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Research Article

Anorexigenic and anxiogenic effects of the plasticiser DEHP (di-2-ethylhexyl phthalate) in goldfish: Involvement of PPAR signalling and feeding-related neuropeptides

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ABSTRACT

Di-2-ethylhexyl phthalate (DEHP), a widely used plasticiser, is a pervasive environmental contaminant with potential detrimental effects on aquatic organisms. The objective of this study was to provide an integrative analysis of how DEHP alters energy balance, temporal homeostasis and fish welfare — interrelated aspects critical to animal survival — to address critical gaps in our understanding of its toxicological effects. Goldfish (*Carassius auratus*) were chronically (14 days) treated with DEHP. Energy balance was assessed through locomotor activity, metabolic rate, feed intake, and growth indices. Daily of locomotor and metabolic rate rhythms were examined to explore potential circadian disruptions. Anxiety-like behaviours were also examined to assess welfare. DEHP decreased feed intake and food-anticipatory activity (FAA), suggesting an anorexigenic effect, which may have been mediated by increased expression of anorexigenic genes in the hypothalamus and liver, along with decreased expression of orexigenic *npy* (neuropeptide Y) gene in the hypothalamus. Growth parameters remained unchanged, probably due to compensatory reductions in energy expenditure, as indicated by decreased locomotor activity and metabolic rate. Daily rhythms in these two parameters were preserved, suggesting no disruption in temporal homeostasis. DEHP increased hepatic expression of peroxisome proliferator-activated receptor (PPAR)-related genes, suggesting that PPARs activation is a potential mode of action for DEHP in fish. Anxiety levels were elevated, as evidenced by increased thigmotaxis and scototaxis in behavioural tests, which may be mediated by changes in hypothalamic neuropeptides. These findings highlight the adverse effects of DEHP on energy regulation and animal welfare, providing novel insights into its broader physiological consequences in fish.

1. Introduction

The use of phthalates as plasticisers in a wide range of industrial and consumer products such as cosmetics, medical devices, building materials, automotive components and food packaging has prompted serious concerns due to their increasing presence in the environment as major organic pollutants (da Costa et al., 2023; Li et al., 2024a). Di-2-ethylhexyl phthalate (DEHP) is one of the most commonly used phthalates, added to plastic products to improve flexibility and durability (Ito et al., 2019; Liu et al., 2024; Wang et al., 2019). As DEHP is not

Abbreviations: A, *amplitude*; *agrp*, *agouti-related peptide*; *actb*, *actin beta*; *bmal1*, brain and muscle Arnt-like protein 1; BW, body weight; BWi, initial body weight; BWf, final body weight; *cartpt1*, cocaine and amphetamine regulated transcript 1; C, control; DEHP, di-2-ethylhexyl phthalate; DMSO, dimethylsulfoxide; *eef1a*, eukaryotic translation elongation factor 1 alpha; F, dilution factor; FAA, food-anticipatory activity; FI, feed intake; GSI, gonadosomatic index; HSI, hepatosomatic index; IP, intraperitoneal; K, condition factor; *lepa1*, leptin a1; Li, initial standard length; Lf, final standard length; M, mesor; MEHP, mono-2-ethylhexyl phthalate; MO_2, oxygen consumption rate; MS222, tricaine methanesulfonate; *npy*, neuropeptide Y; OW, organ weight; *pomca*, proopiomelanocortin a; PPARs, peroxisome proliferator-activated receptors; PPARα, peroxisome proliferator-activated receptor alpha; PPARγ, peroxisome proliferator-activated receptor gamma; qPCR, real-time quantitative PCR; RNasin, RNase inhibitor; SEM, standard error of the mean; SNK, Student-Newman-Keuls; *srebp*, sterol regulatory element-binding protein; t, time; VFI, Perivisceral fat index; Wi, initial weight of the feed; Wf, final weight of the feed; ϕ, acrophase.

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covalently bound to plastic polymers, it can easily diffuse and migrate from plastic products into the environment, where it has been detected in oceans and rivers worldwide, and represents a potential hazard for aquatic organisms (Dueñas-Moreno et al., 2022; Li et al., 2024a; Liu et al., 2024). In fact, DEHP is one of the most prevalent phthalates identified in fish, with exposure occurring through the water column, sediments, and dietary sources (Khoshmanesh et al., 2024; Liu et al., 2024). The bioaccumulation of DEHP in teleost can considerably vary across species due to differences in feeding habits, metabolic capacities, and contaminant bioavailability in their environment (Li et al., 2024a; Liu et al., 2024). Beyond ecological concerns, DEHP accumulation in fish also poses a potential risk to human health, as fish serve as a pathway for this contaminant into the food chain (Eales et al., 2022; da Costa et al., 2023).

DEHP exerts several adverse effects on fish physiology, including oxidative stress, genotoxicity, as well as significant disruptions to the neural, endocrine, immune, metabolic and cardiovascular systems (Liu et al., 2024; Zhang et al., 2021). The zebrafish (*Danio rerio*) has been the predominant model organism for these studies, with many investigations focusing on the embryonic and larval stages (Kwan et al., 2021). Notably this phthalate functions as an endocrine disruptor, altering the thyroid and gonadal axes and interfering with reproduction and development (Adeogun et al., 2018; Golshan and Alavi, 2019; Golshan et al., 2015). DEHP-induced effects on growth also have significant implications for aquatic ecosystems and aquaculture, although findings remain inconsistent. Some studies have indicated that DEHP reduces growth performance (Yang et al., 2018; Zanotelli et al., 2010), while others indicate it promotes growth (Jia et al., 2021; Mo et al., 2019), raising the possibility that this phthalate may act as an obesogen (Buerger et al., 2019). However, these studies have not addressed the underlying mechanisms regulating energy balance. Crucially, there is a notable lack of studies offering a comprehensive analysis of how DEHP affects energy balance in fish, specifically examining both energy intake and expenditure within the same study. This integrated approach is essential to fully understand the potential impact of DEHP on energy homeostasis and growth. Although locomotor alterations have been investigated as an indicator of behavioural toxicity due to DEHP (Huang et al., 2022b; Kwan et al., 2021), these studies have not considered locomotion in the broader context of energy balance. More importantly, no study to date has examined the other fundamental component of energy expenditure — metabolic rate — in fish exposed to DEHP.

The specific mechanism of action of DEHP in fish remains uncertain; however, evidence suggests a possible involvement of nuclear receptors, specifically PPARs. It has been shown that DEHP can up-regulate the hepatic expression of *ppara* (PPARα gene) in zebrafish (Migliarini et al., 2011) and sharptooth catfish (*Clarias gariepinus*; Adeogun et al., 2018), as well as *pparg* (PPARγ gene) in yellow catfish (*Tachysurus fulvidraco*; Meng et al., 2018). These findings suggest that nuclear PPAR receptors may mediate the effects of DEHP in fish, paralleling the mechanism in other vertebrates. Both, DEHP and its primary metabolite, mono-2-ethylhexyl phthalate (MEHP), act as ligands for PPARs, specifically activating PPARα and PPARγ in mammals (Hurst and Waxman, 2003; Ito et al., 2019; Wójtowicz et al., 2023) and birds (Huang et al., 2022a). Many of the pleiotropic effects of DEHP in vertebrates are mediated by the activation of PPARs in the liver, one of its primary target organs (Ito et al., 2019). Given the liver's central role in energy storage and mobilisation, and the involvement of PPAR receptors in metabolic regulation in fish (Boukouvala and Krey, 2024; Gómez-Boronat et al., 2020), it is not surprising that DEHP impairs metabolic functions in fish (Kwan et al., 2021; Jia et al., 2021; Yang et al., 2024). Beyond their well-established role in metabolism, PPARs are also integral components of the molecular machinery of circadian oscillators that regulate temporal homeostasis in organisms (Berthier et al., 2021). In fish, activation of PPARα has been shown to increase hepatic expression of the key clock gene *bmal1* (brain and muscle Arnt-like protein 1) (Gómez-Boronat et al., 2020), reinforcing the connection between metabolic regulation and circadian control. Circadian rhythms orchestrate physiological and behavioural processes essential for maintaining internal stability, thus their proper functioning is crucial in order to preserve energy balance and ensure the overall health of animals (Sato and Sato, 2023). Since PPARs act at the intersection of metabolism and circadian regulation, DEHP-induced PPARs activation could potentially impact not only energy homeostasis, but also temporal homeostasis. Although this connection remains largely unexplored, determining whether DEHP disrupts circadian rhythms is essential, as it could compromise not only energy homeostasis but also overall physiological stability, affecting welfare and survival.

This study provides a comprehensive analysis of how DEHP disrupts energy balance, temporal homeostasis and welfare in *Carassius auratus*, addressing critical gaps in our understanding of its toxicological effects. Unlike previous studies that focus on isolated physiological endpoints, we integrated multiple parameters — including feeding behaviour, metabolic rate and locomotor activity — to assess the two key components of energy balance, intake and expenditure, along with their impact on growth. Additionally, we examined whether DEHP disrupts daily locomotor activity and oxygen consumption rhythms, providing insights into its effects on temporal homeostasis. Finally, given the importance of behavioural responses as indicators of fish welfare, we assessed anxiety-like behaviours using non-invasive tests. To further elucidate the underlying mechanisms, we investigated the potential role of hypothalamic and hepatic signals in mediating DEHP-induced effects. By addressing these interconnected aspects, our study not only enhances our understanding of DEHP's impact on energy and circadian regulation, but also provides novel insights into its broader implications for fish welfare and aquatic ecosystems.

