Grupo de Interesse
Medicamento e Ambiente

Avaliação de efeitos de medicamentos em organismos de diferentes níveis tróficos: do esgoto ao mar

Bruno Nunes

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Grupo de Interesse
Medicamento e Ambiente

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Principal Investigator with Habilitation
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Bachelor's Degree in Pharmaceutical Sciences, FFUP
Ph.D. in Biomedical Sciences, ICBAS-UP
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Expert, Medicines Evaluation Commission, INFARMED
Member of the Medicines and Environment Interest Group, Ordem dos Farmacêuticos (Portuguese Pharmaceutical Society)
https://www.cesam-la.pt/brunonunes/

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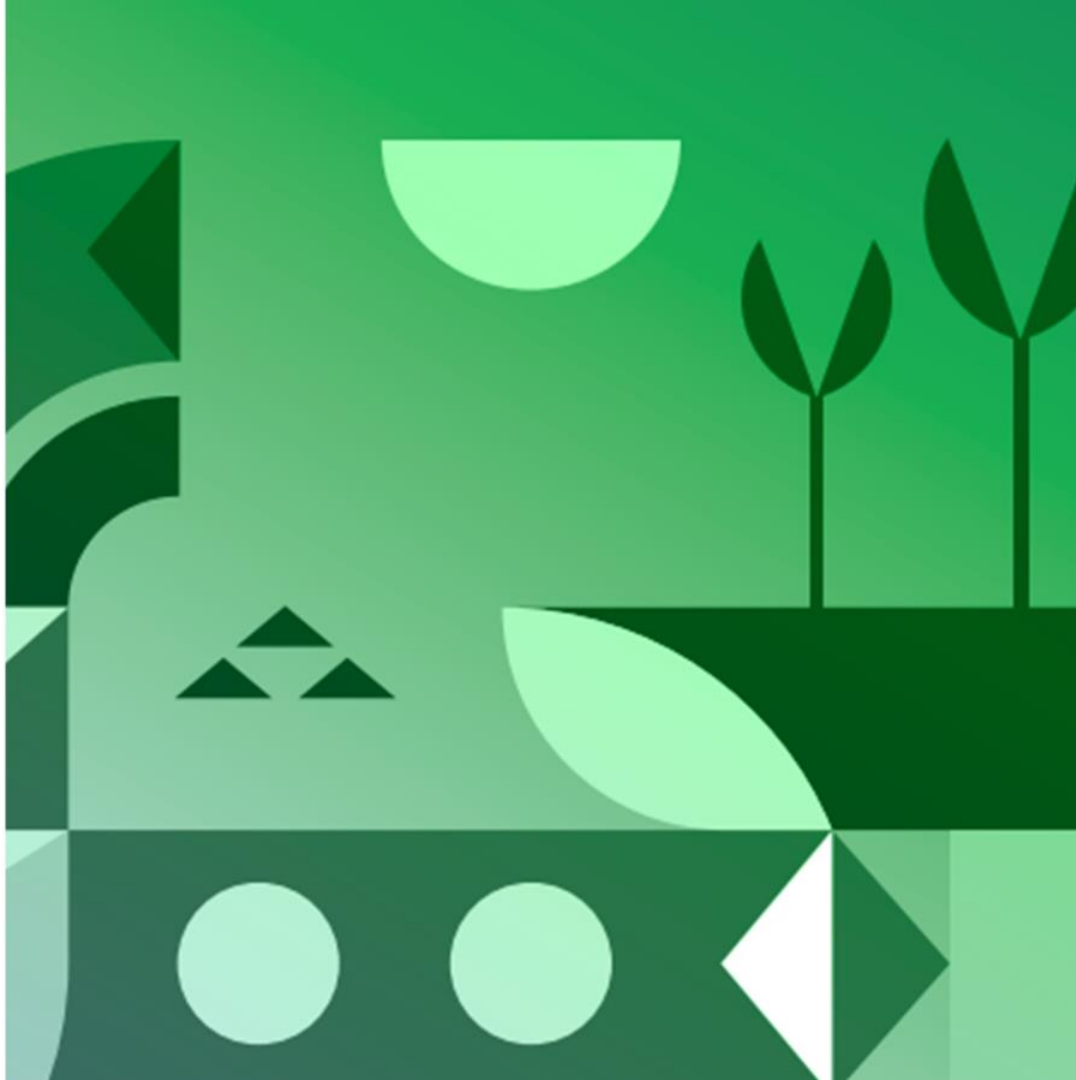

ORDEM DOS FARMACÊUTICOS

Regulatory framework

2006

European Medicines Agency
Pre-Authorisation Evaluation of Medicines for Human Use

London, 20 January 2005
CHMP/SWP/4447/00 draft

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

DRAFT

GUIDELINE ON THE ENVIRONMENTAL RISK ASSESSMENT OF MEDICINAL PRODUCTS FOR HUMAN USE

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 November 2018
EMEA/CHMP/SWP/4447/00 Rev. 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on the environmental risk assessment of medicinal products for human use
Draft

2024

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

22 August 2024
EMEA/CHMP/SWP/4447/00 Rev. 1- Corr.*
Committee for Medicinal Products for Human Use (CHMP)
Guideline on the environmental risk assessment of medicinal products for human use

All medicinal products are subject to an Environmental Risk Assessment prior to being marketed within the EU

Bruno Nunes
CESAM, DBio, University of Aveiro, 20 June 2025

Specific characteristics of pharmaceuticals in the environment

1) High pharmacological potency
2) Lipophilicity + potential effects on non-target organisms
3) Resistant to (bio)degradation, e.g., WWTPs
4) Can take multiple forms with different biological activities
5) Have never been tested in many non-target species
6) What about interactions when present in very complex mixtures?

