongated/filiform (e/f) [34]. The types of feeding group considered were epigrowth (2A), selective deposit (1A), nonselective deposit feeders (1B) and omnivores/predators (2B), based on the characteristics of the mouth opening [35]. The type of life strategy was ranked on a c-p scale as follows: c-p = 1 (i.e., short life cycle, high reproductive rates, tolerant to stress) to c-p = 5 (i.e., long life-cycles, low reproductive output, and sensitive to stress), analogous to the K/r-strategists following [36,37].

2.4. Data Processing

The abundance of nematodes (N), number of species (S), Margalef's species richness (d), Shannon–Wiener index (H'), and evenness (Pielou) (J') were calculated in PRIMER 5. [38,39]. Kolmogorov–Smirnov and Bartlett tests were applied to $\log_{10}(x + 1)$ transformed data [38,40]. One-way ANOVA, followed by Tukey's HSD tests, were used to check for the overall and subsequent multiple comparisons among control and treatments with STATISTICA (v5.1). Square root transformed abundance data and the relative abundance of their functional traits, based on Bray–Curtis similarity measures, were used for nonmetric multidimensional scaling (nMDS, see [41]). SIMPER (i.e., similarity percentage analysis, see [38]) was used afterwards to assess the contribution of species and of their functional traits to the overall average dissimilarity among treatments.

3. Results

3.1. Taxonomic Composition

The initial nematofauna comprised eight orders, 19 families, 24 genera and 26 species. The most diverse families were Xyalidae and Oncholaimidae (Table A1).

At the beginning of the experiment, the nematode community was dominated by *Oncholaimus campylocercoïdes* (19.3 \pm 2.5%) and *Microlaimus honestus* (15 \pm 2%), the other species comprising each less than 10%. By the end of the experiment, the control community was still dominated by *O. campylocercoïdes* (18.6 \pm 4.7%) and *M. honestus* (14.6 \pm 3.5%). The nematode *O. campylocercoïdes* dominated the communities from all microcosms by the end of the experiment. However, the nematodes *Enoplolaimus longicaudatus* represented 15.08 \pm 3.8% and 14.01 \pm 3.4% in P1 and P2, respectively. *Cyartonea germanicum* and *Microlaimus cyatholaimoides* represented 11.3 \pm 1.5% and 11.3 \pm 4.9%, respectively, in D1 and

Trichostrongylus mirabilis $11.3 \pm 1.5\%$ in D2. *E. longicaudatus* ($12.6 \pm 1.9\%$ in P1D1; $13.2 \pm 0.8\%$ in P2D2; $16.7 \pm 0.5\%$ in P1D2) and *T. mirabilis* ($12.5 \pm 4.7\%$ in P1D1, $14.7 \pm 5.8\%$ in P2D2 and $11.6 \pm 4.1\%$ in P1D2) were the co-dominant species in these microcosms. Finally, *M. honestus* represented $13.91 \pm 4.15\%$ in P2D1 (Table A1).

3.2. Abundance and Diversity

The mean abundance of nematodes ranged from 3114 ± 88 (I) and 2906 ± 72 (Utc) individuals in I to 78 ± 7 individuals in P2D2 (Figure 1). Overall, the abundance of nematodes in all types of treatments were significantly lower compared to control ($p < 0.001$, pairwise Tukey-HSD post hoc tests, see Figure 1).

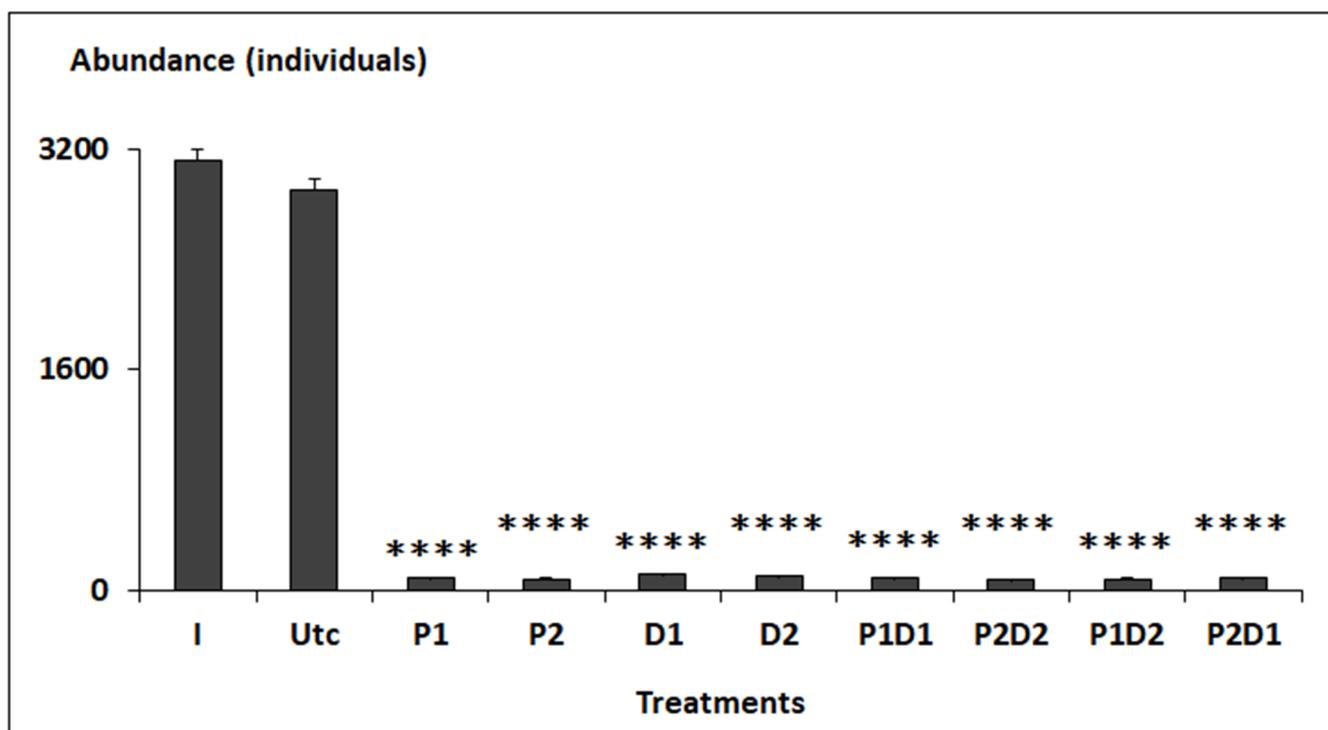


Figure 1. Abundances of nematodes from the initial assemblage (I), and those from uncontaminated (Utc) and contaminated (P1, P2, D1, D2, P1D1, P1D2, P2D1, and P2D2) microcosms. P = prednisolone, D = dexamethasone. Stars above bars indicate significant differences in comparison to the corresponding controls (\log -transformed data) using Tukey's test: $p < 0.0001$ (****).