The goldfish was selected as the model species due to its relevance in both aquaculture and research. This freshwater species is representative of the Cyprinidae family, which constituted the largest group of fish produced in aquaculture in 2022 (FAO, 2024). Furthermore, goldfish are an established model for evaluating fish responses to aquatic toxicity (Blanco and Unniappan, 2022).

2. Materials and methods

All experimental procedures in this study were conducted in accordance with the current regulations of the European Union (2010/63/EU) and Spain (RD 53/2013) on animal welfare protection for scientific purposes. The procedures were approved by the Animal Experimentation Committee of the Complutense University of Madrid and the Community of Madrid (PROEX 317.7/23). All processes were performed in accordance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

2.1. Fish and housing

Juvenile goldfish (*C. auratus,* 10.94 ± 0.34 g body weight, BW) were purchased from ICA (Madrid, Spain), and kept in four 60-L glass tanks with dechlorinated freshwater in a recirculating system (n = 6 fish/tank). These tanks were equipped with mechanical, chemical and biological filtration, as well as continuous aeration. The fish were kept under controlled temperature conditions (21 ± 1 °C) with a photoperiod of 12 h of light (08:00–20:00) and 12 h of darkness (20:00–08:00). Additionally, water quality was monitored weekly for pH (6.8–7.5) and nitrites (0 mg/L). The fish were provided with a daily ration (1.5 % BW) of granulated feed (Sera Pond Granulat; Heinsberg, Germany) at 10:00. All animals were acclimated in these conditions for three weeks before any procedure.

2.2. DEHP administration

Di-2-ethylhexyl phthalate (#36735, Sigma Aldrich, Madrid, Spain) was dissolved in a vehicle consisting of teleost saline (600 mg of sodium

chloride and 15.8 mg sodium bicarbonate in 100 mL of distilled water) and 0.1 % dimethylsulfoxide (DMSO; #D8418, Sigma Aldrich, Madrid, Spain), pH 7.2. The fish were previously anaesthetised with buffered tricaine methanesulfonate (MS222, 0.16 g/L; #E10521, Sigma Aldrich, Madrid, Spain) and intraperitoneally (IP) injected, as previously described (Saiz et al., 2022). The fish were IP injected with 10 μL vehicle/g BW alone (control group) or containing DEHP (10 mg/kg BW). The DEHP dose was chosen based on previous studies on fish (Qu et al., 2014; Uren-Webster et al., 2010). Following the injection, the fish were transferred to fresh water and observed until they regained equilibrium and exhibited normal locomotor activity for a period of 1–2 min.

2.3. Experimental design

For individual identification, the fish were anaesthetised with buffered MS222 (0.16 g/L) and tagged with subcutaneous injections of black ink (Eternal Ink; Brighton, MI, USA). Two groups of goldfish ($n = 12$/group; two tank replicates per group, $n = 6$/tank) were daily IP injected at 12:30 for 14 days with either vehicle or DEHP, as described above. The treatment tanks were the same as the acclimation tanks, with identical volume and conditions. Locomotor activity, feed intake, weight and length were recorded daily throughout the experiment. Anxiety-like behaviours were assessed on day 9, and metabolic rate on day 15, to avoid the potential interference between the two tests, as both were performed individually. Behaviour was examined in advance, to ensure a sufficient time interval before measuring oxygen consumption and to be able to correlate energy expenditure with growth at the end point. On day 16, the fish (after 48 h of fasting and 48 h after the last IP injection) were euthanised using MS222 (0.4 g/L), followed by spinal section. Liver, perivisceral fat, gonads and hypothalamus were collected, snap-frozen in liquid nitrogen and stored at −80 °C until analysis.

2.4. Locomotor activity

Locomotor activity was recorded throughout the acclimation period (7 days) and experimental period (14 days) using 6 photocells (E3S-AD12; Omron Corporation,Kyoto, Japan) attached to the tank walls as previously described (Gómez-Boronat et al., 2018; Saiz et al., 2023b). Two photocells were placed under the feeder in each tank to quantify feeding-associated activity, while four were distributed throughout the lower and middle regions of the tank to measure general locomotor activity. The photocells emitted a continuous infrared beam that was interrupted by fish movements, sending pulse signals recorded every 10 mins by an actimeter connected to a data acquisition software (Adq16; Micronec, Madrid, Spain). The data were analysed with El Temps® software (Prof. Antoni Díez Noguera, University of Barcelona, Spain) to obtain actograms and profiles of averaged daily rhythms. Complete daily locomotor activity data were missing on three days—day 1 (handling), day 9 (behavioural tests) and day 14 (individual feed intake test)—resulting in a total of 11 recorded experimental days.

2.5. Metabolic rate

Intermittent-flow respirometry was used to obtain oxygen consumption rate (MO_2) as a proxy for metabolic rate, as previously described (Herrera-Castillo et al., 2024). The system (Loligo Systems, Viborg, Denmark) comprised four methacrylate chambers submerged in a temperature-regulated (21 ± 1 °C) and oxygenated water bath, with wash and recirculation water pumps. The fish (after 24 h of fasting and 24 h after the last IP injection) were placed individually in the chambers, and their metabolic rate was measured for 24 h. Oxygen optode sensors and a temperature probe were linked to a Witrox 4 module, with data acquisition carried out using AutoResp™ software (version 2.3.0) from Loligo Systems (Viborg, Denmark).

2.6. Feed intake

Feed intake (FI) was daily quantified in groups of 6 fish in each tank, and in individual fish (5 L tanks) on the last day of treatment (day 14), as previously described (Saiz et al., 2022). Briefly, pre-weighted feed in excess (3 % BW) was provided at 10:00, and leftover feed was collected after 2 h and dried for 24 h in a stove. Feed intake was calculated using the following formula: $FI = (Wi - Wf)\ x\ F$, where Wi corresponds to the initial weight of the feed, Wf to the final weight, and F is the dilution factor. The daily feed intake per tank was evaluated on 12 of the 14 days of the experiment, as it could not be evaluated on days 9 and 14.

2.7. Biometric parameters

To evaluate the growth performance of the fish throughout the experimental period, the following biometric parameters were calculated:

- Body Weight Gain (%) $= [(BWf - BWi)/BWi]\ x\ 100$
- Body Length Gain (%) $= [(Lf - Li)/Li]\ x\ 100$
- Specific Growth Rate $(\%/day) = [(LnBWf - LnBWi)/days\ of\ treatment]\ x\ 100$
- Condition Factor $(K) = \left(BWf/Lf^3\right)\ x\ 100$
- Organosomatic Indexes (%) $= (OW/BWf)\ x\ 100$

BWi, initial body weight; BWf, final body weight; Li, initial standard length; Lf, final standard length; OW, organ weight (liver, perivisceral fat or gonads).

2.8. Behavioural tests

Open field and black-white preference tests were conducted to assess anxiety-like behaviours, as previously described (Saiz et al., 2023a). After 8 days of DEHP treatment, each fish (after 29 h of fasting and 26 h after the last injection) was carefully netted from its home tank and placed in a 5-L tank for transport to the test room, where it was acclimated for 5 mins prior to testing. At the start of the open field test, the fish was released in a circular tank (50 cm diameter, 10 cm water depth) near the wall. The open field area was deemed to be the inner part (occupying 75 % of the total area), while the outer area (25 %) was deemed to be "near the wall" (as a proxy for thigmotaxis). After the open field test, each fish was individually returned to the 5-L tank and acclimated to the black-white test room for another 5 mins. Subsequently, the fish were released on the black side of the tank (47 cm length, 10 cm width and 10 cm water depth; with one half of the tank background black and the other half white; Maze Engineers, Skokie, USA). In both tests, the fish movements were recorded for 10 mins with a video camera and behavioural parameters were obtained through automated video tracking with EthoVision XT 17.5 software (Noldus, Wageningen, the Netherlands).

2.9. Gene expression analysis

The relative mRNA abundance of *agrp* (agouti-related peptide), *npy*, *cartpt1* (cocaine and amphetamine regulated transcript 1) and *pomca* (proopiomelanocortin a) in the hypothalamus, and *lepa1* (leptin a1), *bmal1a*, *ppara* and *pparg* in the liver, was determined by real-time quantitative PCR (qPCR), as previously described (Saiz et al., 2022, 2023b). The initial step involved the extraction of total RNA using TRI Reagent (#T9424, Sigma Aldrich, Madrid, Spain), in accordance with the instructions provided by the manufacturer. Any residual DNA in the samples was removed using a DNAse (#M6101, RQ1 RNase-Free DNase; Promega, Madison, USA). Subsequently, 0.5 μg of total RNA was reverse-transcribed into cDNA using random primers (#48190–011, Invitrogen, Carlsbad, USA), an RNase inhibitor (RNasin; #N215B,

Promega, Madison, USA) and the Superscript IV enzyme (#18090010, Invitrogen, Carlsbad, USA). Quantitative PCR was conducted in a 96-well plate, with each well containing 1 μL of cDNA, forward and reverse primers (0.5 μM), 5 μL of the SYBR Green Supermix (#1725124, Bio-Rad Laboratories, Hercules, USA), and topped up to 10 μL with water for the total reaction. The primer sequences (Sigma Aldrich, Madrid, Spain) used for the target and reference genes (*actb,* actin beta*; eef1a,* eukaryotic translation elongation factor 1 alpha) are shown in Table 1. Each sample was measured in duplicate. The qPCR reaction was conducted according to the following protocol: an initial denaturation step (30 s, 95 °C) and 40 denaturation cycles (5 s, 95 °C) followed by the hybridisation and elongation step (30 s, 60 °C). Calibration 4 points curves were generated for each gene and tissue using serial dilutions (1/3) of cDNA. All curves exhibited efficiencies around 95–105 % and $r^2 > 0.98$. Negative controls were run in each plate and included the replacement of cDNA with water and the use of non-retrotranscribed total RNA. The specificity of the amplification was confirmed by the melting temperature of the PCR products measured at the end of all reactions (melting curve: 5 s in the range 70–90 °C every 0.5 °C) and by the product size in an agarose gel. The relative expression of each target gene was calculated using the $2^{-\Delta\Delta Ct}$ method. All data was relativised to the reference genes and subsequently to the control group (Livak and Schmittgen, 2001).