Specific characteristics of pharmaceuticals in the environment

Main sources of medicinal drugs in the wild

1) Domestic sewage after excretion
2) Drugs discarded in the sewage system
3) Urban residues
4) Industrial effluents
5) **Hospital sewage**

Result: presence in the aquatic compartment – adverse effects in aquatic organisms – how to address it?

Developments in the Ecotoxicological Assessment of Drugs

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Ecotoxicology and Environmental Safety 61 (2005) 413–419

Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Acute toxicity of widely used pharmaceuticals in aquatic species: *Gambusia holbrooki*, *Artemia parthenogenetica* and *Tetraselmis chuii*

B. Nunes[a,b,*], F. Carvalho[c], L. Guilhermino[a,b]

[a]*ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Departamento de Estudos de Populações, Laboratório de Ecotoxicologia, Universidade do Porto, Largo Prof. Abel Salazar, 2, 4099-003 Porto, Portugal*
[b]*CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Laboratório de Ecotoxicologia, Rua dos Bragas, 289, 4050-123 Porto, Portugal*
[c]*REQUIMTE, Serviço de Toxicologia da Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal*

Received 3 November 2003; received in revised form 18 August 2004; accepted 25 August 2004
Available online 5 November 2004

Classic ecotoxicological tools – standardized tests; eg.: OCDE – lethality, growth

First approach – calculation of lethality and growth inhibition

Clofibric acid: 526.5 mg/l

Clofibrate: 7.7 mg/l

Diazepam: 12.7 mg/l

SDS: 15.1 mg/l

- Even considering an LC_{50} 7.7mg/l – 3 orders of magnitude above what is reported to occur in the wild
- Low ecological relevance – tools of classic toxicity are **not suited to study medicinal products** – **need to integrate biomarkers**

Developments in the Ecotoxicological Assessment of Drugs

Changing the paradygm

Biomarkers

- Subindividual parameters
- Neurotoxicity, energy and detoxification metabolism
- Energetic balance
- Antioxidant defense and oxidative damage
- Epigenetic changes
- Exposure of test organisms to low levels – similar to those already reported to occur in the environment
- Chronic exposures – similar to what happens in real life
- Integration of data to ascertain impact on ecological relevant traits

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Developments in the Ecotoxicological Assessment of Drugs

Environmental Science and Pollution Research (2021) 28:38792–38808
https://doi.org/10.1007/s11356-021-13200-5

RESEARCH ARTICLE

Check for updates

Dangerous connections: biochemical and behavioral traits in *Daphnia magna* and *Daphnia longispina* exposed to ecologically relevant amounts of paracetamol

Ana Paula Sousa [1] · Bruno Nunes [1,2]

Received: 13 July 2020 / Accepted: 24 February 2021 / Published online: 19 March 2021

Behavioural biomarkers in microcrustacean species


https://cfb.unh.edu/cfbkey/html/Organisms/CCladocera/FDaphnidae/GDaphnia/Daphnia_magna/daphniamagna.html

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Developments in the Ecotoxicological Assessment of Drugs

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Ana Paula Sousa[1] · Bruno Nunes[1,2]

Received: 13 July 2020 / Accepted: 24 February 2021 / Published online: 19 March 2021

Behavioural biomarkers in microcrustacean species

Paracetamol

- Significant behavioral changes in two species of the genus *Daphnia*
- Opposite changes in both species
- Behavioral patterns were distinct according to the levels and durations of exposure
- **Ecological importance**

Table 3 Behavioral changes observed in *D. magna* and *D. longispina* after exposure to paracetamol

Paracetamol											
Concentration (µg/l)		*Daphnia magna*					*Daphnia longispina*				
		5	10	20	40	80	5	10	20	40	80
<24 h	TT-300	*(↑)	*(↑)								
	TT-900	*(↑)	*(↑)				*(↓)				*(↓)
	TT-600	*(↑)	*(↑)								
	TT-1200	*(↑)									
	TD-300		*(↑)			*(↑)	*(↓)	*(↓)			*(↓)
	TD-900						*(↓)	*(↓)		*(↓)	*(↓)
	TD-600						*(↓)	*(↓)		*(↓)	*(↓)
	TD-1200						*(↓)	*(↓)		*(↓)	*(↓)
6 days	TT-300			*(↑)		*(↑)					
	TT-900										
	TT-600					*(↓)					
	TT-1200					*(↓)					
	TD-300										
	TD-900										
	TD-600				*(↓)	*(↓)					
	TD-1200				*(↓)	*(↓)					

TT, total swimming time(s); *TD*, total distance traveled (mm), 300 and 900 (light cycle) and 600 and 1200 (dark cycle); (↑), increase in relation to the control; (↓), decrease in relation to the control; and *, statistically significant differences (Dunnett test, $p < 0.05$) between the different concentrations of paracetamol in relation to the negative control

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Developments in the Ecotoxicological Assessment of Drugs

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Available online at www.sciencedirect.com
ScienceDirect
Ecotoxicology and Environmental Safety 71 (2008) 341–354

Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Behaviour and biomarkers of oxidative stress in *Gambusia holbrooki* after acute exposure to widely used pharmaceuticals and a detergent