The initial diversity significantly decreased in most treatments during the experiment, excepting the control and P1 (Figure 2). At the end of the experiment, the diversity of control community was significantly higher compared to other treatments, excepting P1 and P2 (i.e., P1 vs. PD1, P2D2 and P1D2, as well as between P2 vs. P1D1 and P1D2). The Margalef's species richness (d) showed significant differences among treatments, excepting the final control, P1 and P2 (i.e., P1 vs. P1D1 and P1D2, as well as P2 vs. P1D1 and P1D2, see Figure 2). However, the Shannon–Wiener (H'), and Pielou's evenness (J') indices were similar among all experimental microcosms.

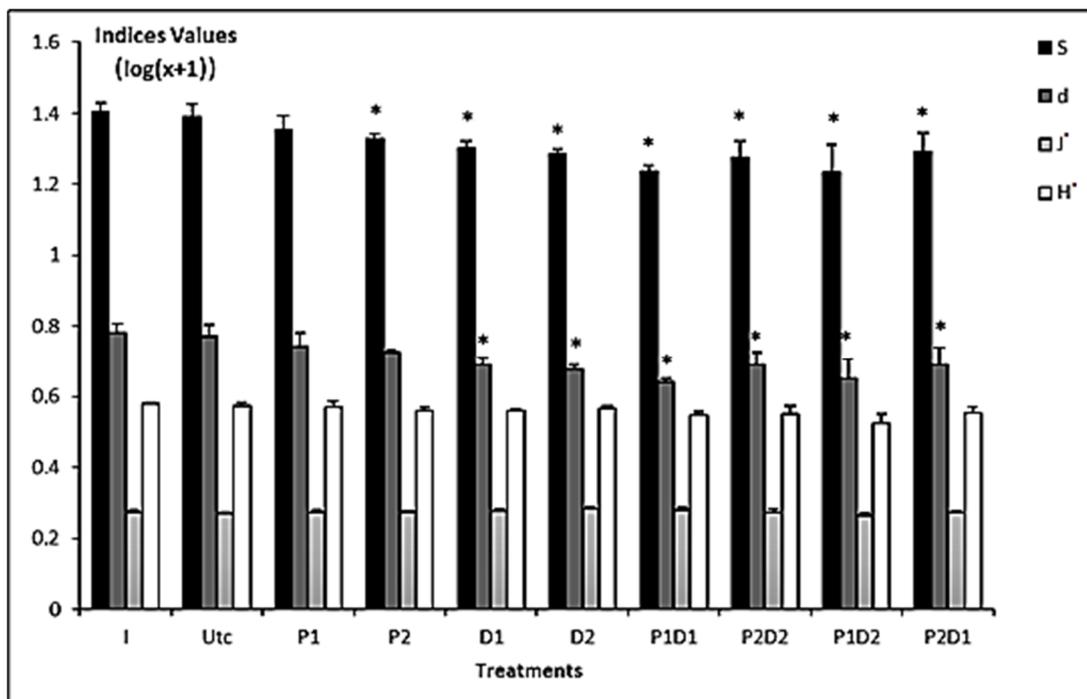


Figure 2. Graphical summary of univariate indices for nematofauna from the initial assemblage (I), and those from uncontaminated (Utc) and contaminated (P1; P2; D1; D2; P1D1; P1D2; P2D1; P2D2) microcosms. P = prednisolone, D = dexamethasone, H' = Shannon–Wiener index, d = species richness, J' = evenness, S = number of species. Stars above bars indicate significant differences in comparison to the corresponding controls (log-transformed data) using Tukey’s test: $p < 0.05$ (*).

3.3. Multivariate Analyses

The nMDS ordination showed a clear differentiation among treatments (stress = 0.17, Figure 3). The initial and final control communities were situated very close in the ordination space. The P and D treatments were fully separated from control microcosms, along with their mixtures, except for P2D1 (Figure 3).

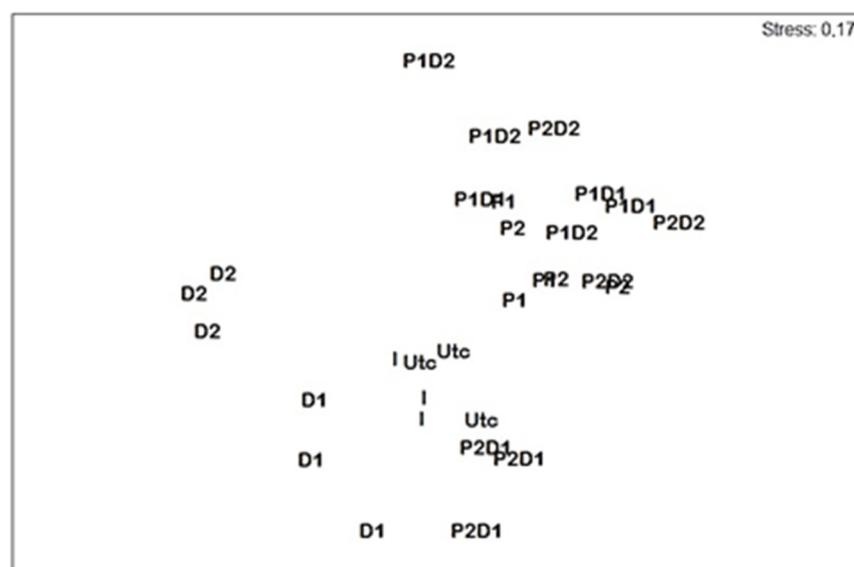


Figure 3. Nonmetric multidimensional scaling (nMDS) 2D plot based on nematode species abundances from the initial assemblage (I), and those uncontaminated (Utc) and contaminated (P1; P2; D1; D2; P1D1; P1D2; P2D1; P2D2) microcosms. P = prednisolone, D = dexamethasone.

