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# assemblages?

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# ABSTRACT

During the summer of 2001, trichoptera larvae were sampled with a kick-net in 15 sites belonging to 3 rivers of the Mondego River basin, in central-north Portugal. Simultaneously, 42 environmental variables were evaluated for each site. Twenty five species and genera of caddisflies were identified. The objective was to assess if trichoptera assemblages within a river tended to be more similar among each other than between rivers, giving the physical continuity of the habitat. Localities showed low segregation between all samples (MDS, CLUSTER and ANOSIM). The Alva River samples had the higher number of taxa and animals while the Ceira River samples had the lowest values. In terms of environmental characteristics, PCA showed high similarities between samples of the same river. However there was not total segregation of rivers. BIOENV analysis identified the set of parameters that best explain trichopteran associations per river. For the rivers, these variables were all related to habitat (e.g. pool quality, depth, substrate quality). The differences between the Alva and Ceira rivers seem to be related to the deterioration of the water quality in the Ceira river (increasing levels of sulphate, chloride and % of industrial, urban and degraded areas), which increased down the river.

Keywords: Trichopteran assemblages, biotic similarities, environmental continuity.

#### RESUMEN

Durante el verano de 2001 se capturaron larvas de tricópteros en 15 puntos de muestreo pertenecientes a 3 ríos de la cuenca hidrográfica del Mondego, en el centro-norte de Portugal. Los muestreos fueron realizados con una red de mano (tipo "kick-net") y, simultáneamente, se evaluaron 42 variables ambientales para cada localidad. En total fueron identificadas 25 especies de tricópteros. El objetivo del estudio era evaluar si, dada la continuidad físico-química del habitat, las asociaciones de tricópteros de las localidades situadas en un mismo río son más semejantes entre sí que las pertenecientes a localidades de ríos diferentes. Los locales se presentaran poco segregados de acuerdo con los análisis MDS, CLUSTER y ANOSIM. Las muestras del río Alva tuvieron un número más elevado de taxa y animales mientras que las muestras del río Ceira tuvieron los valores más bajos. Desde el punto de vista ambiental el PCA reveló que las muestras de un mismo río eran más semejantes entre si. Sin embargo, no hubo segregación completa de ríos, esas variables estaban todas relacionadas con el hábitat (e.g., calidad de las zonas lénticas, calidad de substrato y profundidad). Las diferencias entre los ríos Alva y Ceira parecen ser debidas al empeoramiento de la calidad de las aguas del río Ceira (mayores niveles de sulfato, cloro y porcentaje de degradación industrial y urbana) que aumentan río abajo.

Palabras clave: Comunidades de tricópteros, similitudes bióticas, continuidad ambiental.

# **INTRODUCTION**

The ability to predict aquatic invertebrate communities from environmental information is useful for the management of aquatic ecosystems. Hence the existence of so many studies aimed at finding the best predictors for the communities of each system or key groups. Environmental conditions of rivers exhibit longitudinal changes. Vannote *et al.* (1980) underlined the importance of such gradients in the origin and use of energy sources and for the functional feeding groups of macroinvertebrates. Some studies investigate the influence of specific parameters separately, on the distribution and abundance of macroinvertebrate assemblages. For instance, the watershed vegetation (Hawkins, 1988), or land use (Corkum, 1990), were shown to affect riverine invertebrates. Alternatively, other studies analyse large sets of variables to select the most important for predicting biological assemblages. Variables such as distance to source, altitude, substrate, current velocity, or slope have been selected as relevant and used in the development of many predictive models (Bailey *et al.*, 1998; Reynoldson *et al.*, 1997; Wright, 1995; Wells *et al.*, 2002).

Nevertheless, in all studies a certain amount of variability of the fauna is left unexplained, probably due to the difficulty of covering all of the natural complexity of the streams or due to natural variability. The hypothesis underlying the present work is the concept of a river as a discrete entity showing, in consequence, higher biotic similarities among stretches of the same river than among stretches of different rivers. To test this hypothesis we analysed trichoptera assemblages at multiple sites located in several rivers of the Mondego Basin. We verified whether the ordination of sites in terms of trichoptera composition matched the ordination of sites in terms of environmental conditions and identified the smaller combination of environmental variables that best distinguished the rivers in terms of caddisfly assemblages.