B. Nunes[a,b,*], A.R. Gaio[c], F. Carvalho[d], L. Guilhermino[a,b]

[a]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Departamento de Estudos de Populações, Laboratório de Ecotoxicologia, Universidade do Porto, Largo Prof. Abel Salazar 2, 4099-003 Porto, Portugal
[b]CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Laboratório de Ecotoxicologia, Rua dos Bragas 289, 4050-123 Porto, Portugal
[c]FCUP, Faculdade de Ciências da Universidade do Porto, Departamento de Matemática Pura, Rua do Campo Alegre 687, 4169-007 Porto, Portugal
[d]REQUIMTE, Serviço de Toxicologia da Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha 164, 4099-030 Porto, Portugal

Received 8 June 2006; received in revised form 20 November 2007; accepted 8 December 2007
Available online 19 February 2008

Aquatic Toxicology 144-145 (2013) 218–229

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Contents lists available at ScienceDirect
Aquatic Toxicology
journal homepage: www.elsevier.com/locate/aquatox

Short-term effects of neuroactive pharmaceutical drugs on a fish species: Biochemical and behavioural effects

F.P. Brandão[a,b,1], S. Rodrigues[c], B.B. Castro[a,b], F. Gonçalves[a,b], S.C. Antunes[b,c], B. Nunes[a,b,*]

[a]Department of Biology, University of Aveiro, Portugal
[b]Centre for Environmental and Marine Studies (CESAM), Campus of Santiago, University of Aveiro, Portugal
[c]Department of Biology, Faculty of Sciences, University of Porto, Portugal

Fish Physiol Biochem (2019) 45:1495–1512
https://doi.org/10.1007/s10695-019-00634-5

Behavioral and biochemical effects of the antifouler and antidandruff zinc pyrithione on the freshwater fish *Gambusia holbrooki*

Bruno Falcão · Márcia Marques · Bruno Nunes

Behavioural biomarkers in fish species

Medicinal drugs such as diazepam, paracetamol, clofibric acid, carbamazepine, fenitoine e zinc pyrithione

- Significant behavioral changes in fish
- Changes in swimming patterns
- Thigmotaxis
- Altered feeding
- Increased levels of aggression

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Developments in the Ecotoxicological Assessment of Drugs

Behavioural biomarkers in aquatic organisms (polychaetes)

Environmental Toxicology and Pharmacology 80 (2020) 103505

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Effects of low levels of the antibiotic ciprofloxacin on the polychaete *Hediste diversicolor*: biochemical and behavioural effects

Ana Filipa Nogueira [a,b], Bruno Nunes [a,b,*]

Pharmaceutical drug ciprofloxacine

- Strong behavioural changes in polychaetes
- Increase in burrowing time
- Impairment of mobility
- **High ecological relevance – increase in predation**

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Developments in the Ecotoxicological Assessment of Drugs

Behavioural biomarkers in aquatic organisms (polychaetes)

Ecotoxicology (2021) 30:1841–1853
https://doi.org/10.1007/s10646-021-02444-z

Effect of sublethal concentrations of the antiparasitic ivermectin on the polychaeta species *Hediste diversicolor*: biochemical and behavioral responses

B. Nunes[1,2] · D. Pinheiro[3] · A. Gomes[1]

Accepted: 8 June 2021 / Published online: 5 July 2021

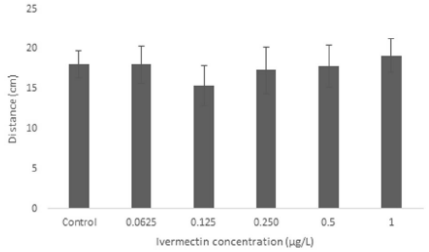

Fig. 1 Displacement of individuals of *H. diversicolor* acutely exposed to IVM. Average distances traveled by different groups as a function of ivermectin concentration, expressed in centimeters. Values are the mean distance, 10 replicates, and corresponding standard error bars

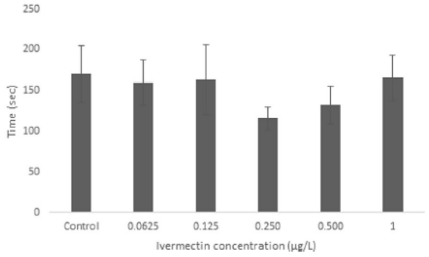

Fig. 2 Burrowing time of individuals of *H. diversicolor* acutely exposed to IVM. Mean time each group took to bury as a function of ivermectin concentration, expressed in seconds. Values are the mean distance, 10 replicates, and corresponding standard error bars

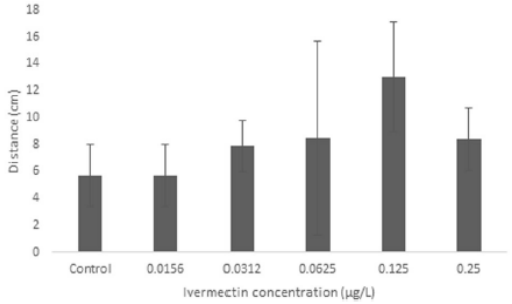

Fig. 3 Displacement of individuals of *H. diversicolor* chronically exposed to IVM. Average distances traveled by different groups as a function of ivermectin concentration, expressed in centimeters. Values are the mean distance, 10 replicates, and corresponding standard error bars

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Developments in the Ecotoxicological Assessment of Drugs

Behavioural biomarkers in aquatic organisms (polychaetes)

Ecotoxicology (2021) 30:1841–1853
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Effect of sublethal concentrations of the antiparasitic ivermectin on the polychaeta species *Hediste diversicolor*: biochemical and behavioral responses

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Accepted: 8 June 2021 / Published online: 5 July 2021