The average dissimilarity was high between the initial and final control nematode communities and other treatments (Table A2). The lowest dissimilarity was between initial and final control (25.1%) and the highest between initial control and P2D2 (48.3%). Despite the overall increase in dissimilarity, the mixture P2D1 showed very low dissimilarity with the initial (30.7%) and final control (27.2%) communities, respectively. The SIMPER results indicated that the dissimilarity between the initial and the final control community was mostly due to the decrease in the *M. honestus* abundance. Moreover, the increase in the abundance of *T. mirabilis* contributed to the dissimilarity observed with the initial community. The fluctuation among treatments of *O. campylocercooides* abundance also contributed to the average dissimilarity.

3.4. Functional Traits

The functional categories within and among treatments communities followed the following patterns (Figure 4):

- The feeding groups of the initial communities were dominated by omnivorous/carnivores (2B) and epistratum-feeders (2A), comprising $36.3 \pm 3.05\%$ and $32.3 \pm 2.3\%$ of the nematofauna. The 1B group significantly increased in final control, D2, P1D1 and P2D2, respectively. Conversely, the 2A group decreased in P, P2, D2, P1D1, P2D2 and P1D2, whereas the 2B group increased in D1 and P2D1. The nMDS results indicated that D2 and P2D2 groups were furthest from initial control. Conversely, the treatments D1 and P2D1 were situated closer to initial and final control, respectively.
- Amphid shapes of the initial community were dominated by circular (cr) and pocket-like (pk) amphids, comprising $47 \pm 1.7\%$ and $32.3 \pm 3.5\%$ of the nematofauna. The cr amphid shape types decreased in P2, D1, P2D2 and P1D2, whereas the id amphid shape significantly increased in P1, P2, P1D1, P2D2, and P1D2. The pk amphid shape significantly increased in P1, D1, P1D1, P2D2, and P2D1. The nMDS results indicated that P1D2 was furthest away from initial control, but P2D1 and final were the closest to initial control.
- Tail shapes were dominated by conical (co) and clavate (cla) types, comprising $47.3 \pm 1.1\%$ and $46 \pm 3.4\%$ of the initial nematofauna, respectively. The contamination induced a significant decrease of co tail shapes in P2, P2D2 and P1D2, as well as of cla tails shape in D1. The co tail shape increased significantly in D1. The nMDS results indicated that D1 was situated the furthest from initial control, but treatments P1 and P1D1 were the closest.
- The initial life history composition was c-p4, followed by c-p3 and c-p2, comprising $41.6 \pm 3.7\%$, $26.3 \pm 0.5\%$ and $25 \pm 4.58\%$ of the nematofauna, respectively. The c-p2 types increased significantly in most treatments, excepting P1D2 and P2D2, as well as c-p5 in P1, P2 and P1D2. Conversely, the results showed a significant decrease of c-p3 in most treatments, except for final control, D1 and P2D1, and of c-p4 in most treatments except for final control and D1. The nMDS results indicated that P1D2 and P2D2 were situated the furthest from initial control, whereas the treatments P2D1 and final control were the closest.
- Body sizes were dominated by 2–4 mm and 1–2 mm species, comprising $57.6 \pm 3.2\%$ and $23.6 \pm 2.5\%$ of the initial nematofauna. The species smaller than 1 mm increased in D1, same for those between 1 and 2 mm interval in D2 and P1D1. Conversely, the species with body sizes between 2 and 4 mm intervals decreased in D1, D2, P1D1 and P2D2. The nMDS ordination indicated that D2 was situated the furthest from initial control, whereas D1 was the closest.