2.10. Statistics

The statistical analysis and data representation were conducted using the software Sigmaplot 12. The data are represented as mean ± SEM (standard error of the mean). Before any analysis, the normality and homoscedasticity of each data set were verified using the Shapiro-Wilk and Levene tests, respectively. When necessary, the data were transformed to a logarithmic or square-root scale. For locomotor activity and oxygen consumption, a two-way ANOVA was performed to assess the main effects of the experimental treatment (control or DEHP) and the phase of the photoperiod (day or night), as well as their interaction. For feed intake in the four tanks, a one-way ANOVA was conducted, followed by a post-hoc Student-Newman-Keuls (SNK) test to compare the 4 groups. For the remaining variables (FAA, individual feed intake, gene expression, biometric and anxiety parameters), a Student's *t*-test was used to compare the two experimental groups (control *vs.* DEHP).

With respect to the locomotor activity average profiles, the rhythm acrophases were calculated by Cosinor analysis, and significance was evaluated *via* the Rayleigh test. The rhythm period was calculated by Sokolove–Bushell periodograms. All significance thresholds were set at $p < 0.05$ (El Temps®). The daily oxygen consumption rhythm was determined using the Cosinor Online application, with the measurements adjusted to a sinusoidal function. The equation used to describe the rhythm is $Y = M + A\,x\cos(t\,x\,\pi/12 - \phi)$, where M represents the mean rhythm value (mesor), A is the amplitude (the difference between the mesor and the maximum or minimum values), t is the time and ϕ is the acrophase (the time at which the peak occurs). The significance was calculated using the zero-amplitude test (Molcan, 2019).

3. Results

3.1. Energy expenditure: Locomotor activity and metabolic rate

The actograms showed that both groups had higher activity during the light phase (08:00–20:00) than during the dark phase (20:00–08:00), and that the fish increased their locomotor activity in the hours prior to the feed arrival (10:00; Figs. 1a,b). These daily profiles are similar to that shown by the same fish over the 7 days prior to the treatment (Figs. S1a,b). This is further evidenced by the significant ($p < 0.001$) 24-h locomotor activity rhythms exhibited by both groups, before (Figs. S1c,d) and during the treatment (Figs. 1c,d). These rhythms had a significant ($p < 0.05$) period of 24 h in all cases. The mesor for the control group was 64.18 pulses/10 mins *vs.* 45.8 pulses/10 mins for DEHP. The rhythm amplitude was 24.86 pulses/10 mins for the control group, and 33.63 pulses/10 mins for the DEHP group. However, DEHP reduced the locomotor activity ($p < 0.001$) both during the photophase and scotophase (Fig. 2a). Moreover, FAA quantified as locomotor activity over the 2 h prior to feeding time was reduced by more than 50 % ($p < 0.001$) in the DEHP-treated group (Fig. 2b).

A significant sinusoidal daily rhythm was also observed in the metabolic rate of goldfish treated with the vehicle or DEHP (Fig. 3a), exhibiting higher values during the photophase than during the scotophase ($p < 0.001$; Fig. 3b). The mesor for the control group was 185.5 mg O_2/kg/h *vs.* 159.9 mg O_2/kg/h for the DEHP group. The rhythm amplitude was also higher in the control group (44.1 mg O_2/kg/h) than in DEHP group (33.9 mgO_2/kg/h). Chronic treatment with DEHP (14 days) significantly reduced both daytime and night-time oxygen consumption levels ($p < 0.001$; Fig. 3b).

3.2. Feed intake and feeding regulators

Chronic administration of DEHP for 14 days significantly reduced the feed intake of the goldfish, both when analysing ingestion in groups of 6 fish (Fig. 4a) or individually (Fig. 4b) on the last day of treatment.

Table 1
Primers access number, sequences, and size of the products used in the qPCR analyses.

Gene symbol	Gene name	Access Number (GenBank)	Sequence (5′ > 3′)	Amplicon (pb)
agrp	agouti-related peptide	AJ555492.1	Forward:ATGGCATCACATCCAAACC Reverse:GCTTTACCCAGATCCTCATCA	152
actb	actin beta	AB039726.2	Forward:CGGGAGTGATGGTTGGCA Reverse: AACACGCAGCTCGTTGTAGA	168
bmal1a	brain and muscle Arnt-like 1	KF840401.1	Forward:ATCGATGAGTCGTTCCCGTG Reverse:AGATTCTGTTCGCTCGGGAG	161
cartpt1	cocaine and amphetamine regulated transcript 1	AY033816	Forward:GTGCCGAGATGGACTTTGAC Reverse:AGCTGCTTCTCGTTGGTCAG	97
eef1a	eukaryotic translation elongation factor 1 alpha	AB056104.1	Forward:CCCTGGCCACCGAGATTTCA Reverse:CAGCCTCGAACTCACCAACA	101
lepa1	leptin a1	FJ534535.1	Forward:AGCTCCTCATAGGGGATC Reverse:TAGATGTCGTTCTTTCCTTA	192
npy	neuropeptide Y	M87297.1	Forward:TTCGTCTGCTTGGGAACTCT Reverse:TGGACCTTTTGCCATACCTC	151
pomca	proopiomelanocortin a	AJ431209	Forward:CTCACCACTGACGAGAACATCTTG Reverse:CGGTTTGCTCCAGCTCAGA	161
ppara	peroxisome proliferator-activated receptor alpha	AY894893.1	Forward:TTCCACAGCTGTCAGTCTCG Reverse:CATGAAGATCTGTCCGTAGG	201
pparg	peroxisome proliferator-activated receptor gamma	AY198322	Forward:GAGCCCAAGTTTCAGTTTGC Reverse:CGACAACGAGTTCCTCTTCC	121

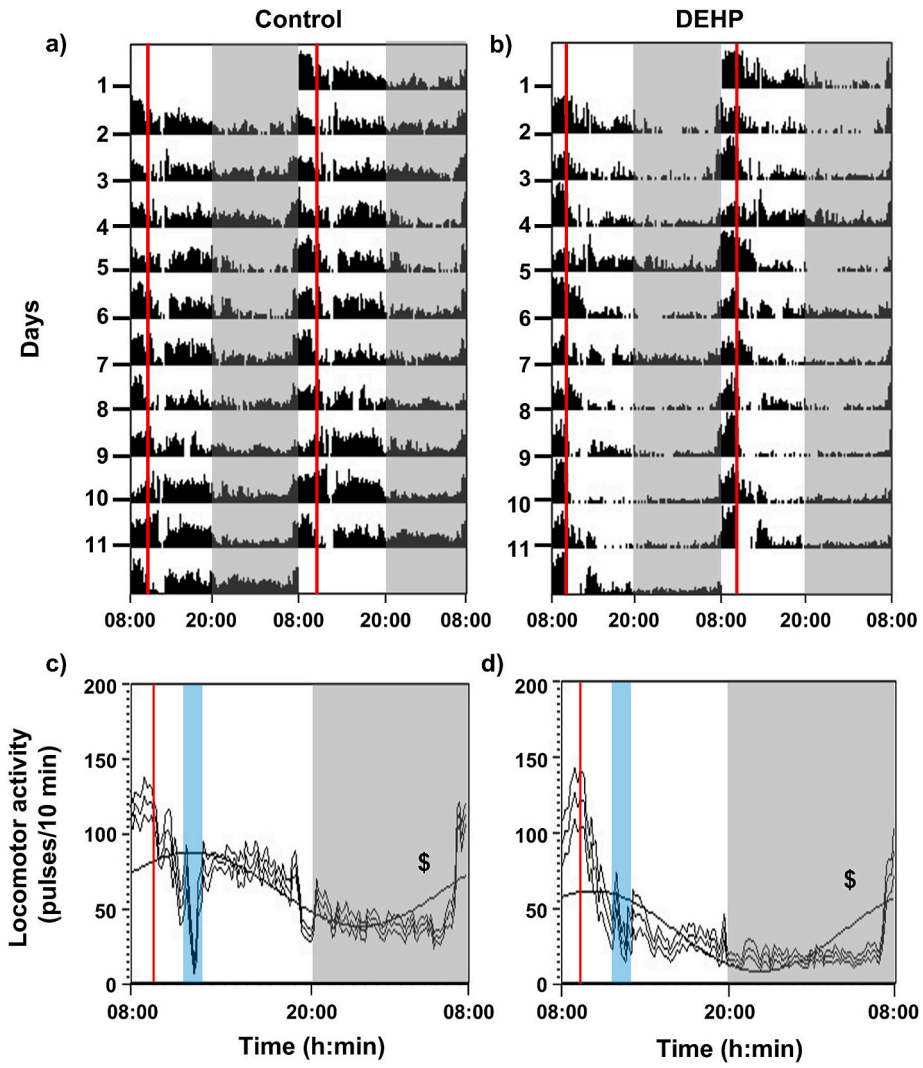

Fig. 1. Locomotor activity of *Carassius auratus* during chronic treatment with vehicle (control) or DEHP. Representative actograms (a,b) are shown in a double plot format (48-h time scale) for better visualization. Corresponding average waveform of locomotor activity (c,d): values are the mean ± SEM ($n = 11$ days per group), bold black line represents periodic sinusoidal function wave. Photophase and scotophase are shown by white and grey areas, respectively. The red line indicates the feeding time (10:00), while the blue shadows denote the injection time (12:30). Significance of the Rayleigh test for a 24-h rhythm are shown ($ $p < 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