Trichoptera were selected because of their high abundance and diversity in rivers and their distribution through a very wide range of freshwater habitats and in functional groups (Faessel, 1985; Morse, 1997). A high number of species (126) and families (20) had been previously reported in the Mondego River Basin by Terra (1994).

#### METHODOLOGY

#### Study area and sampling sites

The Mondego is the largest entirely Portuguese river, located in the centre of Portugal between  $39^{\circ}$  46' and 40° 48'N and 7° 14' and 8° 52'W



Figure 1. Location of the Mondego catchment in Portugal and sampling sites in the Alva, Ceira, and Mondego rivers, in July 2001. Localización de la cuenca del río Mondego en Portugal y puntos de muestreo en los ríos Alva, Ceira y Mondego en Julio de 2001.

(Lima & Lima, 2002). The river flows approximately from NE to SW through 227 km, between *Serra da Estrela* and the Atlantic Ocean at Figueira da Foz (Marques *et al.*, 2002). The highest altitude in the drainage basin is 2000 m in *Serra da Estrela*, and the average altitude is 375 m. The mean annual precipitation in the basin is 1130 mm and the mean annual temperature 13°C, with smaller amplitudes near the coast than inland (Lima & Lima, 2002). The hydrological basin covers around 6670 km<sup>2</sup> (Marques *et al.*, 2002) with a high variability of soil occupation.

The rivers selected for this study were the Mondego and 2 tributaries: the Ceira and the Alva (southern tributaries). These rivers have almost parallel courses through the middle and upper regions of the basin (Fig. 1) and are in very similar geological areas (schist and granites) with good vegetation cover. The areas covered by the studied stretches of the three rivers are mountain areas with an annual precipitation between 600 and 1000 mm, which corresponds to 75 days of rain per year.

The 3 rivers were sampled in summer 2001 (July) in several sites (Fig. 1). The Ceira River was sampled in 5 sites (C1 to C5, with distances to source between 52 and 103 km, respectively), the Alva River was sampled in 6 sites (A1 to A6, with distances to source between 26 and 116 km, respectively) and the Mondego River in 4 sites (M1 to M4, distances to source between 33 and 99 km).

# Field and laboratory work

Invertebrates, including trichoptera larvae, were collected by means of 3 minutes kick-net sampling per site using a hand net with  $0.3 \times 0.3$  m opening and 0.5 mm mesh size. Samples were taken along a transect of each river covering all micro-habitats in the site (e.g. different grain size categories, presence of aquatic vegetation, riffles and pools), preserved in 4 % formalin, sorted, and preserved again in 70 % alcohol.

Trichoptera were generally identified to species level. When larvae were too young to be correctly identified to species level and more than one species of the same genera were present, individuals were identified to the genus level. A species was considered present in a site when more than one individual was found.

The environment was characterized by the variables describing: geographic location, land use in the floodplain and catchment area, site morphology, atmospheric conditions, stream morphology and hydrology, riparian vegetation, water chemistry, characteristics of the aquatic habitat, and human impacts on the stream and floodplain. For each site, 42 environmental parameters were obtained through field measurements, laboratory analysis of collected material (e.g. water and periphyton) or in bibliographic sources such as cartographic material (Table 1).

#### **Data analysis**

All trichopteran counts were converted to individuals/minute (sample unit) and transformed through double square root for multivariate analysis. The following environmental variables were excluded from statistical analysis:  $\% O_2$ (correlated with mg/l of  $O_2$ , 0.757 Pearson correlation), chlorophyll in the periphyton (correlated with the periphyton biomass, 0.625 Pearson correlation), discharge (correlated with current velocity, 0.788 Pearson correlation), total dissolved solids (correlated with conductivity, 1.000 Pearson correlation), days of rainfall (the value was the same for all sites: 75 days/year); nitrite (all sites with <0.001 mg/l), and water and air temperature (they were instantaneous measures, very dependent of the measuring time, which was not the same in all sites).