Pharmaceutical drug ivermectin

- Strong behavioural changes in polychaetes
- Increase in burrowing time
- Impairment of mobility
- **High ecological relevance – increase in predation**

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Developments in the Ecotoxicological Assessment of Drugs

Biomarkers such as epigenetic changes, combined with other effects

Received: 1 March 2019 | Revised: 17 June 2019 | Accepted: 26 June 2019
DOI: 10.1002/tox.22819

RESEARCH ARTICLE

ENVIRONMENTAL TOXICOLOGY WILEY

Embryonic development, locomotor behavior, biochemical, and epigenetic effects of the pharmaceutical drugs paracetamol and ciprofloxacin in larvae and embryos of *Danio rerio* when exposed to environmental realistic levels of both drugs

Ana F. Nogueira[1] | Glória Pinto[1,2] | Barbara Correia[1,2] | Bruno Nunes[1,2]

Medicinal products, such as paracetamol and ciprofloxacine

- Strong behavioural changes in fish
- Epigenetic changes
- Changes in metabolism (detoxification, energy)
- Oxidative stress

Developments in the Ecotoxicological Assessment of Drugs

Physiological biomarkers in plants

Science of the Total Environment 657 (2019) 926–937

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluation of pharmaceutical toxic effects of non-standard endpoints on the macrophyte species *Lemna minor* and *Lemna gibba*

G.D. Alkimin [a,b], D. Daniel [a], S. Frankenbach [a,b], J. Serôdio [a,b], A.M.V.M. Soares [a,b], C. Barata [c], B. Nunes [a,b,*]

[a] Department of Biology, Aveiro University, Campus de Santiago, 3810-193 Aveiro, Portugal
[b] Centre for Environmental and Marine Studies (CESAM), Campus de Santiago, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[c] Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18, 08034 Barcelona, Spain

Medicinal drugs such as carbamazepine, paracetamol and diclofenac

- Reduction in the content of photosynthetic pigments (clorophyls)
- Reduction in auxiliary pigments (charotenoids)
- Changes in growth and in photosynthetic efficiency in aquatic plants of the genus *Lemna*
- **Great ecological relevance – impacts on producers**

Developments in the Ecotoxicological Assessment of Drugs

Biomarkers involved in regulation of inflammatory processes

Environmental Pollution 293 (2022) 118562

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Toxicity of two drugs towards the marine filter feeder *Mytilus* spp, using biochemical and shell integrity parameters☆

David Daniel [a], João C. Campos [b], Paulo C. Costa [b], Bruno Nunes [a,c,*]

[a] Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal
[b] UCIBIO, REQUIMTE, Laboratório de Tecnologia Farmacêutica, Departamento de Ciências Farmacêuticas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[c] Centro de Estudos do Ambiente e do Mar (CESAM), Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

Paracetamol

- Impairment of cycloxigenase (COX) activity in mussels
- COX – involved in the regulation of **inflammatory processes**
- **Impact on celular and tissue homeostasis**

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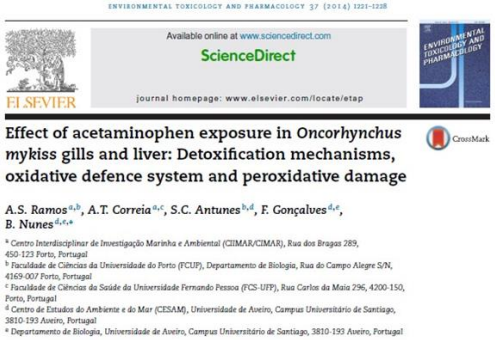
Developments in the Ecotoxicological Assessment of Drugs

Biomarkers of oxidative stress

The case study of paracetamol – a giant headache



ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY 37 (2014) 1221–1228

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/etap

ELSEVIER

Effect of acetaminophen exposure in *Oncorhynchus mykiss* gills and liver: Detoxification mechanisms, oxidative defence system and peroxidative damage

A.S. Ramos[a,b], A.T. Correia[a,c], S.C. Antunes[b,d], F. Gonçalves[d,e], B. Nunes[d,e,*]

[a] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Rua dos Bragas 289, 450-123 Porto, Portugal
[b] Faculdade de Ciências da Universidade do Porto (FCUP), Departamento de Biologia, Rua do Campo Alegre S/N, 4169-007 Porto, Portugal
[c] Faculdade de Ciências da Saúde da Universidade Fernando Pessoa (FCS-UFP), Rua Carlos da Maia 296, 4200-150, Porto, Portugal
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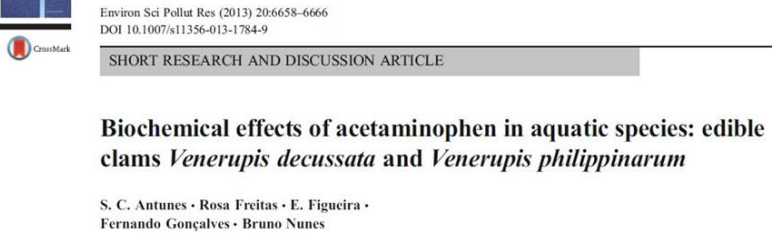
[e] Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal



Environ Sci Pollut Res (2013) 20:6658–6666
DOI 10.1007/s11356-013-1784-9

SHORT RESEARCH AND DISCUSSION ARTICLE

Biochemical effects of acetaminophen in aquatic species: edible clams *Venerupis decussata* and *Venerupis philippinarum*

S. C. Antunes · Rosa Freitas · E. Figueira · Fernando Gonçalves · Bruno Nunes

Ecotoxicology and Environmental Safety 107 (2014) 178–185

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

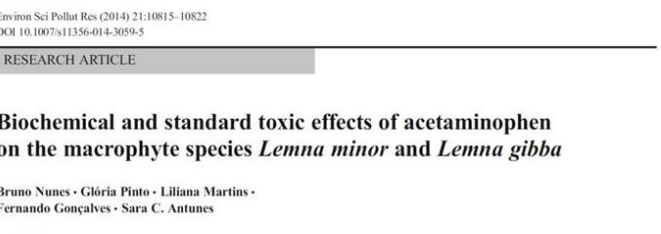
journal homepage: www.elsevier.com/locate/ecoenv

ELSEVIER

Toxic potential of paracetamol to freshwater organisms: A headache to environmental regulators?