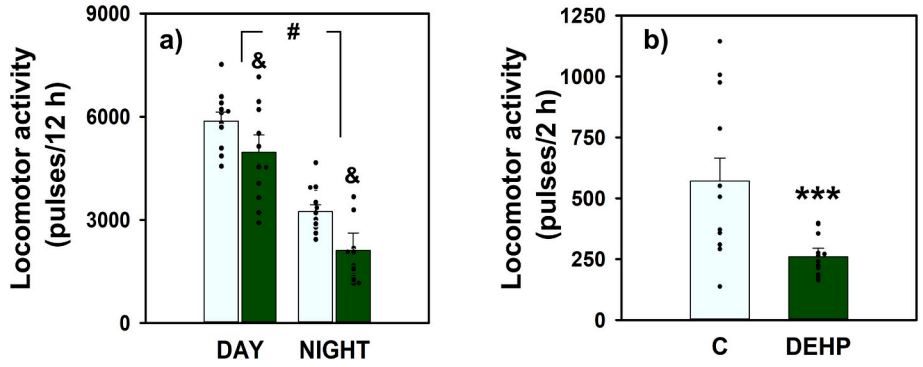

Fig. 2. Effects of chronic treatment DEHP on locomotor activity in *Carassius auratus*. a) General locomotor activity. Two-way ANOVA: & $p < 0.001$, control (white) *versus* DEHP (green); # $p < 0.001$ day *versus* night. b) Food-anticipatory activity (measured during 2 h prior to the feeding time, 8:00–10:00). Student's *t*-test, *** $p < 0.001$ compared to the control (C) group. Data are represented as mean + SEM ($n = 11$/group) and individual values are plotted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

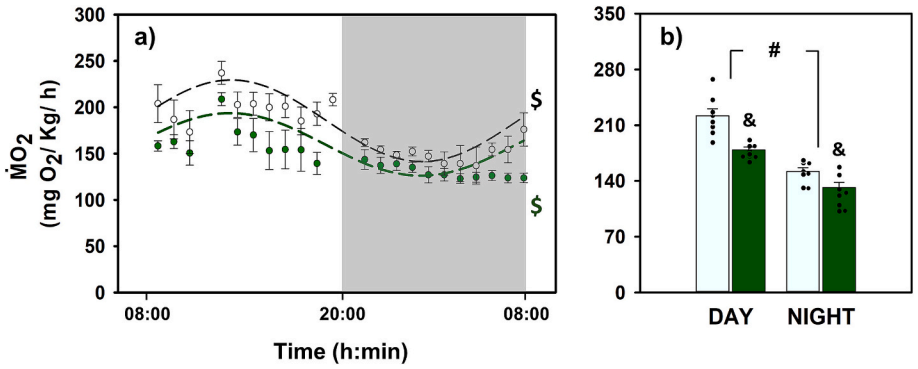

Fig. 3. Oxygen consumption after chronic treatment with vehicle or DEHP in *Carassius auratus*. a) Cosinor analysis of data (mean ± SEM) of the metabolic rate during 24 h (data are grouped by hours); white points for the control group and green ones for the DEHP group. Zero-amplitude test, $ $p < 0.001$; the sinusoidal periodic function is represented as a black (control) or green (DEHP) dashed line. b) Mean + SEM of the oxygen consumption in photophase and scotophase ($n = 8$/group) and individual values are plotted. Two-way ANOVA: & $p < 0.001$, control (white) *versus* DEHP (green); # $p < 0.001$ day *versus* night. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

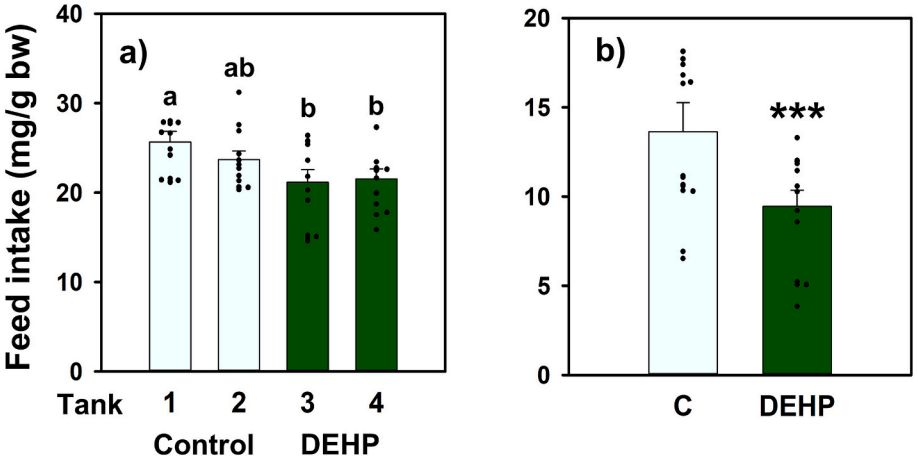

Fig. 4. Effect of the chronic treatment with DEHP on feed intake in *Carassius auratus*. a) Average intake by the group of 6 fish per tank over the 14-day treatment period. One-way ANOVA, SNK, different letters indicate significant differences ($n = 12$/tank). b) Individual feed intake ($n = 12$/group) and individual values are plotted. Student's *t*-test, *** $p < 0.001$ compared to the control group (C). Data are represented as mean + SEM.

Fig. 5 shows the mRNA abundance of feeding regulatory neuropeptides in the hypothalamus. DEHP reduced the expression of orexigenic peptides, significantly ($p < 0.01$) in the case of *npy* (Fig. 5a); the trend did not reach statistical significance in the case of *agrp* (Fig. 5b). Regarding the anorexigenic peptides, the mRNA levels of *pomca* and *cartpt1* were significantly increased ($p < 0.05$) in the DEHP group compared to control (Figs. 5c,d).

The hepatic expression of genes related to feeding and metabolism is illustrated in Fig. 6. DEHP significantly ($p < 0.05$) increased the mRNA abundance of *lepa1* and *ppara* (Figs. 6a,c), with a near-significant ($p = 0.06$) increase in *bmal1a* and *pparg* (Figs. 6b,d).

3.3. Growth and organosomatic indexes

A significant decrease in the condition factor was observed after chronic treatment with DEHP, without significant differences in body weight and length gains, or in standard growth rate (Table 2). The perivisceral fat and the gonadosomatic indexes (VFI and GSI, respectively) were significantly reduced by DEHP administration, while no changes in the hepatosomatic index (HSI) were detected (Table 2).

3.4. Anxiety-like behaviour

Fig. 7 summarises the results from the black-white and open field tests. All the animals spent less time in the aversive zones in both behavioural tests (Figs. 7a,b), as expected. Representative swimming trajectories for these tests are shown in Fig. 8. After 8 days of DEHP treatment, there was a significant decrease ($p < 0.05$) in the time DEHP-treated fish spent in the aversive zones, both white (Fig. 7a) and open field (Fig. 7b). In addition, the number of entries into these aversive zones was significantly lower ($p < 0.05$) in the DEHP group (Figs. 7c,d). There were no significant differences in the time required to enter the white zone (*i.e.*, latency; Fig. 7e), athough the velocity in this zone significantly increased ($p < 0.01$) following DEHP treatment (Fig. 7g). However, no significant differences in either parameter were observed in the open field test (Figs. 7f,h).

4. Discussion

This study provides new insights into the impact of a commonly used plasticiser, DEHP, on energy homeostasis (*i.e.,* energy expenditure and feed intake), growth and fish welfare, offering valuable contributions to understanding the physiological and behavioural responses of aquatic organisms to phthalates.

4.1. Energy expenditure

The significant reduction in locomotor activity and metabolic rate

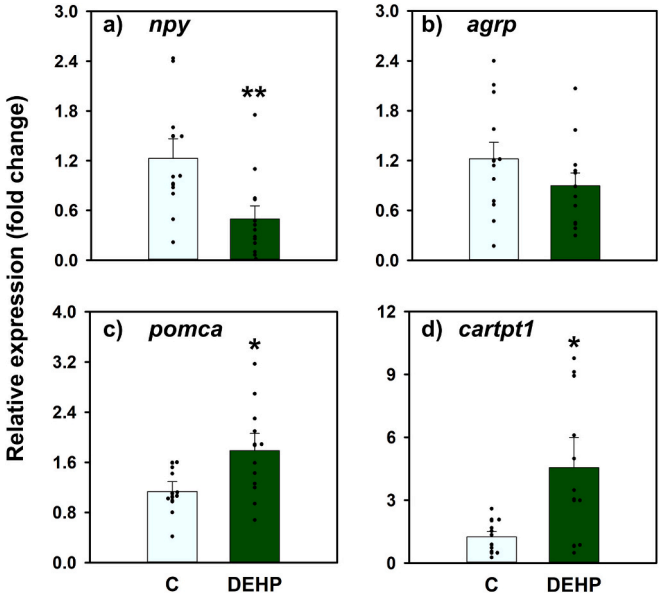

Fig. 5. Relative mRNA abundance of hypothalamic feeding regulators in *Carassius auratus* after chronic treatment with vehicle (control, C) or DEHP. Data are represented as mean + SEM (n = 11–12/group) and individual values are plotted in relative units ($2^{-\Delta\Delta Ct}$). Student's t-test, * $p < 0.05$, ** $p < 0.01$.