The data were analyzed by multivariate statistical methods with PRIMER software (version 5.2.6, PRIMER-E Ltd). To check for similarity in patterns among sites located in the same river (i.e. whether multiple river sites are replicates or different entities) the 15 sites were ordinated by a non-metric MDS (Multidimensional Scaling, 10 restarts) and classified with CLUS-TER analysis, after a similarity matrix (Bray-Curtis coefficient), using the river name as a

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Table1. Environmental parameters obtained for each sampling site, description and sources. Parámetros ambientales obtenidos para cada punto de muestreo, descripción y fuentes.

Environmental Variables	Description and Source
Stream Order	Military maps 1:250 000 (Strahler system) (Inst. Geográfico do Exército)
Distance to Source (km)	Digital military maps (1: 25 000: DRAOT-Centro)
Decimal Latitude and Decimal Longitude	GPS (GARMIN) and digital military maps (1:25 000; DRAOT-Centro)
Altitude (m)	idem
Valley Form	Field observations; Categories: 1 for V shapes; 2 for U shape,
•	meander and plain floodplain)
Mean Annual Temperature (°C)	Atlas Digital do Ambiente - DGA (data from 1931-1960).
Mean Annual Total Precipitation (mm)	Idem
Mean Annual Precipitation (days/year)	Idem
Mean Stream Width (m)	Field measurements (6 measurements each transept)
Mean Stream Depth (m)	Idem
Current Velocity (ms <sup>-1</sup> )	6 field measurements (VALEPORT 15277)
Mean Discharge (m <sup>3</sup> s <sup>-1</sup> )	Stream width x Stream Depth X Current Velocity (n=6)
Water Temperature (°C)	Field measurement (WTW OXI 92)
pH	Field measurement (JENWAY 3310)
Conductivity (uS cm <sup>-1</sup> )	Field measurement (WTW LF 330)
O <sub>2</sub> (mg l <sup>-1</sup> ) and O <sub>2</sub> (%)	Field measurement (WTW OXI 92)
Total Dissolved Solids (TDS, mg l <sup>-1</sup> )	Field measurement (WTW LF 330)
Chloride (mg l <sup>-1</sup> )	Ion Chromatograph Dionex DX-120
Nitrate (mg l <sup>-1</sup> )	Idem
Nitrite (mg l <sup>-1</sup> )	Idem
Sulphate (mg l <sup>-1</sup> )	Idem
P-Phosphate (mg l <sup>-1</sup> )	Idem
N-Ammonia (mg l <sup>-1</sup> )	Idem
Alkalinity (mg l <sup>-1</sup> )	Titration to an end pH of 4.5 (A.P.H.A., 1995)
FPOM <1mm (AFDM, g)	Collected in benthos samples, dried and burned to ashes (500°C, 2h). AFDM= Dry mass – Ashes mass
CPOM >1mm (AFDM, g)	Idem
Chlorophyll in Periphyton (mg m <sup>-2</sup> )	Collection by stone scraping; washed with 300 ml of water and kept in
	WHATMAN GFC fibre-glass filters. Analysis according to A.P.H.A., 1995.
Biomass of Periphyton (g l <sup>-1</sup> )	Same collection procedure. Biomass =(dry mass filters + periphyton) – dry
	mass of filters
Substrate Quality	Field observation. Categories: 1: poor;2:marginal;3:sub-optimal; 4:optimal.
	Based in Barbour <i>et al.</i> 1999
Mean Substrate Size (mm)	Field measurements of 18 average stones.
Habitat Complexity	Field observation. Categories: 1: poor;2:marginal;3:sub-optimal;
	4:optimal. Based in Barbour <i>et al.</i> 1999
Pool Quality	Field observation. Categories: 1: poor;2:marginal;3:sub-optimal;4:optimal.
T*/L-1	Based in Barbour et al. 1999
Lithology	Atlas Digital do Ambiente - DGA (1982). Categories: 1= sedimentary;
	2= sedimentary + metamorphic;3= plutonic rocks
Riparian vegetation (total width; m)	Field measurement.
woody vegetation (%)	Field observation. Woody vegetation in the riparian corridor.
Shaung at Zentin (70)	Measured in the area of a sirely of 1km radius marked around each
FUICSL (70)	sompling site
	Sampling She. Data from Plano de Racia Hidrográfica de Mondege (MAOT 2002)
Fucelyntus (%)	Idem
Eucarypius (70) Industrial urban and dogradod aroos (0/)	Idem
Industrial, ut ball and degraded areas $(\%)$	Idem
Agriculture (70)	100111



number of individuals/sample unit (log x+1)



Figure 2. Number of taxa and number of individuals for all samples gathered from the 3 rivers of the Mondego catchment. Número de taxones y número de individuos encontrados en las muestras recogidas en los 3 ríos de la cuenca del Mondego.

factor. The one-way ANOSIM test (Analysis of similarities, 999 permutations) was also used to evaluate the similarities between streams. This test uses permutation/randomisation methods on a similarity matrix.