Bruno Nunes[a,b], Sara C. Antunes[a,d,*], Joana Santos[b], Liliana Martins[c], Bruno B. Castro[a,b]

[a] Centro de Estudos do Ambiente e do Mar (CESAM), Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[b] Departamento de Biologia da Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[c] Faculdade de Ciências da Saúde da Universidade Fernando Pessoa (FCS-UFP), Porto, Portugal
[d] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal



Environ Sci Pollut Res (2014) 21:10815–10822
DOI 10.1007/s11356-014-3059-5

RESEARCH ARTICLE

Biochemical and standard toxic effects of acetaminophen on the macrophyte species *Lemna minor* and *Lemna gibba*

Bruno Nunes · Glória Pinto · Liliana Martins · Fernando Gonçalves · Sara C. Antunes

The Impact of Paracetamol on Selected Biomarkers of the Mollusc Species *Corbicula fluminea*

Fátima Pinto Brandão,[1] Joana Luísa Pereira,[1] Fernando Gonçalves,[1] Bruno Nunes

[1]CESAM – Centro de Estudos do Ambiente e do Mar/ Departamento de Biologia da Universidade de Aveiro, Campus Santiago 3810-193 Aveiro, Portugal

[2]CIAGEB – Centro Interdisciplinar de Alterações Globais, Energia e Bioengenharia, FCS-UFP – Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal

Environ Sci Pollut Res
DOI 10.1007/s11356-015-4329-6

RESEARCH ARTICLE

Biochemical effects of the pharmaceutical drug paracetamol on *Anguilla anguilla*

Bruno Nunes[1] · Maria Francisca Verde[1] · Amadeu M. V. M. Soares[1]

Developments in the Ecotoxicological Assessment of Drugs

Biomarkers of oxidative stress

The case study of paracetamol – a giant headache

The assessment of oxidative stress caused by paracetamol was conducted in multiple aquatic species

- Bivalves: *Corbicula fluminea*, *Ruditates decussatus*, *Ruditapes philipinarum*
- Fish: *Onchorrynchus mykiss*, *Gambusia holbrooki*, *Danio rerio*, *Anguilla anguilla*, *Phaloceros harpagos*
- Aquatic plants: *Lemna gibba*, *Lemna minor*
- Microcrustaceans: *Daphnia magna*, *Daphnia longispina*, *Ceriodaphnia dubia*
- Polychaetes: *Hediste diversicolor*, *Diopatra neapolitana*

All of these species, without exception, demonstrated that paracetamol, at concentrations similar to those already reported in the environment, can exert adverse effects, namely by inducing oxidative stress

Developments in the Ecotoxicological Assessment of Drugs

Biomarkers of oxidative stress

The case study of paracetamol – a giant headache




Developments in the Ecotoxicological Assessment of Drugs

Biomarkers of oxidative stress

The case study of paracetamol – a giant headache

In addition to the oxidative stress caused by acetaminophen, an unexpected pattern of toxicity was observed in crustacean species

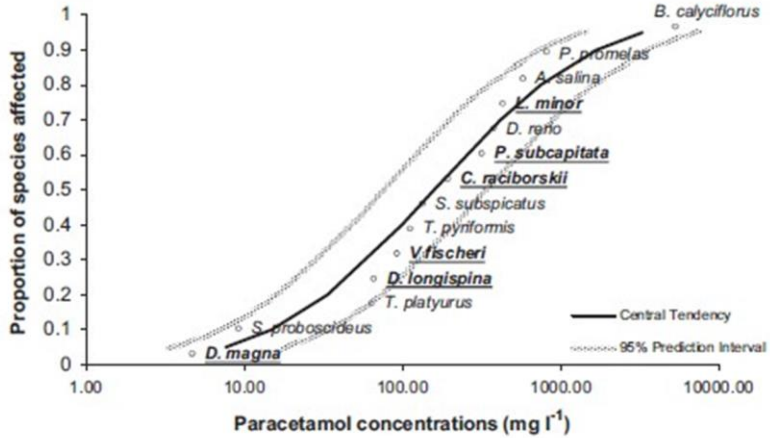


Fig. 1. Species sensitivity distribution (SSD) plot, showing the distribution of EC_{50}s for organisms acutely exposed to paracetamol, with 95 per cent confidence intervals (dotted lines). Data were obtained from the literature and from this study (highlighted in bold and underlined).