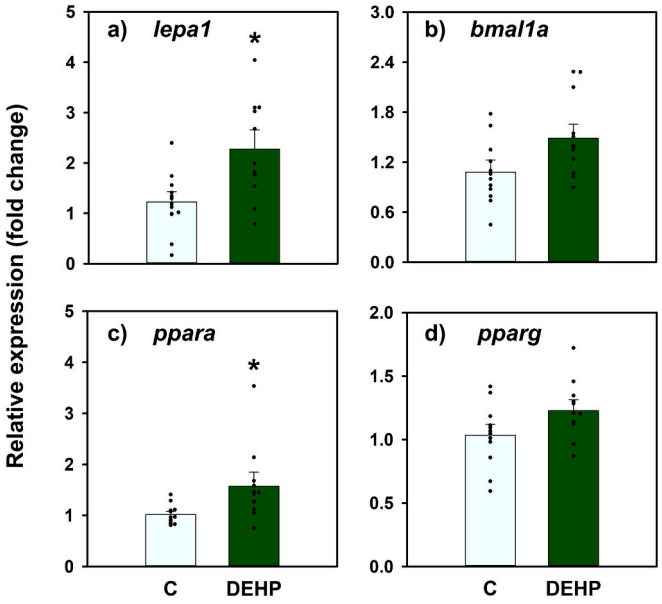

Fig. 6. Relative mRNA abundance of hepatic feeding regulators in *Carassius auratus* after chronic treatment with vehicle (control, C) or DEHP. Data are represented as mean + SEM (n = 11–12/group) and individual values are plotted in relative units ($2^{-\Delta\Delta Ct}$). Student's t-test, * $p < 0.05$.

observed in fish treated with DEHP for 14 days suggests a decrease in energy expenditure. Numerous studies have shown that this phthalate lowers locomotor activity in zebrafish (Huang et al., 2022b; Kaplan et al., 2024; Lu et al., 2021; Qian et al., 2020; Sarangi et al., 2024). The potentially altered mechanisms include dopamine reduction (Huang et al., 2022b), increased acetylcholinesterase (Kaplan et al., 2024) and/or transcriptional alterations of genes related to skeletal and muscle development (Qian et al., 2020). This study is the first to show that DEHP reduces oxygen consumption in goldfish, a proxy for energy expenditure, in addition to locomotor activity. This reduction in oxygen consumption could be related to lower mitochondrial oxygen

Table 2
Effect of DEHP on biometric parameters in *Carassius auratus*.

	Control	DEHP	Student's t-test (p-value)
Initial Body Weight (g)	12.06 ± 0.49	12.07 ± 0.57	$p = 0.991$
Final Body Weight (g)	13.06 ± 0.50	12.89 ± 0.58	$p = 0.827$
Body Weight Gain (%)	8.35 ± 0.53	6.90 ± 0.71	$p = 0.124$
Initial Body Length (cm)	6.86 ± 0.07	7.01 ± 0.10	$p = 0.256$
Final Body Length (cm)	7.09 ± 0.07	7.23 ± 0.10	$p = 0.284$
Body Length Gain (%)	3.32 ± 0.20	3.17 ± 0.29	$p = 0.677$
Specific Growth Rate (%/day)	0.73 ± 0.04	0.6 ± 0.06	$p = 0.124$
Condition Factor	**3.62 ± 0.08**	**3.39 ± 0.05**	**$p = 0.024$** *
Hepatosomatic Index (%)	3.37 ± 0.18	3.43 ± 0.28	$p = 0.860$
Perivisceral Fat Index (%)	**0.82 ± 0.25**	**0.21 ± 0.05**	**$p = 0.010$** *
Gonadosomatic Index (%)	**1.20 ± 0.11**	**0.62 ± 0.12**	**$p = 0.011$** *
Survival Rate (%)	92	100	

Data are represented by mean ± SEM (n = 10–12/group). Student's t-test, significant differences are represented by * and bold numbers.

consumption, as has been shown in mice after MEHP exposure (Traore et al., 2021). Additionally, DEHP-induced toxicity and histopathological changes in gills (Bisai et al., 2022; Desai et al., 2023; Liu et al., 2022) may alter respiration and oxygen uptake, contributing to the reduced oxygen consumption observed in the goldfish.

Goldfish treated with DEHP displayed a reduction in both locomotor activity and oxygen consumption during both the daytime and nighttime periods, while maintaining the daily rhythmicity of both parameters. Both rhythms exhibited a 24-h period with higher values during the day, as previously described for this diurnal species (Gómez-Boronat et al., 2018; Herrera-Castillo et al., 2024; Saiz et al., 2022). Additionally, all the fish, regardless of the treatment, showed an increase in locomotor activity a few hours before feeding time (FAA), as expected with restricted feeding schedules. These results suggest that DEHP does not disrupt circadian rhythms related to locomotor activity and metabolic rates in goldfish under our experimental conditions, though other potential effects on the circadian system cannot be ruled out.

4.2. Feed intake

The reduction in feed intake caused by DEHP supports an anorexigenic action of this phthalate in goldfish. This effect was accompanied by a reduction in the FAA, suggesting that DEHP influences both the consummatory phases of feed intake, but also the preceding phases of feed-seeking, as occurs for other anorexigenic factors in this species (Gómez-Boronat et al., 2020; Saiz et al., 2022). A similar reduction in feed intake was observed in adult zebrafish after 7 days of DEHP exposure (Migliarini et al., 2011). However, other study has reported different responses. For instance, juvenile yellow catfish showed an increase in feed intake at higher doses of DEHP after 56 days, with no changes at the lowest dose (Yuan et al., 2017). Such variations in results following DEHP treatment may be attributed to differences in the type of administration, phthalate dose, treatment duration, species, age and environmental factors such as temperature, among others (Yuan et al., 2017; Zanotelli et al., 2010).

To better understand the observed anorexigenic effect of DEHP, we focused on the hypothalamus, which integrates neuroendocrine signals involved in feeding regulation in fish. There are two populations of mutually inhibiting neurons, one is orexigenic, producing the neuropeptides NPY and AgRP (eating stimulators), and the other is anorexigenic, producing the neuropeptides POMC and CART (eating inhibitors). The balance between these signals allows fish to regulate feed intake according to their metabolic needs and nutritional status (Delgado et al.,

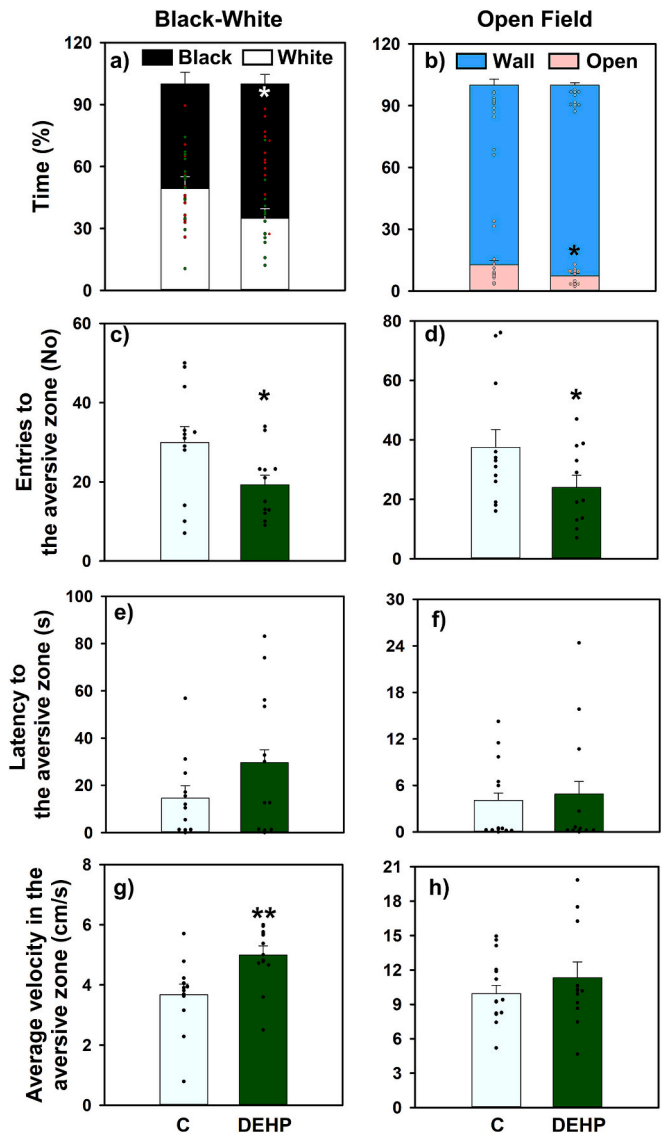

Fig. 7. Anxiety-like behaviour after chronic treatment with vehicle (control, C) or DEHP in *Carassius auratus*. Left column: black-white preference test. Right column: open field test. Aversive zone: white (a, c, e, g) and open (b, d, f, h). Data are represented as mean + SEM (n = 11–12/group). Bars represent the percentage of time spent in each area, with 100 % corresponding to the total time spent in both black and white zones (a),and both wall and open areas (b). Individual values for time in each zone are shown as follows: in the black-white preference test (a), red indicates time spent in the black zone, and green in the white zone; in the open field test (b), blue indicates time spent in the wall area, and pink in the open area. Student's *t*-test, * $p < 0.05$, ** $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2017; Soengas et al., 2024). The present study shows that DEHP up-regulated the anorexigenic signals *pomc* and *cartpt* (genes encoding POMC and CART) in the hypothalamus of the goldfish. Consistently, there was a significant decline in the mRNA abundance of orexigenic *npy*, and a downward trend in *agrp* expression. Although protein levels have not been measured, mRNA abundance suggests that the balance between orexigenic and anorexigenic peptides shifts toward the inhibition of feed intake. These findings align with those observed in zebrafish, where DEHP reduced expression of orexigenic signals (*i.e.*, NPY, orexin, and cannabinoid receptor) in whole brains (Migliarini et al., 2011). DEHP also appears to interfere with peripheral feeding regulation, as hepatic expression of *lepa* and *ppara* (genes encoding