The Principal Components Analysis (PCA, normalised data) was performed in order to evaluate the similarities of sites between and within rivers and environmental conditions.

To select the variables that best explain the patterns of the trichopteran communities of each river, the BIOENV procedure (BIOta ENVironment matching, Bray-Curtis similarity coefficient, Spearman rank correlation method, 10 restarts) was used. This procedure maximizes the rank correlation between the respective similarity matrices and all permutations of trial variables are tried. The process was repeated 3 times, one for each river, with their respective environmental and biological data.

#### RESULTS

A total of 25 species of trichoptera were identified in the Mondego River basin in the 15 samples/sites of the 3 rivers. The trichoptera species found in the studied rivers belonged to 10 families: Hydropsychidae, Calamoceratidae, Hydroptilidae, Lepidostomatidae, Leptoceridae, Philopotamidae, Polycentropodidae, Psychomyiidae, Rhyacophilidae, and Sericostomatidae. The number of taxa in the samples ranged from 1 to 10 and varied considerably within streams. Nevertheless, the mean number of taxa/sample of the same river was lowest in Ceira ( $4.8 \pm 1.5$ ) and highest in Alva  $(7.0 \pm 2.4)$ . The Mondego River had an intermediate value  $(6.0 \pm 4.2)$ . These mean values correspond to the observed graphic tendency (Fig. 2A). The abundance of individuals varied even greatly within rivers but the highest values were found again in the Alva River with a



**Figure 3.** Ordination by non-metric MDS (A) and classification by CLUSTER analysis (B) based on trichopteran assemblages in multiple sites of the 3 rivers of the Mondego catchment. Samples M1-M4 represent Mondego sites, A1- A6 Alva sites, and C1-C5 Ceira sites. Ordenación por MDS no métrico (A) y clasificación por análisis de CLUSTER (B) basados en agrupaciones de Tricópteros en varias localidades de 3 ríos de la cuenca hidrográfica del Río Mondego. Muestras M1-M4 representan Mondego, A1-A6 Alva and C1-C5 Ceira.

total mean of captures of  $152 \pm 283$  collected by sample unit (Fig. 2B). This value and variability was partially due to one exceptionally high value of 723 individuals/ sample unit (mainly *Chimarra marginata*, *Hydropsyche incognita* and *Cheumatopsyche lepida*) captured in one site of the low reach (Vimieiro, A5). The lowest mean value corresponded to Ceira ( $11 \pm 13$ ) and the intermediate one to Mondego  $(18 \pm 15)$ . Therefore, and analysing figures 2A and 2B, a direct relation between the number of captures and the number of taxa identified is observed.

MDS (3D minimum stress: 0.09; 2D: 0.17; Fig. 4A) analysis cross-checked with the CLUS-TER analysis (Fig. 3B) showed that sites were not clearly aggregated in any way. The ANO-



**Figure 4.** PCA with habitat data from several sites on each river, using the variables that better explain the distribution patterns of two tricopterans on the three rivers. The arrows next to the axis represent the variables that most contribute to the ordination of sites along that axis. *PCA con datos de hábitat de varias localidades en cada río usando las variables que explicaran mejor los patrones de la distribución dos tricópteros de los 3 ríos. Las flechas junto a los ejes indican las variables que más contribuyen para la ordenación de los sitios a lo largo de ese eje.* 

SIM global test (global r=-0.01; p=0,487) and pairwise tests (Ceira, Alva: r=0.008, p=0.394; Ceira, Mondego: r=-0.041; p=0.548; Alva, Mondego: r=0.004; p=0.405) also indicates that sites of different rivers are not segregated.