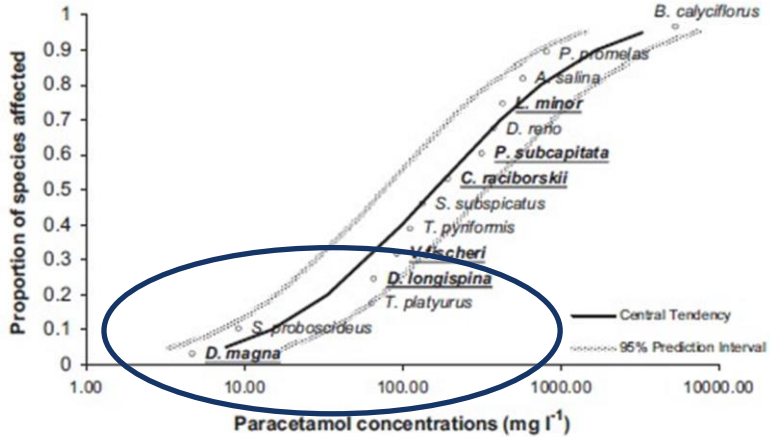
- Crustaceans appear to be consistently **more sensitive** than other groups of organisms to exposure to acetaminophen
- Acetaminophen is an **endocrine disruptor for crustaceans**
- It disrupts the physiological function of an enzyme that produces a **hormonal precursor for molting**

Developments in the Ecotoxicological Assessment of Drugs

Biomarkers of oxidative stress

The case study of paracetamol – a giant headache

In addition to the oxidative stress caused by acetaminophen, an unexpected pattern of toxicity was observed in crustacean species



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Grupo de Interesse
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Developments in the Ecotoxicological Assessment of Drugs

Contamination by medicinal drugs, and their effects in a context of global change

Comparative Biochemistry and Physiology, Part C 179 (2016) 116–124

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Oxidative effects of the pharmaceutical drug paracetamol on the edible clam *Ruditapes philippinarum* under different salinities

Bárbara Correia, Rosa Freitas, Etelvina Figueira, Amadeu M.V.M. Soares, Bruno Nunes *

Department of Biology, Centro de Estudos do Ambiente e do MAR (CESAM), University of Aveiro, Portugal

- In the aquatic environment, there are **two key factors** that may influence the ecotoxicity of multiple xenobiotics, including pharmaceuticals, in the future
- **Temperature** and **salinity** are key factors

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Grupo de Interesse
Medicamento e Ambiente

Developments in the Ecotoxicological Assessment of Drugs

Contamination by medicinal drugs, and their effects in a context of global change

Comparative Biochemistry and Physiology, Part C 179 (2016) 116–124

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Oxidative effects of the pharmaceutical drug paracetamol on the edible clam *Ruditapes philippinarum* under different salinities

Bárbara Correia, Rosa Freitas, Etelvina Figueira, Amadeu M.V.M. Soares, Bruno Nunes *

Department of Biology, Centro de Estudos do Ambiente e do MAR (CESAM), University of Aveiro, Portugal

- Patterns of toxic responses varied with salinity, accompanied by increased oxidative stress
- Will organisms facing global changes face an additional threat?
- What will be the ecological consequences of this new reality?

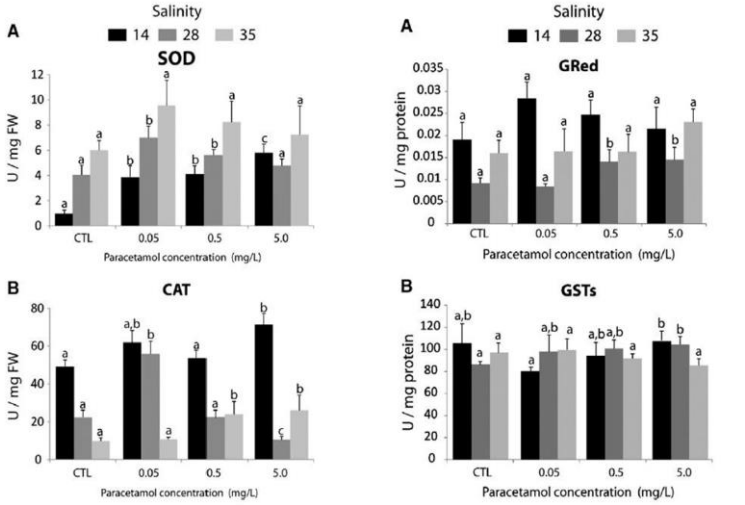

Fig. 1. (A) Superoxide dismutase (SOD) and (B) catalase (CAT) activities in *R. philippinarum* exposed to the control and paracetamol conditions (CTL, 0.05, 0.5 and 5 mg/L) under the salinities of 14, 28 and 35 (black, dark gray, and light gray bars, respectively). Values are the mean of seven replicates ± standard error bars. Significant differences between paracetamol concentrations (0.05, 0.5 and 5 mg/L) and the control condition (0 mg/L) are represented with different letters (a–c).

Fig. 2. (A) Glutathione reductase (GRed) and (B) glutathione S-transferases (GSTs) activities in *R. philippinarum* exposed to the control and paracetamol conditions (CTL, 0.05, 0.5 and 5 mg/L) under the salinities of 14, 28 and 35 (black, dark gray, and light gray bars, respectively). Values are the mean of seven replicates ± standard error bars. Significant differences between paracetamol concentrations (0.05, 0.5 and 5 mg/L) and the control condition (0 mg/L) are represented with different letters (a–b).