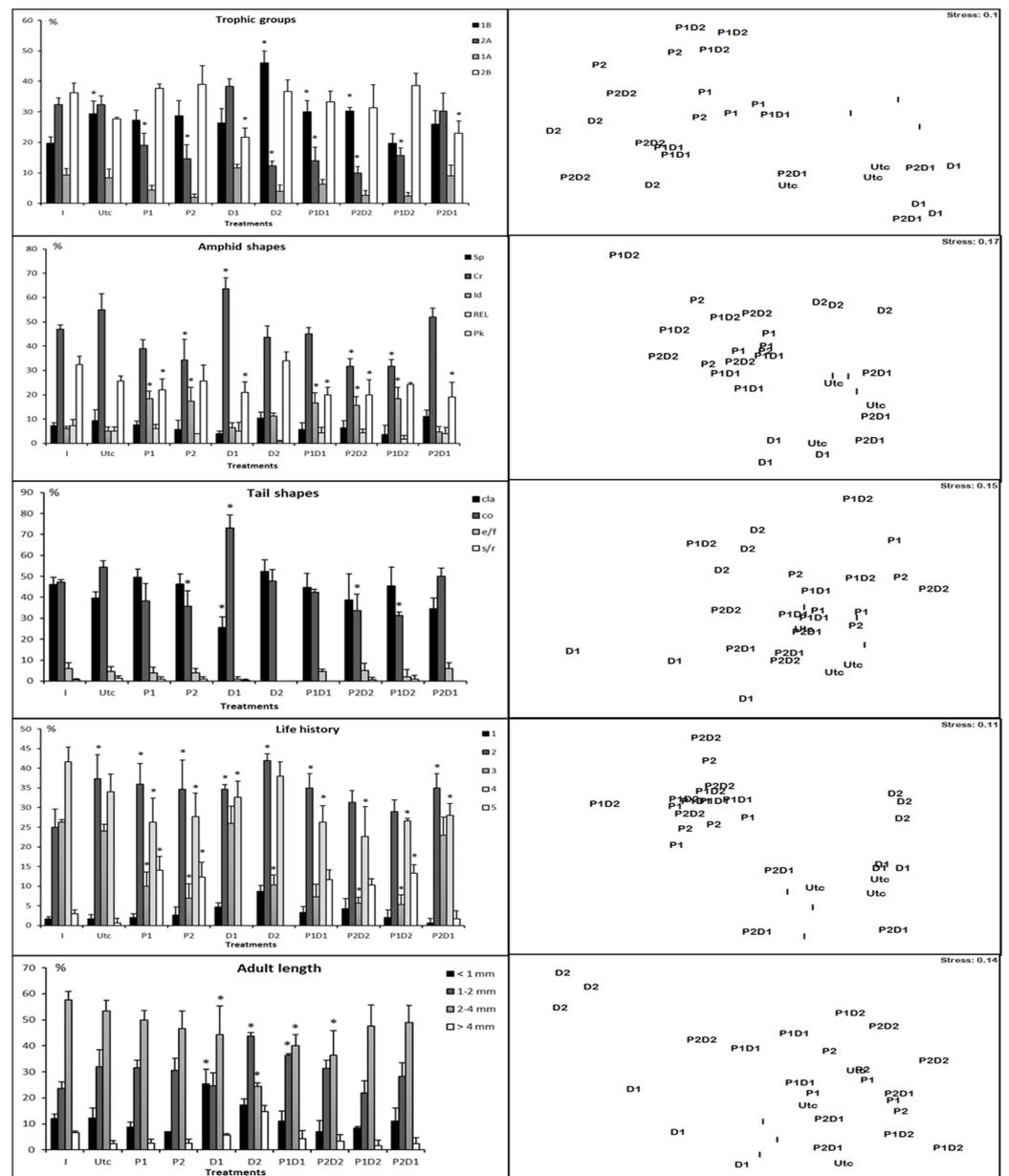


Figure 4. Nonmetric multidimensional scaling (nMDS) 2D plots (left) and graphical summary (right) based on abundances of functional groups of nematode assemblages from each microcosm. P = prednisolone, D = dexamethasone. Selective deposit feeders (1A); nonselective deposit feeders (1B); epigrowth feeders (2A); omnivores–carnivores (2B); short/round (s/r); elongated/filiform (e/f); conical (co); clavate/conical-cylindrical (cla); spiral (sp); pocket-like (pk); indistinct (id); circular (cr). Stars indicate significant differences with the initial nematofauna (*).

The dissimilarity values of all functional traits were lower than 30% compared to initial conditions, except for that of adults length in D2 (Table A2). SIMPER results highlighted significant decreases of 2A and 2B feeding groups, with 2–4 mm body-size interval, and of c-p3 life history type in most treatments compared to the initial conditions. Moreover, a significant modification of the co and cla tail shapes and cr and pk amphid shapes, respectively, were observed in treatments compared to initial conditions, but similar to final control.

The nMDS second-stage ordination showed that the responses of nematodes to different treatments depended mainly on life history (86.71%), amphid shape (84.17%), adult body size (81%) and trophic groups (80.12%) (Figure 5).

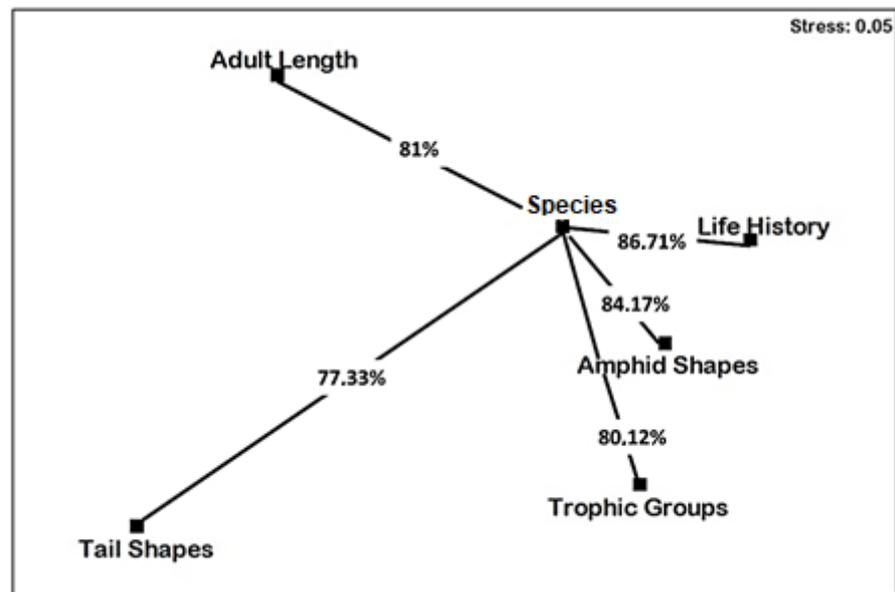


Figure 5. Nonmetric multidimensional scaling (nMDS) second-stage ordination of intermatrix rank correlations. For matrices included. See Figure 3 (species) and Figure 4 (functional traits). Values indicate average similarity percentages between nMDS related to species and those related to functional traits.

4. Discussion

The knowledge of ecotoxicological effects of glucocorticoids in aquatic habitats, including the marine milieu, is scarce. The main objective of this experiment was to partially cover this knowledge gap by assessing the impact of prednisolone and dexamethasone on free-living marine nematodes. As such, several community-based indices were quantified following exposure of this phylogenetic group to various single and combined concentrations of these two glucocorticoids.

The decrease in the abundance of nematodes following their exposure to both dexamethasone and prednisolone, as well as to their mixtures, was in line with previous findings who assessed similar responses of nematofauna following exposure to hydrocarbons [42], heavy metals [43], and pesticides [44].

The initial community was dominated by *O. campylocercoides* ($19.3 \pm 2.5\%$) and *M. honestus* ($15 \pm 2\%$), respectively, by the end of the experiment and was similar after 30 days, indicating that the control community did not change during the experiment, assuring the representativeness of multiple comparisons with other treatments.

The contamination with prednisolone showed significant changes in diversity of nematofauna only for the highest concentration, P2. According to SIMPER results, the dissimilarity between the initial and final control and P2 treatments was mainly due to the disappearance of *M. honestus* and increased abundance of *E. longicaudatus*, suggesting sensitivity for the former and tolerance for the latter species to prednisolone. This pattern was also paralleled by changes in the composition of functional traits, showing a decrease in 2A feeding group frequency, cr amphid shapes and co tail shapes, but an increase in 2B feeding groups and id amphid shapes. A previous study suggested low acute toxicity of prednisolone, due to its photo-degradation byproducts [23]. Such byproducts may have occurred during the 30 days exposure and are potentially responsible for the decrease in abundance of intermediate size species (i.e., 1–4 mm) and the disappearance of *M. honestus*.

The contamination with both dosages of dexamethasone showed significant effects on the initial and control communities, suggesting higher toxicity compared to prednisolone. Additionally, according to SIMPER results, the dexamethasone impacted differently the initial nematofauna, by inducing a decrease in *O. campylocercoides* and an increase in *Microaimus cyatholaimoides* abundances in D1 treatment, whereas in D2, the composition switch was due to the decrease in *M. honestus* and increase in *Trichotheistus mirabilis* abundances.