The PCA shows higher similarity of environmental characteristics between sites of the same river than the biological data (Fig. 4). Table 2 shows the coefficients of the linear combinations of variables making up the PC's. Sites in the Ceira River are distributed in the graph according to their distance to the source, from the more distant (C5) to the less distant, on the left (C1). The distances to the source seem to correspond to gradients in conductivity, chloride, sulphate, periphyton, % of industrial, urban, and degraded areas, with the highest values in the site most distant to the source (C5). For the Alva River these gradients are not so evident, but the sites, although not very different from each other, could be divided into two groups: A1, A2, and A3, the sites more distant to the source and A4, A5, and A6, the sites more distant to source. The first group is therefore, and not surprisingly, characterised by higher altitudes and greater substrate elements. These characteristics are shared with sites M3 and M4 of the Mondego River and site C1 of the Ceira River. For the Mondego River there is a clear group of 2 sites (sites M3 and M4) distant to source and with intermediate characteristics from the two Alva sub-groups and sites, M1 and M2 with higher latitude, altitude, and mean substrate size. Yet, site M1 is different from M2 mainly due to a greater stream width and current velocity.

The BIOENV analysis for matrices shows the sets of variables with highest influence in the trichoptera distribution of the 3 rivers (Table 3). For the Mondego River (4 sites) the variables substrate quality, pool quality, chloride concentration in the water, and % of industrial, urban and degraded areas were correlated with the caddisfly assemblage patterns. For the Ceira River, the variables pool quality, depth, and pH explain 85 % of the distribution of the trichoptera species in the 5 sites. In the Alva River

**Table 2.** Coefficients in linear combinations of variables constituting axes 1 and 2 of the PCA. *Coefficientes en combinaciones lineares de variables que constituyen los ejes 1 y 2 del PCA.* 

Variables	PC1	PC2
stream order	0.227	0.231
distance to source (km)	0.217	0.147
decimal lat	-0.223	0.223
decimal long	0.288	-0.095
substrate quality	-0.190	0.138
mean substrate size	-0.215	-0.166
habitat complexity	-0.156	0.122
pool quality	-0.047	0.056
altitude(m)	-0.288	0.015
width(m)	0.045	0.231
depth(m)	-0.029	-0.327
current velocity (m/s)	0.083	0.319
pH	0.161	-0.067
conductivity (uS/cm)	0.266	0.036
$O_2(mg/l)$	0.022	-0.143
chloride (mg/l)	0.264	0.006
nitrate (mg/l)	0.255	-0.080
sulphate (mg/l)	0.284	-0.071
ammonium (mg/l)	-0.064	0.077
alkalinity (mg/l)	0.190	0.060
afdm <1mm(g)	0.056	-0.075
afdm>1mm(g)	-0.049	-0.225
periphyton (g dry mass /l)	0.240	-0.114
shading (%)	-0.024	-0.265
riparian veg.(m)	0.031	-0.077
woody veg. (%)	0.056	0.124
% Florest	-0.117	-0.298
% Eucaliptus	0.110	0.084
% industrial. urban	0.238	0.052
and degraded areas		
% agriculture	-0.026	0.211
rain fall (total; mm)	0.074	-0.359
lithology	-0.179	0.202

(6 sites), pool quality, current velocity, chloride and sulphate concentrations, and fine particulate organic matter (<1 mm) explained 77 % of the caddisflies' distribution patterns.

# DISCUSSION

The three studied rivers are different in the total number of trichoptera taxa and individual abundances. Differences in invertebrates' species richness between streams must be deter-

Table 3. Results of BIOENV analysis, indicating which of the measured environmental parameters best explain the caddisflies community patterns in each river and the respective correlation. *Resultados del análisis BIOENV indicando cuáles de los parámetros ambientales medidos explican mejor los patrones de las comunidades de tricópteros en cada río y las respectivas correlaciones.* 

