New Tools to Analyse the Ecological Status of Mediterranean Wetlands and Shallow Lakes

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Abstract The efforts done in Catalonia (Spain) to assess the ecological status of Mediterranean wetlands and shallow lakes are described. The term wetland includes all shallow lentic waterbodies, temporary or permanent, where light reaches the bottom allowing the development of primary producers at the maximum water depth. Two water quality indexes and one habitat condition rapid assessment were developed. The first quality index ($QAELS^{e}_{2010}$) is based on the sensitivity of microcrustaceans (cladocerans, copepods and ostracods) and the richness of crustaceans and insects found in these habitats; the second one (*EQAT*) uses the composition of Chironomidae pupal exuviae. Rapid assessment of habitat condition (*ECELS* index) considers wetland hydromorphological aspects, the presence of human pressures in the surroundings and the conservation status of the wetland vegetation. Some data of the current ecological status of Mediterranean wetlands in Catalonia are also provided.

Keywords Chironomidae, Crustaceans, *ECELS*, *EQAT*, Habitat condition, Insects, *QAELS*, Shallow lakes, Transitional waters, Wetlands - WFD

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Contents

1 Introduction

Since the implementation of the Water Framework Directive in 2000 (WFD, Directive 2000/60/EC), several efforts have been done in the description of parameters related to the ecological status of shallow lakes and wetlands and the design of efficient tools based on biological elements for its assessment (e.g. [1–7]). During this process, some difficulties arose in the development of criteria and methodological standards on good environmental status of shallow waters. Sediment proximity makes nutrient concentrations often more dependent on water – sediment equilibria than on nutrient inputs [8], making difficult to distinguish between anthropogenic eutrophication and natural eutrophication [9]. In Mediterranean wetlands, water level fluctuations and the lack of water inputs during most of the year cause an endorheic process of nutrient accumulation [10, 11], accentuated in temporary habitats during desiccation. Moreover, in Mediterranean transitional waters (i.e. estuaries, lagoons and coastal wetlands), the low tidal influence favours water confinement, making nutrient contents and nutrient balances more dependent on internal loading than on external water inputs [12–14].

Following the guidelines of the WFD, several indices have been developed using aquatic invertebrate fauna as indicators to assess the ecological status of Mediterranean shallow lentic ecosystems. Some of them use the sensitivity of species composition (e.g. [15]) or are based on higher taxonomic levels (e.g. [16–19]). Other approaches use alternatives to invertebrate species composition, such as body size [20, 21] or percentages of some functional groups [22]. Within aquatic invertebrates, several properties make crustaceans and insects suitable for their use in the ecological status assessment of wetlands and shallow lakes [23]: they appear in all lentic environments in fresh and transitional waters and are easy to capture; their assemblages vary with differences in trophic state; they respond rapidly to disturbance; and the relationships between their assemblages and both phytoplankton and macrophytes are well documented [24–30].

In this chapter, we summarise the efforts done in Catalonia (Spain) to assess the ecological status of Mediterranean wetlands and shallow lakes. We describe two water quality indexes: the first one ($QAELS^{e}_{2010}$) is an improvement of a water quality index already published [23], based on the sensitivity of microcrustaceans (cladocerans, copepods and ostracods) and the richness of crustaceans and insects; the second one (*EQAT*) is a proposal based on the composition and sensitivity of Chironomidae assemblages through the use of pupal exuviae described in Cañedo-Argüelles et al. [31]. We also include a rapid assessment method to determine the habitat condition of wetlands, developed by Sala et al. [32].

2 Typologies and Reference Conditions

The spatial approach is the underlying methodological principle of the WFD for the development of biotic indices to assess the ecological status of surface waters. The concept is that waterbodies can be classified into units with homogenous characteristics, thus belonging to a similar functional type with comparable biological communities. The principle behind this approach is that the less the functional and biotic heterogeneity within identified types, the higher the accuracy of the employed biological indicators. The WFD offers two options to classify waterbodies of surface waters, both of them use only abiotic descriptors to define typologies. The resulting classification of surface waterbodies is based on the assumption that an abiotic typology is adequate to stratify biological communities. However, there are few examples of efforts to validate this assumption in wetlands [23, 33]. Moreover, several proposals exist for Mediterranean lentic and shallow waters using abiotic variables, chlorophyll-*a* abundance or vegetation composition (e.g. [34–37]).