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MDPI

Article

Assessment of Paracetamol Toxic Effects under Varying Seawater pH Conditions on the Marine Polychaete *Hediste diversicolor* Using Biochemical Endpoints

David Daniel [1,2], Bruno Nunes [1,3,*], Edgar Pinto [4,5], Isabel M. P. L. V. O. Ferreira [5] and Alberto Teodorico Correia [2,6,7]

Changes in **water pH**

- Change absorption of drugs, e.g.: paracetamol
- Change the patterns of toxic response of acidic drugs
- Will organisms facing global changes face an additional threat?
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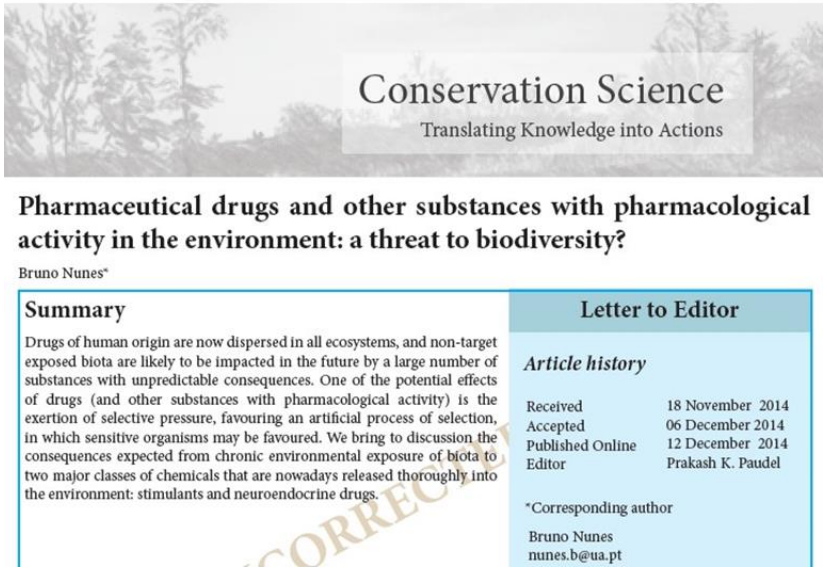
Developments in the Ecotoxicological Assessment of Drugs

Biodiversity and medicinal drugs in the environment



Conservation Science
Translating Knowledge into Actions

Pharmaceutical drugs and other substances with pharmacological activity in the environment: a threat to biodiversity?

Bruno Nunes*

Summary

Drugs of human origin are now dispersed in all ecosystems, and non-target exposed biota are likely to be impacted in the future by a large number of substances with unpredictable consequences. One of the potential effects of drugs (and other substances with pharmacological activity) is the exertion of selective pressure, favouring an artificial process of selection, in which sensitive organisms may be favoured. We bring to discussion the consequences expected from chronic environmental exposure of biota to two major classes of chemicals that are nowadays released thoroughly into the environment: stimulants and neuroendocrine drugs.

Letter to Editor

Article history

Received	18 November 2014
Accepted	06 December 2014
Published Online	12 December 2014
Editor	Prakash K. Paudel

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Could pharmaceuticals pose a threat to biodiversity, with some species being more sensitive than others?

Which parts of the world are likely to be most affected?

How will wildlife cope with pharmaceutical contamination in the context of global change?

Developments in the Ecotoxicological Assessment of Drugs

Biomarkers of effects of pharmaceutical drugs and populational effects

Environ Sci Pollut Res (2014) 21:4418–4429
DOI 10.1007/s11356-013-2339-9

RESEARCH ARTICLE

Environmental effects of anticholinesterasic therapeutic drugs on a crustacean species, *Daphnia magna*

R. Rocha · F. Gonçalves · C. Marques · B. Nunes

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High ecological relevance

Clearly established relationship – exposure – cholinesterasic inhibition - behavior impairment – reduced amount of food intake – lesser body growth – population effects (decrease)

Developments in the Ecotoxicological Assessment of Drugs

Transgenerational effects of pharmaceutical drugs and populational effects

Science of the Total Environment 831 (2022) 154677

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluation of parental and transgenerational effects of clotrimazole in *Daphnia magna* — A multi-parametric approach

David Ferreira [a], Gilberto Dias de Alkimin [a,b,e,*], Bruna Neves [b,c], Tiago Conde [b,c,d], M. Rosário Domingues [b,c], Bruno Nunes [a,b]

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[e] Centre for Marine and Environmental Research (CIMA), Universidade do Algarve, Campus de Gambelas, 8000-139, Faro, Portugal

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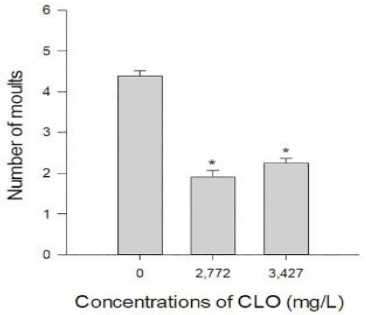

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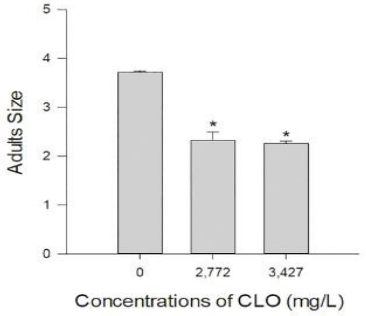

High ecological relevance

- Reduction of the molts
- Reduction in size of adults
- Potential populational effects