STREAM	VARIABLES	CORRELATION
CEIRA	pool quality depth pH	0.853
ALVA	pool quality chloride sulphate FPOM	0.770
MONDEGO	substrate quality pool quality chloride % industrial, urban and degrade areas	1.000

mined primarily by variability in resources and environmental characteristics, although the connection is not always obvious (Jacobsen, 1999). In this study, the Alva River showed higher species richness than the other rivers (Ceira and Mondego), despite the high variability between samples. Looking into the PCA results (Fig. 4), the main environmental differences between Alva and Ceira (which had the lowest mean species richness) are connected to pollution and other anthropogenic disturbances (sulphate, chloride, industrial percentage, urban and degraded areas), with higher values in the Ceira River. In this way, the studied streams, although similar in a wider, geographical scale, are chemically different and show different degrees of disturbance. The influence of chloride concentration and other chemical parameters such as phosphate, nitrate, and alkalinity are known to directly and indirectly limit species richness (Wright et al., 1994, 1998, Richards et al., 1993, Jacobsen, 1999). Therefore, this seems to be a plausible justification for the differences in species richness between the Alva and Ceira rivers, and maybe even to the differences in individual abundances, since physical factors like pool quality,

substrate quality, habitat complexity, or current velocity did not discriminate streams.

However, the hypothesis that sites of the same river have a more similar taxonomical composition when compared with sites of different rivers is inconsistent with our results. Other authors have reported higher similarities within rivers than among rivers (Graça *et al.*, 1989), but the rivers in those studies greatly differed in water chemistry and physical composition.

Indeed, relevant information can only be provided by studies on compatible rivers. Wright et al. (1984) sampled 268 sites in the U.K., distributed among more than 50 rivers. In most of those rivers, the sites within a river were classified into different groups based on their macroinvertebrate assemblages, showing great variability among sites. This idea was also confirmed by comparing rivers sampled for that study in nearby areas and of similar dimensions, like the Blithe and Dove rivers in the center of England and the Yare and Wensum rivers in the east coast. Furthermore, in proximate areas the rivers also differed taxonomically, showing a spatial heterogeneity. Similarly, Zamora-Muñoz and Alba-Tercedor (1996) reported that sites of two tributaries of the Genil River, in Spain, were included in different groups, after a classification based on their invertebrates.

For our streams, the environmental gradients verified for sites of the same river (from source to mouth), especially in the case of the Ceira River (Fig. 4), did not have a correspondence in the trichopteran assemblages (Fig. 3). A study on trichoptera in Danish streams showed that taxa richness increases from upland to lowland (Wieber-Larsen et al., 2000), which is in agreement with the River Continuum Concept predicting maximum species richness in 4th-6th order streams with high environmental variability (Vannote et al., 1980, Minshall et al., 1985). Therefore, in our study, and since samples do not seem to be aggregated by stream, we hypothesise that samples from the upper reaches would be more similar and have higher species richness than samples from the lower reaches. Yet according to figures 2 and 3, neither of the two situations is verified: samples of the same reach are not clearly similar in caddisflies assemblages and also samples from the same reach do not show similar species richness (e.g., M4, C2 and A1 in Fig. 3B). Our data agrees with other studies (Wieber-Larsen et al., 2000, Schmera & Erős, 2004) that refer the importance of the spatial variability, in diversity of pools and rifles, for the caddisflies. The BIOENV results show that spatial diversity of the habitat, in pool and substrate quality was correlated with the trichoptera community patterns. Yet, for the stretches of the rivers studied these factors seem to be more site dependent than related to the distance from the source.

In conclusion, our analysis shows that rather than being related to geographical variables, such as latitude, altitude, stream order, rainfall or the stream itself, the caddisflies' assemblages in the rivers studied, depended on habitat characteristics, such as pool quality, substrate type diversity, current velocity, and quantity of organic matter. The result should not be surprising, since these characteristics, which are very site specific, vary considerably and unpredictably in the studied rivers. These characteristics are also largely affected by human activities such as damming and riparian vegetation cutting.

# ACKNOWLEDGMENTS

We are grateful to Dr. Marcos González and Dr. Fernando Cobo from the Dept. of Animal Biology of the University of Santiago de Compostela; to Dr. K.R. Clarke from the Plymouth Marine Laboratory; to Veronica Ferreira, Cláudia Mieiro, and Elsa Rodrigues from the Dept. of Zoology of the University of Coimbra; to Draot-Centro; and to Dr. Leonor Varela for reviewing the English of this paper. We also thank the *Fundação para a Ciência e Tecnologia, Ministério da Ciência e do Ensino Superior* and *Fundo Social Europeu* (Praxis XXI/BD/21702/99 and project POCTI/BSE/32389/2000), and to IMAR for the financial support.

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