For the identification of types in Catalan wetlands, we follow Boix et al. [23]. This classification splits wetlands according to salinity and water permanence, and its effectivity to identify different invertebrate communities has been validated [38]. Salinity discriminates between meso-hyperhaline waters (conductivity $> 5\ \mathrm{mS \cdot cm^{-1}}$) and fresh oligohaline waters (conductivity $< 5\ \mathrm{mS \cdot cm^{-1}}$). Meso-hyperhaline wetlands are different if salinity comes from marine origin (thalassohaline wetlands) or from endorheic concentration of salts in arid or semiarid regions (athalassohaline wetlands). Regarding fresh oligohaline wetlands, permanent and temporary waterbodies contain different invertebrate fauna. Thus, four wetland types were discriminated: 1-thalassohaline (TA), 2-athalassohaline, 3-freshwater permanent (PF) and

4-freshwater temporary (TF). Athalassohaline wetlands are very scarce in Catalonia and are not considered further.

For each waterbody type, the basic functional unit, reference conditions are formulated and the deviation from these conditions provides the measure of the ecological status. The reference conditions can be defined in different ways [39]. If reference conditions are not available (the most common situation in the case of wetlands), one option is to use best available least-disturbed conditions resulting in unequal thresholds for less and more impacted biological assemblage types. However, the WFD requires standardised reference conditions showing no, or only minor, anthropogenic alterations. Another way of defining reference conditions is the availability of historical data when anthropogenic impacts were nonexistent or very low. In both cases, present and historical data to define reference conditions and information on pressures is necessary to distinguish between reference and impacted sites and for calibrating or scoring of metrics. This information should be expressed by different variables that should quantify the environmental quality of the surface waterbodies taking into account different types of pressures and impacts (water pollution, hydromorphological quality, etc.). It is, thus, easy to understand that classifying waterbodies and defining reference conditions should be two independent procedures; otherwise, the response of biological indicators to pressures and impacts will not be accurate. Therefore, pressure or impact variables should not be used to define typologies (i.e. the waterbodies' typology should be done with undisturbed waterbodies' datasets and using exclusively variables that cannot be modified by anthropogenic activities). Only then, proper reference conditions can be formulated for each waterbody type.

Wetlands have been lost and disturbed more rapidly than other ecosystems, and much of the global wetland area that remains is degraded (Millennium Ecosystem Assessment [40, 41]). Worldwide, an estimated half of the total wetland area has been lost due to anthropogenic activities [41]. Moreover, the historical information on wetlands is very scarce and often nonexistent, especially in Mediterranean areas. Thus, developing a wetland typology is a challenging task. One of the most reasonable ways to cope with these difficulties is to use expert judgement to define and evaluate the relevant abiotic variables, which, as previously said, should not be the same used to evaluate anthropogenic impacts and pressures. This approach supposes a deep knowledge of the ecological functioning of the wetlands to be assessed. Other more objective approaches analyse the influence of wetland environmental variables on the spatiotemporal patterns of their fauna [37, 38, 42, 43].

3 Water Quality Assessment Using Crustaceans and Insects: The $QAELS^{e}_{2010}$ Index

3.1 Background

Boix et al. [23] developed the *QAELS* water quality index for wetlands and shallow lakes carried out in Catalonia, based on microcrustacean sensitivity complemented with richness of crustaceans and insects. Later some improvements were done in the quality coefficients of the different species and in the definition of the quality category thresholds. Here we describe the resulting $QAELS^{e}_{2010}$ index.

3.2 Sampling Procedure

For the construction of the water quality $QAELS^{e}_{2010}$ index, we used data of 200 Mediterranean wetlands located throughout Catalonia (Fig. 1). This includes wetlands, shallow lakes, lagoons, ponds and pools, that is, all lentic waterbodies, temporary or permanent, that are shallow enough that light reaches the bottom allowing the presence of macrophytes or other primary producers at the maximum water depth [33, 44]. From here on, we will use the term "wetlands" to refer these shallow water ecosystems. Wetlands were sampled once (132 waterbodies) in late spring or twice a year (68 waterbodies) in late winter and late spring. All wetlands sampled were below 800 m a.s.l. to ensure they were under Mediterranean climatic conditions. Therefore, those located in mountain and alpine climatic areas, above