Furthermore, the SIMPER results highlighted the contribution to the dissimilarity with final control community of *Nudora gerlachi* and *Metoncholaimus pristiurus* in D1 and D2, respectively. Despite the significant effect of dexamethasone on diversity indices, the nMDS ordination placed the D1 and D2 treatments close to the initial and final control microcosms, indicating a smaller change in the overall community composition compared to other treatments. Little is known about the fate and toxicity of dexamethasone on aquatic invertebrates, including meiofauna. To our knowledge, previous studies reported that the exposure to dexamethasone led to early hatching of zebra fish embryos [45]—impacting the overall ontogenetic development [46]—as well as to reducing *C. dubia* population growth by 50% at concentrations of 0.05 mg·L⁻¹ [23].

All types of mixtures had a significant impact on nematofauna compared to initial and final control microcosms. However, the mixtures with the highest concentrations of prednisolone showed less effect on Margalef's index, especially P2D1. Furthermore, according to SIMPER results, P2D1 recorded the lowest dissimilarity with the initial and control communities. The other mixtures led to higher dissimilarities compared to single glucocorticoid contamination. The species responsible for the average dissimilarity in P2D1 were the decreased abundances of *T. mirabilis* and *O. campyloceroïdes*, but with different rates in different mixtures and also due to the elimination of *M. honestus* and increased abundance of *E. longicaudatus*. The same conclusion is applicable for the functional traits, as reflected in the nMDS ordination of the P2D1 cluster, which was close to the initial and final control communities. The nMDS ordination and the opposite trends of the relative abundances of *M. honestus* and *E. longicaudatus* following exposure to the mixtures P1D1, P2D2, P1D2, and P2D1 support a potential antagonist interaction between glucocorticoids, as well as potentially higher toxicity of dexamethasone compared to prednisolone for the nematodes. The latter speculation seems also to be supported by a less severe impact on the nematofauna in P2D1 compared to P1D2.

Besides community-based indices, the functional traits also differed among treatments. The initial community was dominated by c-p4 species throughout the experiment in control microcosms. However, the emergence of c-p2 species was noticeable by the end of the experiment in control microcosms, slightly exceeding that of c-p4 species. The contamination induced a significant increase of c-p5 species, except for D1, D2 and P2D1 treatments, respectively. The only species belonging to c-p5 type in the current study was *E. longicaudatus*. This species has K type reproductive strategy and thrived in most treatments, excepting those contaminated only with D. These results suggest a potential higher tolerance of *E. longicaudatus* to prednisolone, but sensitivity to dexamethasone. Thus, this species of nematode could be considered as a positive bioindicator for prednisolone pollution and negative for dexamethasone.

The biomonitoring of marine areas is a controversial topic but necessary in establishing blue economy strategies [47,48]. In the case of meiobenthic nematodes, taxonomic diversity was commonly included, whereas functional tools such as Maturity index or Index of Trophic Diversity were less considered. In fact, it is not possible for the moment to develop a generally usable ecotoxicological model because experiments' outcomes are different depending on the type of chemicals. Bioassays on nematodes, including the current one, belong to a primordial step with a main objective to classify taxa and/or functional groups in tolerant or sensitive categories. For several reasons, no absolute results are associated to taxa or functional traits [49–51]. First, the Maturity Index was developed by Bongers [52,53] and employed, like most functional descriptors, at a generic level. In our opinion, this could be the main reason of conflicting results regarding its usefulness (see [54–56]). Second, the response of any given species to stress will depend on its physiological, morphological and behavior characteristics. Several closely related (i.e., appurtenant to the same genus or family) nematode species from Tunisian waters have different responses to pollutants [57] due distinct tail shapes (e.g., *O. campyloceroïdes* vs. *O. brevicaudatus*), the presence/absence of sexual dimorphism (e.g., *M. pristiurus* vs. *M. demani*) and the presence/absence of long somatic setae (e.g., *Spirinia gerlachi* vs. *S. parasitifera* or *T. mirabilis* vs. *Theristus modicus*

or *Setosabatieria hilarula* vs. *Sabatieria granifer*), etc. Third, during the last two decades, the use of molecular investigations in the systematics of nematodes became a very useful technique [58]. In particular, the results of [59–61] and [62], based on DNA barcoding of the mitochondrial marker Cytochrome *c* Oxidase I (COI), demonstrated for the marine nematode *Pellioiditis marina* the existence of divergent lineages or haplotypes with different potentials for colonization/persistence and a wide spectrum of tolerance/sensitivity to pollutants. This body of evidence leads us to the conclusion that a second step is needed in the future in order to establish standard rearing methods for species with large spectra of tolerance or sensitivity and to launch bioassays on confirmed models and cell lines.

5. Conclusions

The current study explored for the first time the toxic effects of glucocorticoids on meiobenthic fauna, with a main focus on free-living marine nematodes. Therefore, single and mixtures of two commonly used glucocorticoids, prednisolone and dexamethasone, were used to study such toxicological interactions.

The results highlighted a significant toxic effect on nematode abundances. Diversity indices indicated higher toxicity of dexamethasone compared to prednisolone on nematofauna. SIMPER analysis showed significant modifications in the nematode's communities, as well as on their functional traits. Thus, the results of the current experiment reveal the sensitivity of *M. honestus* to prednisolone. On the other hand, the results showed that *E. longicaudatus* was highly tolerant to prednisolone, but potentially sensitive to dexamethasone. Finally, the contradictory results obtained from the P2D1 treatment compared to P1D2 suggest potential antagonistic interactions between these pharmaceuticals, as well as potentially higher toxicity of dexamethasone compared to prednisolone for nematodes.

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Appendix A

Table A1. Species list and functional traits of nematode species identified in the initial assemblage (I), and those from uncontaminated (Utc) and contaminated (P1, P2, D1, D2, P1D1, P2D2, P2D1, and P1D2) microcosms. Prednisolone (P); dexamethasone (D); Colonizers-Persisters scores (c-p); tail shape (TI): conical (co), elongated/filiform (e/f), clavate (cla); amphid shape (Am): pocket-like (pk), indistinct (id), spiral (sp), circular (cr), Rounded or Elongate Loop (REL); feeding groups (FG): selective deposit-feeders (1A), non-selective deposit-feeders (1B), epistratum-feeders (2A), omnivores carnivores (2B), adult length (AL).