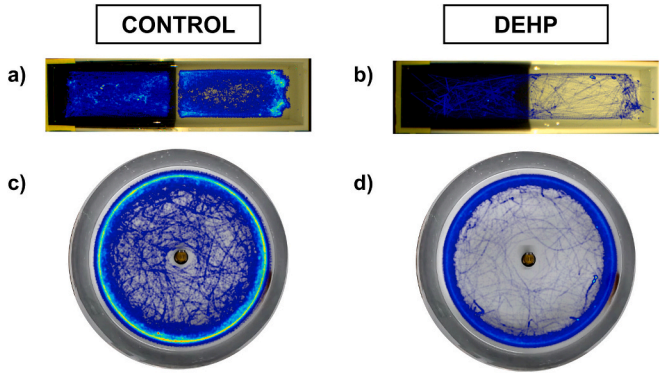

Fig. 8. Representative swimming trajectories of *Carassius auratus* in control and DEHP groups. a-b) Black-white preference test (average of 6 fish per group). c-d) Open field test (average of 12 fish per group).The movement of fish was monitored over a period of 10 min, with the data obtained using EthoVision XT 17.5 tracking software.

leptin and PPARα) were significantly increased, both being anorexigenic factors in goldfish (de Pedro et al., 2006; Gómez-Boronat et al., 2020). Leptin, synthetised mainly in the liver of fish, acts at the hypothalamic level, activating POMC/CART neurons and inhibiting NPY/AgRP neurons (Blanco and Soengas, 2021). This mechanism might explain our results: DEHP increased *leptin*, leading to reduced *npy* and increased *pomc* and *cartpt*, ultimately reducing feed intake. This hypothesis is supported by the hepatic increase in *ppara* observed in our study, as activation of this nuclear receptor with SR9009 agonist has an anorectic effect, associated with increased hepatic *lepa* and reduced hypothalamic *npy* in goldfish (Gómez-Boronat et al., 2020).

The activation of PPAR receptors and increased expression seems to be a generalized effect of DEHP in mammals and birds (Huang et al., 2022a; Ito et al., 2019; Wójtowicz et al., 2023). Similar results have been reported in various fish species, including zebrafish (Migliarini et al., 2011), sharptooth catfish (Adeogun et al., 2018) and yellow catfish (Meng et al., 2018), consistent with the data obtained in goldfish in this study. Such PPARα activation induced by DEHP could explain the increase in *bmal* mRNA abundance observed in this work, as *bmal* expression increases following PPARα activation in goldfish (Gómez-Boronat et al., 2020). It is well documented that DEHP significantly affects lipid metabolism in fish by regulating PPARs (Kwan et al., 2021; Yang et al., 2024). Thus, it is hypothesised that DEHP may influence hepatic lipid metabolism by modulating PPAR signalling in goldfish too. However, the specific effects of increased hepatic expression of *ppars* and *bmal* on lipid metabolism in goldfish remain to be elucidated, as does whether these transcriptional changes result in modifications at the protein level. These intriguing questions warrant further investigation.

4.3. Growth performance

Although DEHP-treated animals showed decreased feed intake, 14 days of exposure did not affect body weight and length gain, growth rate or HSI in goldfish. This may be attributed to the fact that the reduction in feed intake was compensated by a decrease in energy expenditure, maintaining the final energy balance. Similarly, no changes in length, body weight, and/or HSI have been previously reported after the same experimental period of DEHP exposure in goldfish (Golshan et al., 2015) and sharptooth catfish (Adeogun et al., 2018), as well as in zebrafish after 3 days of exposure (Lu et al., 2021). However, different results have been reported in another teleost. A reduction in growth, indicated by the decreased length and body weight, was reported in medaka (*Oryzias latipes;* Yang et al., 2018), and guppy fish (*Poecilia reticulata*; Zanotelli et al., 2010). On the other hand, DEHP increased weight gain and HSI in juvenile yellow catfish (Mo et al., 2019) and zebrafish (Jia et al., 2021), with longer exposure periods (2 and 3.5 months, respectively).

Particularly, in zebrafish, 7.5 months of DEHP exposure in zebrafish decreased physical fitness and lipid utilization, preventing body mass loss and suggesting an obesogenic effect (Buerger et al., 2019). This obesogenic action is linked to the up-regulation of *srebp* (sterol regulatory element-binding protein) and *pparα* in zebrafish liver, potentially leading to fatty liver (Migliarini et al., 2011). In fact, DEHP increased HSI and induced hepatic steatosis by fat accumulation in the liver of yellow catfish (Mo et al., 2019). If lipid accumulation also occurs in the liver of goldfish, it could explain why HSI, although not increasing, at least did not decrease, unlike other organosomatic indices (GSI and VFI) observed in this study. Moreover, DEHP reduced VFI in goldfish, as with decreased adipose tissue in rats (Martinelli et al., 2010), suggesting possible alterations in lipid metabolism. The reduction in GSI observed in goldfish has been widely documented in other fish species exposed to DEHP, along with reproductive alterations (Bisai et al., 2023; Kwan et al., 2021; Zhu et al., 2016).

4.4. Behaviour and welfare

Several findings from the present study indicate that DEHP impairs the well-being of goldfish. Notably, the condition factor was diminished in goldfish chronically treated with DEHP, similar to the effects observed in zebrafish (Jia et al., 2021), suggesting that these fish were not in an optimal state of fitness and welfare (Neethiselvan et al., 2023). This result aligns with the behavioural tests conducted, where DEHP-treated goldfish exhibited increased thigmotaxis and scototaxis in the open field and black-white tests, respectively, both indicative of heightened anxiety-like behaviours (Saiz et al., 2023a). Recent studies in zebrafish have demonstrated that DEHP induces neurobehavioural modifications, affecting scototaxis, bottom-dwelling, exploratory activity and promoting aggressive behaviours (Sarangi et al., 2024). All these behavioural disruptions caused by DEHP in zebrafish were associated with increased oxidative stress and neurodegeneration. Although studies in fish are still scarce, our results support the anxiogenic effects of DEHP, a finding widely documented in rodent models (Carbone et al., 2019; Eleiwa et al., 2024; Li et al., 2024b). The exact mechanism of this effect is not yet fully understood. However, considering that DEHP modulates hypothalamic neuropeptides, as demonstrated in the present study, this could be one potential mechanism. Many brain neuropeptides systems involved in energy homeostasis also play a key role in anxiety regulation (Matsuda et al., 2011; Satao and Doshi, 2024). Specifically, an increase in *npy* and *agrp* expression, alongside a decrease in *pomc*, has been associated with anxiolytic effects in medaka (Lu et al., 2023). In our study, DEHP treatment led to a significant decrease in *npy* expression, while *pomc* and *cartpt* were up-regulated in the hypothalamus. These changes suggest that DEHP may induce an anxiogenic state by altering hypothalamic signalling pathways. The observed behavioural effects are therefore consistent with neuropeptide-mediated anxiogenic responses (Lu et al., 2023; Matsuda et al., 2011), reinforcing the link between DEHP exposure, hypothalamic dysregulation and altered anxiety-like responses. Further research is needed to elucidate the precise mechanisms by which phthalates influence neuropeptide signalling and induce neurobehavioural changes in fish. Future studies incorporating region-specific analyses of additional brain areas, such as the telencephalon or limbic structures, may provide deeper insights into the pathways mediating these effects.

5. Conclusion

DEHP impairs feed intake in goldfish by altering central and peripheral feeding regulation, resulting in an imbalance in favour of anorexigenic signals. Despite this, body weight and growth are not reduced, probably due to decreased energy expenditure, as DEHP also reduces locomotor activity and oxygen consumption. Additionally, the decrease in condition factor and increase in anxiety-like behaviours indicate that this phthalate is detrimental to fish well-being. These findings highlight the physiological and behavioural disruptions caused by DEHP, and provide valuable insights into the possible regulatory mechanisms involved.

CRediT authorship contribution statement

Lisbeth Herrera-Castillo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Claudia Hernández-Villasevil:** Writing – original draft, Methodology, Investigation. **André Barany:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Miguel Gómez-Boronat:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Esther Isorna:** Writing – review & editing, Funding acquisition, Conceptualization. **Nuria de Pedro:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2025.111878.

Data availability

Data are free available in the UCM Data base DOCTA (https://hdl.handle.net/20.500.14352/114762).

References

Adeogun, A.O., Ibor, O.R., Imiuwa, M.E., Omogbemi, E.D., Chukwuka, A.V., Omiwole, R. A., Arukwe, A., 2018. Endocrine disruptor responses in African sharptooth catfish (*Clarias gariepinus*) exposed to di-(2-ethylhexyl)-phthalate. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 213, 7–18. https://doi.org/10.1016/J.CBPC.2018.07.001.

Berthier, A., Johanns, M., Zummo, F.P., Lefebvre, P., Stael, B., 2021. PPARs in liver physiology. Biochim. Biophys. Acta - Mol. Basis Dis. 1867, 166097. https://doi.org/10.1016/j.bbadis.2021.166097.

Bisai, K., Behera, B.K., Nayak, S.K., Pati, M.K., 2022. Effect of di-(2-ethylhexyl) phthalate on certain haematological and histological parameters of common carp (*Cyprinus carpio*). Toxicol. Int. 29, 227–237. https://doi.org/10.18311/ti/2022/v29i2/29129.