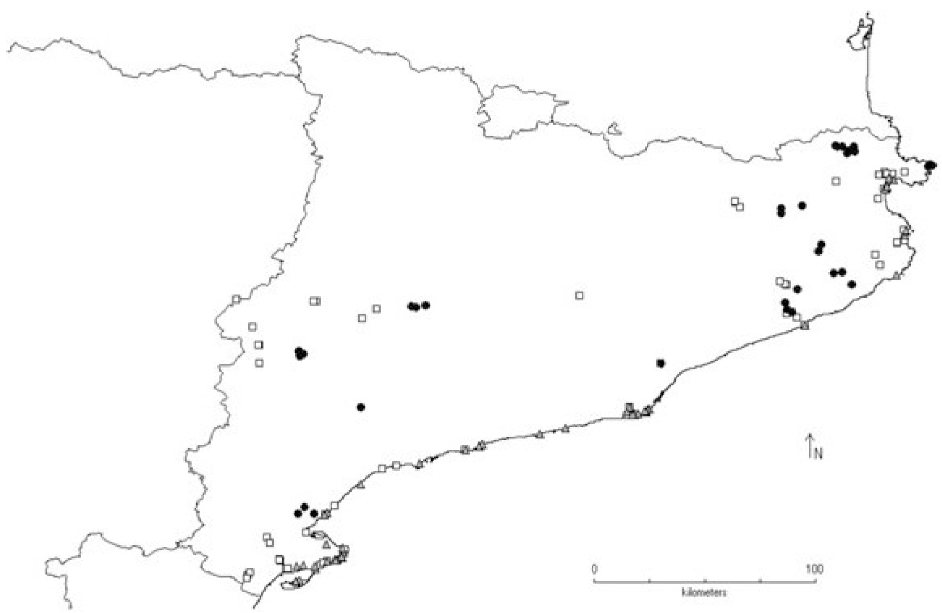

Fig. 1 Location of the studied wetlands (*triangles*, thalassohaline wetlands; *white squares*, permanent freshwater wetlands; *black points*, temporary freshwater wetlands)

800 m a.s.l., were not considered. Wetlands were previously classified by types following Boix et al. [23]. Thus 76 waterbodies were thalassohaline, 79 were freshwater permanent and 45 were freshwater temporary. Temperature, conductivity, percentage of oxygen saturation and pH were measured in situ. Chlorophyll-*a* was extracted using 80% methanol, after filtering water samples (Whatman GF/C filters), and measured following Talling and Driver [45]. Analyses of dissolved inorganic nutrients (ammonium, nitrite, nitrate and phosphate) were carried out from filtered samples and total nutrients (total nitrogen and total phosphorus) from unfiltered samples, following Grasshoff et al. [46].

Invertebrate sampling was performed as described in Boix et al. and ACA [23, 47], using a 20 cm diameter dip-net (mesh size 250 μm). At each wetland, three sweeps of dip-net "pushes" per visit were carried out along transects. Each sweep consisted of 20 dip-net "pushes" in rapid sequence, to cover all the different habitats in the littoral zone of the wetland. Only the organisms from the first sweep were used to estimate the relative abundances of microcrustaceans, whereas all sweeps were used to calculate the taxon richness. Samples were preserved in 10% formalin. All crustaceans and insects were identified to species level, or to the lowest taxonomic level possible, except for dipterans, which were always identified to family level.

3.3 *Building* $\mathrm{QAELS}^{e}_{2010}$ *Index*

The $QAELS^{e}_{2010}$ index consists of two components: the first one is obtained from the composition of microcrustaceans and the sensitivity of their different species to water quality ($ACCO_{2010}$ value); the second one is related to crustacean and insect richness (*RIC* value). Microcrustaceans and macroinvertebrates strongly differ in abundance, and a correct estimation of the abundance of both faunal groups may be highly time-consuming. Thus the $ACCO_{2010}$ value only considers microcrustacean taxa, because a rapid estimation of abundance is preferred in bio-assessment indices. However, when estimating richness, it is better to include as many faunal groups as possible [23], since a large number of taxa offer a spectrum of responses to environmental stresses [48]. That's why the *RIC* value includes crustacean and insect richness.