	TI	Am	FG	c-p	AL	I	Utc	P1	P2	D1	D2	P1D1	P2D2	P1D2	P2D1
<i>Bathylaimus</i> sp.	cla	sp	1B	2	1–2 mm	1.33 ± 1.15	1.66 ± 1.52	0.74 ± 0.64		1.33 ± 1.52	3.66 ± 1.15		0.38 ± 0.67		1.82 ± 1.21
<i>Cyartonema germanicum</i>	co	cr	1A	4	<1 mm	6.33 ± 1.52	7 ± 2.64	3.64 ± 1.41	0.71 ± 1.21	11.33 ± 1.52	4 ± 2	5.76 ± 1.29	1.73 ± 0.81	2.25 ± 1.81	8.92 ± 4.21
<i>Daptonema fallax</i>	cla	cr	1B	2	1–2 mm	0.66 ± 0.57	0.66 ± 0.57			0.66 ± 1.15	5.33 ± 2.08		0.45 ± 0.79		1.45 ± 1.63
<i>Enoplolaimus longicaudatus</i>	cla	id	2B	5	2–4 mm	3 ± 1	0.66 ± 1.15	15.08 ± 3.85	14.01 ± 3.45			12.64 ± 1.93	13.21 ± 0.81	16.69 ± 0.53	1.81 ± 2.22
<i>Halalaimus gracilis</i>	e/f	REL	1A	4	2–4 mm	3 ± 1	1.33 ± 0.57	1.04 ± 1.06	1.53 ± 0.64	0.33 ± 0.57		1.09 ± 0.07	1.61 ± 1.76	0.76 ± 1.32	1.11 ± 1.08
<i>Longicyatholaimus longicaudatus</i>	e/f	sp	2A	3	2–4 mm	3 ± 1	3.33 ± 2.08	3.13 ± 1.94	2.93 ± 2.07	0.66 ± 0.57		3.99 ± 1.21	4.73 ± 4.12	1.53 ± 2.65	5.44 ± 2.77
<i>Metoncholaimus pristiurus</i>	cla	pk	2B	4	>4 mm	3.33 ± 0.57	0.66 ± 1.15			0.66 ± 0.57	8.33 ± 1.52				
<i>Microlaimus cyatholaimoides</i>	co	cr	2A	2	2–4 mm	5.66 ± 3.05	7 ± 1.73	8.95 ± 1.36	7.31 ± 2.41	11.33 ± 4.93	2 ± 1	7.22 ± 3.01	5.66 ± 1.88	10.54 ± 1.13	8.15 ± 3.09
<i>Microlaimus honestus</i>	co	cr	2A	3	2–4 mm	15 ± 2	14.66 ± 3.51			15 ± 2	5.33 ± 0.57				13.91 ± 4.15
<i>Nudora gerlachi</i>	co	cr	2A	3	<1 mm	2.66 ± 2.08	1 ± 1.73	1.12 ± 1.16	1.71 ± 2.96	7.66 ± 2.51	2 ± 2	0.68 ± 1.19	0.45 ± 0.79	2.26 ± 2.2	
<i>Odontophora villoti</i>	co	REL	1B	2	1–2 mm	0.33 ± 0.57	1.33 ± 0.57	1.85 ± 1.41	1.96 ± 0.81	3 ± 2	0.33 ± 0.57	2.25 ± 2.35	3.86 ± 0.33	0.77 ± 0.67	
<i>Oncholaimellus calvadocicus</i>	cla	pk	2B	4	1–2 mm	3.33 ± 0.57	2 ± 1	1.73 ± 1.61	2.41 ± 2.1	1 ± 1	5.33 ± 1.52	1.71 ± 2.97	1.93 ± 3.35	1.16 ± 1.14	0.71 ± 1.24
<i>Oncholaimus campylocercoides</i>	cla	pk	2B	4	2–4 mm	19.33 ± 2.51	18.66 ± 4.72	16.96 ± 3.32	20.15 ± 5.83	13 ± 2.64	14 ± 2.64	15.31 ± 3.94	16.07 ± 4.43	24.52 ± 3.11	17.54 ± 4.26
<i>Paramonohystera proteus</i>	cla	cr	1B	2	1–2 mm	7.33 ± 0.57	7.33 ± 2.08	8.69 ± 2.67	7.46 ± 3.63	4 ± 1	8 ± 2	7.88 ± 2.76	7.28 ± 0.82	5.67 ± 2.69	7.363 ± 5.46
<i>Parasphaerolaimus paradoxus</i>	cla	cr	2B	3	1–2 mm	2.33 ± 0.57	2.33 ± 1.52	5.09 ± 1.63	3.01 ± 1.04	2 ± 2	1 ± 1	8.85 ± 2.79	4.66 ± 2.93	4.01 ± 2.29	2.58 ± 1.31
<i>Phanoderma</i> sp.	s/r	pk	2A	4	2–4 mm	0.66 ± 0.57	1.33 ± 1.15	1.12 ± 1.16	1.11 ± 1.13	0.33 ± 0.57			0.77 ± 1.34	1.19 ± 2.06	
<i>Prochromadorella longicaudata</i>	co	id	2A	2	<1 mm	1.33 ± 0.57	2.66 ± 1.52	2.46 ± 1.12	2.66 ± 0.45	1.66 ± 1.52	2.66 ± 2.08	1.73 ± 2.13	1.36 ± 2.37	3.71 ± 0.93	2.64 ± 2.9
<i>Rhabditis</i> sp.	co	id	1B	1	<1 mm	1.66 ± 0.57	1.66 ± 1.15	2.12 ± 1.01	3.023 ± 2.33	4.66 ± 1.15	8.66 ± 1.52	3.65 ± 1.66	5.63 ± 3.56	2.32 ± 2.29	0.71 ± 1.24
<i>Sabatiera splendens</i>	cla	sp	1B	2	1–2 mm	1.33 ± 1.52	1.66 ± 0.57	1.46 ± 0.074	1.08 ± 1.05	1 ± 1	4 ± 2	0.34 ± 0.59	0.77 ± 1.34	0.49 ± 0.84	2.18 ± 1.03
<i>Spirinia parasitifera</i>	co	REL	2A	3	2–4 mm	4 ± 1	2.33 ± 1.52	3.48 ± 2.05	1.12 ± 1.06	1.66 ± 1.52	0.33 ± 0.57	1.51 ± 1.3		0.39 ± 0.68	3.29 ± 1.85
<i>Synonchiella edax</i>	cla	sp	2B	3	2–4 mm	1.66 ± 1.15	2.66 ± 1.15	2.84 ± 0.046	2.28 ± 1.03	1 ± 1.73	2.66 ± 0.57	1.82 ± 2.22	2.07 ± 1.83	2.32 ± 2.29	2.65 ± 3.71
<i>Thalassironus britannicus</i>	co	pk	2B	4	>4 mm	3.33 ± 0.57	1.66 ± 1.15	2.81 ± 1.55	3.14 ± 1.99	5 ± 1	6.33 ± 1.52	4.81 ± 3.37	4.46 ± 3.55	2.34 ± 3.11	2.55 ± 2.49
<i>Theristus modicus</i>	co	cr	1B	2	1–2 mm	1.66 ± 0.57	3.66 ± 1.52	3.55 ± 1.46	4.14 ± 2.03	1.66 ± 1.52	4 ± 1.73	3.54 ± 2.09	2.06 ± 1.23	2.92 ± 0.53	2.95 ± 0.7
<i>Theristus pertenuis</i>	co	cr	1B	2	1–2 mm	2 ± 1.73	2 ± 1	3.18 ± 0.85	2.34 ± 1.19	0.66 ± 1.15	0.66 ± 1.15	2.57 ± 2.69	2.27 ± 2.85	0.383 ± 0.66	1.1 ± 1.07
<i>Thoonchus inermis</i>	cla	pk	2B	4	2–4 mm	2.33 ± 0.08	1.33 ± 1.15	1.12 ± 1.16	3.07 ± 1.29	1 ± 1.73			2.063 ± 1.23	1.65 ± 0.57	
<i>Trichotheristus mirabilis</i>	co	cr	1B	2	1–2 mm	2 ± 1	7.33 ± 3.21	6.32 ± 2.8	9.81 ± 2.54	5.33 ± 2.88	11.33 ± 1.51	12.57 ± 4.74	14.71 ± 5.81	11.64 ± 4.14	7.37 ± 3.57
<i>Valvaelaimus maior</i>	co	cr	1B	2	1–2 mm	1.33 ± 1.15	2 ± 1	1.41 ± 1.63	2.96 ± 1.51	4 ± 1			1.72 ± 2.05	0.39 ± 0.68	3.66 ± 1.61