Bisai, K., Kumar, V., Roy, A., Parida, S.N., Dhar, S., Das, B.K., Behera, B.K., Pati, M.K., 2023. Effects of di-(2-Ethylhexyl) phthalate (DEHP) on gamete quality parameters of male koi carp (*Cyprinus carpio*). Curr. Issues Mol. Biol. 45, 7388–7403. https://doi.org/10.3390/cimb45090467.

Blanco, A.M., Soengas, J.L., 2021. Leptin signalling in teleost fish with emphasis in food intake regulation. Mol. Cell. Endocrinol. 526, 111209. https://doi.org/10.1016/j.mce.2021.111209.

Blanco, A.M., Unniappan, S., 2022. Goldfish (*Carassius auratus*): Biology, husbandry, and research applications. In: D'Angelo, L., Girolamo, P. (Eds.), Laboratory Fish in

Biomedical Research. Academic Press, London, pp. 373–408. https://doi.org/10.1016/B978-0-12-821099-4.00012-2.
Boukouvala, E., Krey, G., 2024. The peroxisome proliferator-activated receptors of ray-finned fish: unique structures. Elusive Functions. Biomolecules 14, 634. https://doi.org/10.3390/biom14060634.
Buerger, A.N., Schmidt, J., Chase, A., Paxaio, C., Patel, T.N., Brumback, B.A., Kane, A.S., Martyniuk, C.J., Bisesi, J.H., 2019. Examining the responses of the zebrafish (*Danio rerio*) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environ. Pollut. 245, 1086–1094. https://doi.org/10.1016/J.ENVPOL.2018.11.032.
Carbone, S., Ponzo, O.J., Gobetto, N., Samaniego, Y.A., Reynoso, R., Moguilevsky, J.A., Cutrera, R.A., 2019. Effect of di(2-ethylhexyl) phthalate on the neuroendocrine regulation of reproduction in adult male rats and its relationship to anxiogenic behavior: participation of GABAergic system. Hum. Exp. Toxicol. 38, 25–35. https://doi.org/10.1177/0960327118774868.
da Costa, J.M., Kato, L.S., Galvan, D., Lelis, C.A., Saraiva, T., Conte-Junior, C.A., 2023. Occurrence of phthalates in different food matrices: a systematic review of the main sources of contamination and potential risks. Compr. Rev. Food Sci. Food Saf. 22, 2043–2080. https://doi.org/10.1111/1541-4337.13140.
de Pedro, N., Martínez-Álvarez, R., Delgado, M.J., 2006. Acute and chronic leptin reduces food intake and body weight in goldfish (*Carassius auratus*). J. Endocrinol. 188, 513–520. https://doi.org/10.1677/joe.1.06349.
Delgado, M.J., Cerdá-Reverter, J.M., Soengas, J.L., 2017. Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: involvement in the control of food intake. Front. Neurosci. 11, 354. https://doi.org/10.3389/fnins.2017.00354.
Desai, A., Pathan, F., Yadav, R., Yogi, D., Nanajkar, M.R., 2023. Phthalate induced hormetic effect reveals susceptibility of gill compared to muscle tissue after depuration in commercially important fish (*Etroplus suratensis*). Mar. Pollut. Bull. 194, 115238. https://doi.org/10.1016/J.MARPOLBUL.2023.115238.
Dueñas-Moreno, J., Mora, A., Cervantes-Avilés, P., Mahlknecht, J., 2022. Groundwarer contamination pathways of phthalates and bisphenol a: origin, characteristics, transport, and fate – a review. Environ. Int. 170, 107550. https://doi.org/10.1016/j.envint.2022.107550.
Eales, J., Bethel, A., Galloway, T., Hopkinson, P., Morrissey, K., Short, R.E., Garside, R., 2022. Human health impacts of exposure to phthalate plasticisers: an overview of reviews. Environ. Int. 158, 106903. https://doi.org/10.1016/j.envint.2021.106903.
Eleiwa, N.Z., Elsayed, A.S., Said, E.N., Metwally, M.M., Abd-Elhakim, Y.M., 2024. Di (2-ethylhexyl) phthalate alters neurobehavioral responses and oxidative status, architecture, and GFAP and BDNF signaling in juvenile rat's brain: protective role of Coenzyme10. Food Chem. Toxicol. 184, 114372. https://doi.org/10.1016/j.fct.2023.114372.
FAO, 2024. The State of World Fisheries and Aquaculture 2024 - Blue Transformation in Action; Rome.
Golshan, M., Alavi, S.M.H., 2019. Androgen signaling in male fishes: examples of anti-androgenic chemicals that cause reproductive disorders. Theriogenology 139, 58–71. https://doi.org/10.1016/j.theriogenology.2019.07.020.
Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I.A.E., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S.M.H., 2015. Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquat. Toxicol. 163, 16–26. https://doi.org/10.1016/J.AQUATOX.2015.03.017.
Gómez-Boronat, M., Sáiz, N., Delgado, M.J., de Pedro, N., Isorna, E., 2018. Time-lag in feeding schedule acts as a stressor that alters circadian oscillators in goldfish. Front. Physiol. 9. https://doi.org/10.3389/fphys.2018.01749.
Gómez-Boronat, M., Isorna, E., Conde-Sieira, M., Delgado, M.J., Soengas, J.L., de Pedro, N., 2020. First evidence on the role of palmitoylethanolamide in energy homeostasis in fish. Horm. Behav. 117, 104609. https://doi.org/10.1016/J.YHBEH.2019.104609.
Herrera-Castillo, L., Vallejo-Palma, G., Saiz, N., Sánchez-Jiménez, A., Isorna, E., Ruiz-Jarabo, I., de Pedro, N., 2024. Metabolic rate of goldfish (*Carassius auratus*) in the face of common aquaculture challenges. Biol 13, 804. https://doi.org/10.3390/biology13100804.
Huang, Y.-Q., Tang, Y.X., Qiu, B.H., Talukder, M., Li, X.N., Li, J.L., 2022a. Di-2-ethylhexyl phthalate (DEHP) induced lipid metabolism disorder in liver via activating the LXR/SREBP-1c/PPARα/γ and NF-κB signaling pathway. Food Chem. Toxicol. 165, 113119. https://doi.org/10.1016/j.fct.2022.113119.
Huang, W., Xiao, J., Shi, X., Zheng, S., Li, H., Liu, C., Wu, K., 2022b. Effects of di-(2-ethylhexyl) phthalate (DEHP) on behavior and dopamine signaling in zebrafish (*Danio rerio*). Environ. Toxicol. Pharmacol. 93, 103885. https://doi.org/10.1016/J.ETAP.2022.103885.
Hurst, C.H., Waxman, D.J., 2003. Activation of PPARα and PPARγ by environmental phthalate monoesters. Toxicol. Sci. 74, 297–308. https://doi.org/10.1093/toxsci/kfg145.
Ito, Y., Kamijima, M., Nakajima, T., 2019. Di(2-ethylhexyl) phthalate-induced toxicity and peroxisome proliferator-activated receptor alpha: a review. Environ. Health Prev. Med. 24, 1–9. https://doi.org/10.1186/s12199-019-0802-z.
Jia, P.P., Junaid, M., Xin, G.Y., Wang, Y., Ma, Y.B., Pei, D.S., 2021. Disruption of intestinal homeostasis through altered responses of the microbial community, energy metabolites, and immune system in zebrafish after chronic exposure to DEHP. Front. Microbiol. 12, 729530. https://doi.org/10.3389/fmicb.2021.729530.
Kaplan, G., Beler, M., Ünal, I., Karagöz, A., Eğilmezer, G., Üstündağ, Ü.V., Cansız, D., Alturfan, A.A., Emekli-Alturfan, E., 2024. Diethylhexyl phthalate exposure amplifies oxidant and inflammatory response in fetal hyperglycemia model predisposing insulin resistance in zebrafish embryos. Toxicol. Ind. Health 40, 232–243. https://doi.org/10.1177/07482337241238475.
Khoshmanesh, M., Farjadfard, S., Ahmadi, M., Ramavandi, B., 2024. Review of toxicity and global distribution of phthalate acid esters in fish. Sci. Total Environ. 953, 175966. https://doi.org/10.1016/J.SCITOTENV.2024.175966.
Kwan, W.S., Roy, V.A.L., Yu, K.N., 2021. Review on toxic effects of Di(2-ethylhexyl) phthalate on zebrafish embryos. Toxics 9, 193. https://doi.org/10.3390/TOXICS9080193.
Li, L., Guo, Z., Deng, R., Fan, T., Dong, D., Dai, Y., Li, C., 2024a. The concentrations and behavior of classic phthalates and emerging phthalate alternatives in different environmental matrices and their biological health risks. Environ. Sci. Pollut. Res. Int. 31, 46790–46805. https://doi.org/10.1007/s11356-024-34213-w.
Li, L., Su, Y., Wang, S., Wang, C., Ruan, N., Hu, Z., Cheng, X., Chen, J., Yuan, K., Peijun, L., Fan, P., 2024b. Neonatal di-(2-ethylhexyl) phthalate exposure induces permanent alterations in secretory CRH neuron characteristics in the hypothalamus paraventricular region of adult male rats. Experim. Neurol. 372, 114616. https://doi.org/10.1016/j.expneurol.2023.114616.
Liu, Y., Chen, Z., Li, S., Ding, L., Wei, X., Han, S., Wang, P., Sun, Y., 2022. Multi-omics profiling and biochemical assays reveal the acute toxicity of environmental related concentrations of Di-(2-ethylhexyl) phthalate (DEHP) on the gill of crucian carp (*Carassius auratus*). Chemosphere 307, 135814. https://doi.org/10.1016/J.CHEMOSPHERE.2022.135814.
Liu, Y., Wu, N.-N., Xu, R., Li, Z.-H., Xu, X.-R., Liu, S., 2024. Phthalates released from microplastics can't be ignored: sources, fate, ecological risks, and human exposure risks. TrAC Trends Anal. Chem. 179, 117870. https://doi.org/10.1016/J.TRAC.2024.117870.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−DDCT method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.
Lu, C., Luo, J., Liu, Y., Yang, X., 2021. The oxidative stress responses caused by phthalate acid esters increases mRNA abundance of base excision repair (BER) genes in vivo and in vitro. Ecotoxicol. Environ. Saf. 208, 111525. https://doi.org/10.1016/j.ecoenv.2020.111525.
Lu, K., Jia, X., Wu, J., Wang, Q., Liang, X.F., 2023. Neuropeptide Y receptor Y2 (npy2r) deficiency reduces anxiety and increases food intake in Japanese medaka (*Oryzias latipes*). Front. Cell Dev. Biol. 11, 1273006. https://doi.org/10.3389/fcell.2023.1273006.
Martinelli, M.I., Mocchiutti, N.O., Bernal, C.A., 2010. Effect of di(2-ethylhexyl) phthalate (DEHP) on lipolysis and lipoprotein lipase activities in adipose tissue of rats. Hum. Exp. Toxicol. 29, 739–745. https://doi.org/10.1177/0960327110361750.
Matsuda, K., Kang, K.S., Sakashita, A., Yahashi, S., Vaudry, H., 2011. Behavioral effect of neuropeptides related to feeding regulation in fish. Ann. N. Y. Acad. Sci. 1220, 117–126. https://doi.org/10.1111/j.1749-6632.2010.05884.x.
Meng, F.X., Li, M., Song, M.Z., Yuan, L.X., Gong, Y.F., Qian, Y.X., Shi, G., Wang, R.X., 2018. Di-2-ethylhexyl phthalate (DEHP) exposure disturbs lipid metabolism in juvenile yellow catfish *Tachysurus fulvidraco*. J. Fish Biol. 92, 85–93. https://doi.org/10.1111/JFB.13493.
Migliarini, B., Piccinetti, C.C., Martella, A., Maradonna, F., Gioacchini, G., Carnevali, O., 2011. Perspectives on endocrine disruptor effects on metabolic sensors. Gen. Comp. Endocrinol. 170, 416–423. https://doi.org/10.1016/J.YGCEN.2010.11.025.
Mo, N., Zhang, M., Wang, R., Xia, S., Meng, F., Qian, Y., Li, M., 2019. Effects of α-ethinyl estradiol (EE2) and diethylhexyl phthalate (DEHP) on growth performance, antioxidant status and immune response of juvenile yellow catfish *Pelteobagrus fulvidraco*. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 226, 108615. https://doi.org/10.1016/J.CBPC.2019.108615.
Molcan, L., 2019. Time distributed data analysis by Cosinor Online application. bioRxiv 805960. https://doi.org/10.1101/805960.
Neethiselvan, N., Jayakumar, N., Sudhan, C., Umamaheswari, T., Padmavathy, P., Ravikumar, T., 2023. Length-weight relationships and Fulton's condition factor (K) for 29 demersal reef fishes caught by longline. Int. J. Mar. Sci. 39, 1263–1270. https://doi.org/10.1007/s41208-023-00588-9.
Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., et al., 2020. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 18, e3000411. https://doi.org/10.1371/journal.pbio.3000411.
Qian, L., Liu, J., Lin, Z., Chen, X., Yuan, L., Shen, G., Yang, W., Wang, D., Huang, Y., Pang, S., Mu, X., Wang, C., Li, Y., 2020. Evaluation of the spinal effects of phthalates in a zebrafish embryo assay. Chemosphere 249, 126144. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126144.
Qu, R., Feng, M., Sun, P., Wang, Z., 2014. A comparative study on antioxidant status combined with integrated biomarker response in *Carassius auratus* fish exposed to nine phthalates. Environ. Toxicol. 2015 (10), 1125–1134. https://doi.org/10.1002/tox.21985.
Saiz, N., Herrera-Castillo, L., Isorna, E., Delgado, M.J., Conde-Sieira, M., Soengas, J.L., de Pedro, N., 2022. REV-ERBα agonist SR9009 promotes a negative energy balance in goldfish. Int. J. Mol. Sci. 23, 2921. https://doi.org/10.3390/ijms23062921.
Saiz, N., Herrera-Castillo, L., de Pedro, N., Delgado, M.J., Arvidsson, S.D., Marugal-López, M.Á., Isorna, E., 2023a. Assessing Chronodisruption distress in goldfish: the importance of multimodal approaches. Animals 13, 2481. https://doi.org/10.3390/ani13152481.
Saiz, N., Herrera-Castillo, L., Gómez-Boronat, M., Delgado, M.J., Isorna, E., de Pedro, N., 2023b. Daily rhythms of REV-ERBα and its role as transcriptional repressor of clock genes in fish hepatic oscillator. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 283, 111458. https://doi.org/10.1016/j.cbpa.2023.111458.
Sarangi, P., Sahoo, P.K., Pradhan, L.K., Bhoi, S., Sahoo, B.S., Chauhan, N.R., Raut, S., Das, S.K., 2024. Concerted monoamine oxidase activity following exposure to di-2-ethylhexyl phthalate is associated with aggressive neurobehavioral response and