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High ecological relevance

- Reduction of feeding
- Adults and neonates respond differently

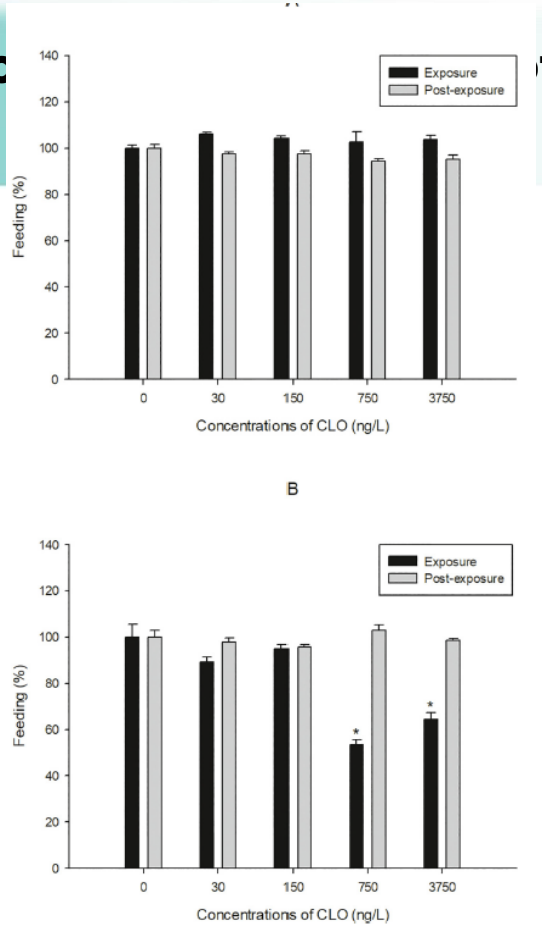

Fig. 2. Effects of clotrimazole on the feeding of *D. magna*. A) Feeding rate for individuals with 5 days and 24 h of exposure + 4 h of recovery; B) Feeding rate for neonates <24 h and 120 h of exposure + 4 h of recovery. Bars and error bars correspond to the mean ± SE (N = 5). * means statistically significant differences from the control, after an ANOVA followed by a Dunnett test (or another equivalent non-parametric test).

Developments in the Ecotoxicological Assessment of Drugs

Adverse effects of pharmaceutical in hospital effluents

Environmental Science and Pollution Research (2022) 29:19132–19147
https://doi.org/10.1007/s11356-021-16977-7

RESEARCH ARTICLE

Check for updates

A multi-biomarker approach for the early assessment of the toxicity of hospital wastewater using the freshwater organism *Daphnia magna*

Sabrine Afsa[1] · Madalena Vieira[2,3] · Ana Filipa Nogueira[2,3] · Hedi ben Mansour[1] · Bruno Nunes[2,3]

Received: 22 April 2021 / Accepted: 6 October 2021 / Published online: 28 October 2021

Objectives

- Characterize the toxicity of an untreated general hospital effluent
- Biomarkers measured in *D. magna* neonates

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Chemical composition of this effluent

- NSAIDs (mainly ketoprofen and salicylic acid, up to 18.1 µg/L and 120 ng/L, respectively)
- Neuroactive drugs (caffeine and carbamazepine, up to 902 µg/L and 4.5 µg/L, respectively)
- Antibiotics (up to 39.3 µg/L for sulfadiazine)
- β-blockers (atenolol up to 12.9 µg/L)

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Main results

- Decrease in CAT and GSTs activities – impact on the antioxidant defense and phase II metabolism
- Increase in GPx activity – impact on the antioxidant defense
- Reduction of lipoperoxidation
- Cholinesterasic inhibition
- Increase in COX, inflammatory response

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Main implications

- Highly complex and variable matrices – difficult to ascertain causal relationships between chemical contaminants (e.g.: drugs) and effects
- Activation of antioxidant response – may lead to inflammatory conditions
- Co-occurrence of organically bound halogens (AOX, disinfectants) – lead to the activation of antioxidant response
- ChE inhibition – AOX, ROS produced during the metabolism of drugs, direct effect of neuroactive drugs

Take home messages

- Drugs are substances that need to be studied when they are in the environment
- It is necessary to develop concrete, systematic monitoring programs and **regulatory frameworks focused on the toxicological and pharmacological effects of these substances, with multiple species**
- These biomarkers should be studied after **long-term exposure**, with organisms exposed to **low, environmentally realistic levels of drugs**, similar to those already reported in the environment
- It will be very important to study **pathways that do not exist in vertebrates**

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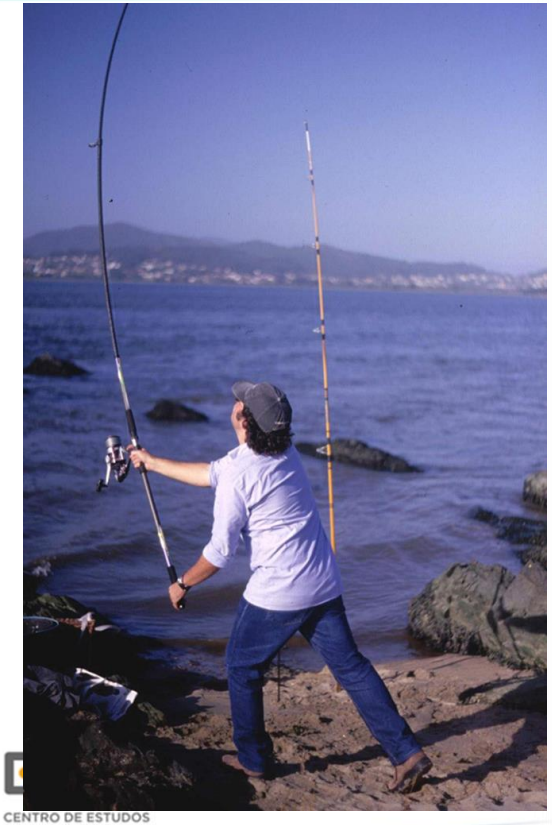
Acknowledgements

Thank you for your attention



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