Table A2. Dissimilarity percentages (bold values) between the initial assemblage (I) and treatments and results of Similarity Percentage analysis (SIMPER) based on square-root transformed data. Species and functional groups accounting for ~70% of overall dissimilarity are ranked in order of importance of their contribution. Untreated control (Utc); Prednisolone (P); dexamethasone (D); Colonizers-Persisters scores (c-p); conical (co); elongated/filiform (e/f); clavate (cla); pocket-like (pk); indistinct (id); spiral (sp); circular (cr); Rounded or Elongate Loop (REL); selective deposit-feeders (1A); non-selective deposit-feeders (1B); epistratum-feeders (2A); omnivores carnivores (2B); more abundant (+); less abundant (−); eliminated (elim); no change (=).

	I vs. P1D1	I vs. P2D2	I vs. P1D2	I vs. P2D1
Species	44.87% <i>Microlaimus honestus</i> (17.45%) elim <i>Trichotheistis mirabilis</i> (10.95%) + <i>Enoplolaimus longicaudatus</i> (10.02%) + <i>Parasphaerolaimus paradoxus</i> (6.64%) + <i>Oncholaimus campylocercoides</i> (6.21%) − <i>Metoncholaimus pristiurus</i> (3.88%) elim <i>Microlaimus cyatholaimoides</i> (3.47%) + <i>Oncholaimellus calvadosicus</i> (3.25%) − <i>Spirinia parasitifera</i> (3.08%) − <i>Thalassironus britanicus</i> (2.96%) +	48.32% <i>Microlaimus honestus</i> (17.46%) elim <i>Trichotheistis mirabilis</i> (10.91%) + <i>Enoplolaimus longicaudatus</i> (8.49%) + <i>Oncholaimus campylocercoides</i> (7.86%) − <i>Cyartonema germanicum</i> (5.81%) − <i>Spirinia parasitifera</i> (4.66%) elim <i>Metoncholaimus pristiurus</i> (3.88%) elim <i>Oncholaimellus calvadosicus</i> (3.26%) − <i>Rhabditis</i> sp. (3.12%) + <i>Odontophora villoti</i> (3.1%) +	44.44% <i>Microlaimus honestus</i> (18.83%) elim <i>Enoplolaimus longicaudatus</i> (12.87%) + <i>Trichotheistis mirabilis</i> (8.88%) + <i>Cyartonema germanicum</i> (5.8%) − <i>Spirinia parasitifera</i> (4.61%) − <i>Metoncholaimus pristiurus</i> (4.18%) elim <i>Microlaimus cyatholaimoides</i> (3.9%) + <i>Paramonohystera proteus</i> (3.45%) − <i>Oncholaimellus calvadosicus</i> (2.97%) − <i>Halalaimus gracilis</i> (2.96%) −	30.72% <i>Trichotheistis mirabilis</i> (7.98%) − <i>Oncholaimus campylocercoides</i> (6.9%) − <i>Paramonohystera proteus</i> (6.41%) − <i>Microlaimus honestus</i> (6.3%) − <i>Metoncholaimus pristiurus</i> (5.69%) + <i>Microlaimus cyatholaimoides</i> (5.15%) − <i>Oncholaimellus calvadosicus</i> (4.57%) + <i>Nudora gerlachi</i> (4.55%) + <i>Longicyatholaimus longigandatus</i> (4.53%) − <i>Cyartonema germanicum</i> (4.42%) − <i>Thoonchus inermis</i> (3.99%) + <i>Synonchiella edax</i> (3.83%) − <i>Thalassironus britanicus</i> (3.61%) −
Feeding groups	19.87% 2A −	27.48% 2A − 1B +	17.41% 2A −	14.95% 2B −
Tail shape	6.91% cla −	16.71% co −	16% co −	9.6% cla −
Amphid shape	16.62% pk −	24.2% cr − pk −	25.36% cr − id +	14.5% pk − cr +
Adult length	18.89% 2–4 mm −	21.26% 2–4 mm −	12.77% 2–4 mm − > 4 mm −	11.92% 2–4 mm − 1–2 mm +
c-p score	30.13% c-p3 − c-p4 −	32.86% c-p3 − c-p4 −	30.16% c-p3 − c-p4 −	16.92% c-p4 − c-p2 +
	Utc vs. P1	Utc vs. P2	Utc vs. D1	Utc vs. D2
Species	34.62% <i>Microlaimus honestus</i> (21.97%) elim <i>Enoplolaimus longicaudatus</i> (19.95%) + <i>Oncholaimus campylocercoides</i> (6.12%) − <i>Cyartonema germanicum</i> (5.46%) − <i>Trichotheistis mirabilis</i> (4.69%) − <i>Parasphaerolaimus paradoxus</i> (3.53%) + <i>Paramonohystera proteus</i> (3.02%) + <i>Longicyatholaimus longicaudatus</i> (2.84%) −	36.76% <i>Microlaimus honestus</i> (21.37%) elim <i>Enoplolaimus longicaudatus</i> (16.85%) + <i>Cyartonema germanicum</i> (9.27%) − <i>Oncholaimus campylocercoides</i> (6.42%) − <i>Trichotheistis mirabilis</i> (4.48%) − <i>Paramonohystera proteus</i> (3.39%) − <i>Longicyatholaimus longicaudatus</i> (2.93%) − <i>Microlaimus cyatholaimoides</i> (2.59%) − <i>Theristus modicus</i> (2.57%) =	32% <i>Nudora gerlachi</i> (10.42%) + <i>Oncholaimus campylocercoides</i> (9.2%) − <i>Cyartonema germanicum</i> (6.77%) + <i>Microlaimus cyatholaimoides</i> (6.77%) + <i>Thalassironus britanicus</i> (5.21%) + <i>Trichotheistis mirabilis</i> (5.21%) − <i>Paramonohystera proteus</i> (5.21%) + <i>Rhabditis</i> sp. (4.69%) + <i>Microlaimus honestus</i> (4.34%) + <i>Longicyatholaimus longicaudatus</i> (4.17%) − <i>Theristus modicus</i> (3.47%) − <i>Synonchiella edax</i> (3.3%) +	41.67% <i>Microlaimus honestus</i> (11.2%) − <i>Metoncholaimus pristiurus</i> (9.2%) + <i>Rhabditis</i> sp. (8.4%) + <i>Oncholaimus campylocercoides</i> (6.13%) − <i>Microlaimus cyatholaimoides</i> (6%) − <i>Thalassironus britanicus</i> (5.6%) + <i>Daptonema fallax</i> (5.6%) + <i>Trichotheistis mirabilis</i> (5.33%) − <i>Cyartonema germanicum</i> (4.13%) elim <i>Longicyatholaimus longicaudatus</i> (4%) + <i>Oncholaimellus calvadosicus</i> (4%) +