neurodegeneration in zebrafish brain. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 283, 109970. https://doi.org/10.1016/J.CBPC.2024.109970.
Satao, K.S., Doshi, G.M., 2024. Anxiety and the brain: neuropeptides as emerging factors. Pharmacol. Biochem. Behav. 173878. https://doi.org/10.1016/j.pbb.2024.173878.
Sato, T., Sato, S., 2023. Circadian regulation of metabolism: commitment to health and diseases. Endocrinology 164, bqad086. https://doi.org/10.1210/endocr/bqad086.
Soengas, J.L., Comesaña, S., Blanco, A.M., Conde-Sieira, M., 2024. Feed intake regulation in fish: implications for aquaculture. Rev. Fish. Sci. Aquac. 1–53. https://doi.org/10.1080/23308249.2024.2374259.
Traore, K., More, P., Adla, A., Dogbey, G., Papadopoulos, V., Zirkin, B., 2021. MEHP induces alteration of mitochondrial function and inhibition of steroid biosynthesis in MA-10 mouse tumor Leydig cells. Toxicol 463, 152985. https://doi.org/10.1016/J.TOX.2021.152985.
Uren-Webster, T.M., Lewis, C., Filby, A.L., Paull, G.C., Santos, E.M., 2010. Mechanisms of toxicity of di(2-ethylhexyl) phthalate on the reproductive health of male zebrafish. Aquat. Toxicol. 99, 360–369. https://doi.org/10.1016/j.aquatox.2010.05.015.
Wang, Y., Zhu, H., Kannan, K., 2019. A review of biomonitoring of phthalate exposures. Toxics 7, 21. https://doi.org/10.3390/TOXICS7020021.
Wójtowicz, A.K., Sitarz-Głownia, A.M., Wnuk, A., Kajta, M., Szychowski, K.A., 2023. Involvement of the peroxisome proliferator-activated receptor gamma (Pparγ) and matrix metalloproteinases-2 and-9 (Mmp-2 and-9) in the mechanism of action of di (2-ethylhexyl) phthalate (DEHP) in cultured mouse brain astrocytes and neurons. Toxicol. In Vitro 92, 105639. https://doi.org/10.1016/j.tiv.2023.105639.
Yang, W.K., Chiang, L.F., Tan, S.W., Chen, P.J., 2018. Environmentally relevant concentrations of di(2-ethylhexyl) phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Sci. Total Environ. 640–641, 512–522. https://doi.org/10.1016/J.SCITOTENV.2018.05.312.
Yang, Y., Tao, Y., Yang, R., Yi, X., Zhong, G., Gu, Y., Zhang, Y., 2024. Ca^{2+} homeostasis imbalance induced by Pparg: a key factor in di (2-ethylhexyl) phthalate (DEHP)-induced cardiac dysfunction in zebrafish larvae. Sci. Total Environ. 918, 170436. https://doi.org/10.1016/J.SCITOTENV.2024.170436.
Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R., 2017. Growth, blood health, antioxidant status, immune response and resistance to *Aeromonas hydrophila* of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comp. Biochem. Physiol. C Toxicol. Pharmacol. 202, 79–84. https://doi.org/10.1016/J.CBPC.2017.08.004.
Zanotelli, V.R.T., Neuhauss, S.C.F., Ehrengruber, M.U., 2010. Long-term exposure to bis (2-ethylhexyl) phthalate (DEHP) inhibits growth of guppy fish (*Poecilia reticulata*). J. Appl. Toxicol. 30, 29–33. https://doi.org/10.1002/jat.1468.
Zhang, Y., Jiao, Y., Li, Z., Tao, Y., Yang, Y., 2021. Hazards of phthalates (PAEs) exposure: a review of aquatic animal toxicology studies. Sci. Total Environ. 771, 145418. https://doi.org/10.1016/j.scitotenv.2021.145418.
Zhu, Y., Hua, R., Zhou, Y., Li, H., Quan, S., Yu, Y., 2016. Chronic exposure to mono-(2-ethylhexyl)-phthalate causes endocrine disruption and reproductive dysfunction in zebrafish. Environ. Toxicol. Chem. 35, 2117–2124. https://doi.org/10.1002/etc.3369.