Table A2. Cont.

	I vs. P1D1	I vs. P2D2	I vs. P1D2	I vs. P2D1
Feeding groups	16.9% 2A –	21.78% 2A –	10.3% 2B – 2A +	25.52% 2A –
Tail Shape	15.3% co –	15.25% co –	18.78% co +	12.89% cla +
Amphid Shape	20.32% cr –	23.47% cr –	12.44% cr + sp –	17% cr – pk +
Adult Length	7.9% 1–2 mm + 2–4 mm –	10.39% 2–4 mm – 1–2 mm –	17.89% < 1 mm + 2–4 mm –	29% 2–4 mm –
c-p score	22.75% c-p3 – c-p5 +	24.35% c-p3 – c-p5 +	8% c-p2 – c-p4 –	16.39% c-p3 – c-p1 +
	Utc vs. P1D1	Utc vs. P2D2	Utc vs. P1D2	Utc vs. P2D1
Species	40.94%	44.68%	40.54%	27.28%
	<i>Microlaimus honestus</i> (18.7%) elim <i>Enoplolaimus longicaudatus</i> (13.96%) + <i>Parasphaerolaimus paradoxus</i> (7.28%) + <i>Oncholaimus campylocercoides</i> (6.51%) – <i>Trichotheistis mirabilis</i> (6.02%) + <i>Thalassironus britannicus</i> (3.99%) + <i>Cyartonema germanicum</i> (3.27%) – <i>Microlaimus cyatholaimoides</i> (3.24%) – <i>Oncholaimellus calvadosicus</i> (2.96%) – <i>Paramonohystera proteus</i> (2.86%) =	<i>Microlaimus honestus</i> (18.46%) elim <i>Enoplolaimus longicaudatus</i> (12.12%) + <i>Oncholaimus campylocercoides</i> (7.93%) – <i>Cyartonema germanicum</i> (7.13%) – <i>Trichotheistis mirabilis</i> (6.19%) + <i>Rhabditis</i> sp. (3.66%) + <i>Microlaimus cyatholaimoides</i> (3.32%) – <i>Longicyatholaimus longicaudatus</i> (3.24%) + <i>Spirinia parasitifera</i> (2.94%) elim <i>Oncholaimellus calvadosicus</i> (2.92%) –	<i>Microlaimus honestus</i> (20.18%) elim <i>Enoplolaimus longicaudatus</i> (17.32%) + <i>Cyartonema germanicum</i> (7.28%) – <i>Oncholaimus campylocercoides</i> (5.19%) + <i>Paramonohystera proteus</i> (4.38%) – <i>Trichotheistis mirabilis</i> (4.16%) + <i>Longicyatholaimus longicaudatus</i> (3.71%) – <i>Spirinia parasitifera</i> (2.76%) – <i>Parasphaerolaimus paradoxus</i> (2.58%) + <i>Theristus pertenuis</i> (2.31%) –	<i>Oncholaimus campylocercoides</i> (9.45%) – <i>Microlaimus honestus</i> (7.72%) – <i>Paramonohystera proteus</i> (7.65%) – <i>Trichotheistis mirabilis</i> (6.4%) – <i>Cyartonema germanicum</i> (6.23%) – <i>Longicyatholaimus longicaudatus</i> (5.32%) – <i>Synonchiella edax</i> (4.93%) – <i>Microlaimus cyatholaimoides</i> (4.07%) – <i>Prochromadorella longicaudata</i> (4.06%) – <i>Enoplolaimus longicaudatus</i> (3.19%) – <i>Oncholaimellus calvadosicus</i> (3%) + <i>Spirinia parasitifera</i> (2.98%) – <i>Thalassironus britannicus</i> (2.98%) +
Feeding groups	16.81% 2A –	21% 2A –	24.96% 2A – 2B +	9.84% 1B – 2A –
Tail Shape	10.91% co –	19.24% co –	20.43% co –	7.81% cla –
Amphid Shape	17.69% id + cr –	26.28% cr –	26.71% cr –	10.63% pk – cr –
Adult Length	13.69% 2–4 mm –	17.1% 2–4 mm –	13.3% 1–2 mm –	9.82% 1–2 mm – 2–4 mm –
c-p score	23.55% c-p3 – c-p5 +	28.67% c-p3 – c-p4 –	28.06% c-p3 – c-p5 +	9.82% c-p4 – c-p2 –

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