Microcrustacean sensitivities to build the $ACCO_{2010}$ value were obtained by means of a partial canonical correspondence analysis (PCCA). A different PCCA analysis was carried out for each wetland type. In the microcrustacean matrix, the relative abundance of each species was square-root transformed and rare species were downweighted in order to reduce their influence in the analysis. The water quality variables matrix used in PCCA was composed by a unique variable, the *TRIX* index, described by Vollenweider et al. [49]:

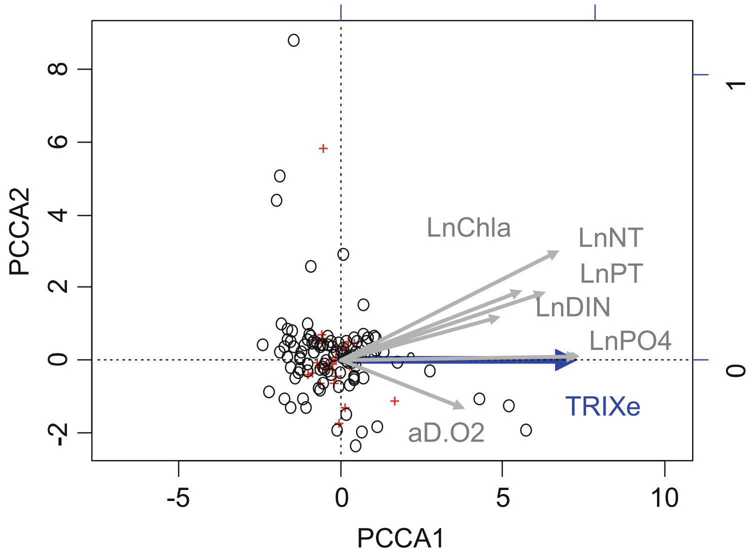

Fig. 2 Results of the PCCA analysis using the *TRIX* index (*blue arrow*) as variable indicative of water quality in permanent freshwater wetlands. Other variables related to water quality are not considered in the analysis, but included in the plot (*grey arrows*) as supplementary variables (*Chla* chlorophyll-*a*, *aD.O2* absolute deviation of 100% of oxygen saturation (see text), *DIN* dissolved inorganic nitrogen, *NT* total nitrogen, *PO4* soluble reactive phosphate, *PT* total phosphorus). *Circles* and *crosses* represent samples and species position, respectively. Similar plots were built for other wetland types (thalassohaline and temporary freshwater wetlands)

$$TRIX = \frac{[\log_{10}(\mathrm{Chla} \cdot \mathrm{aD.O2} \cdot \mathrm{DIN} \cdot \mathrm{Pt}) + 1.5]}{1.2}, \tag{1}$$

where Chla, DIN and Pt are the chlorophyll-*a*, the dissolved inorganic nitrogen and the total phosphorus concentrations ($\mathrm{mg} \cdot \mathrm{L}^{-1}$) and aD.O2 is the absolute deviation of the percentage of oxygen saturation (i.e. the absolute value of 100% O_2 saturation). This index has been widely used in water quality assessment, especially in transitional waters [50–52]. Variability caused by variables not necessarily related to water quality, such as temperature or conductivity, was removed from the PCCA analysis by entering them as covariables.

The first PCCA axis was strongly related to *TRIX* index in each of the three different wetland types. Other environmental variables related to water quality were included as supplementary variables, such as chlorophyll-*a*, total and dissolved nitrogen and phosphorus, related to the same first PCCA axis (Fig. 2). Thus, we used the microcrustacean species scores in this first PCCA axis as a measure of species sensitivity. Only species with occurrences >1% were considered indicator species. Microcrustacean indicator species were sorted by their scores in the first PCCA axis. Scores were distributed in ten categories, and a value between 1 and 10 was assigned to each indicator species. This rescaled score is the "quality coefficient" used for the computation of the $ACCO_{2010}$ value. Extreme and anomalous scores for the interval (values >1.5 times the interquartile range) were not taken into account for the creation of the ten categories. Quality coefficients for a given species differ among wetland types, and some taxa may be indicator in some types and not in others. The final $ACCO_{2010}$ value is obtained by means of the following equation:

$$ACCO = \sum_{i=1}^{j} k_i \cdot n_i; \quad n_i = \frac{N_i}{N_{\text{tot}}}, \tag{2}$$

where

i = each taxon with a weight in the analysis >1% (indicator species)
j = number of taxa with a weight in the analysis >1%
n_i = relative abundance of the species i
k_i = quality coefficient of the species i
N_i = abundance of the species i
N_{tot} = sum of the abundance of the species with a weight in the analysis >1%

To determine species quality coefficients and their robustness, for each microcrustacean species, we did 100 additional iterations of the same PCCA analyses (one per each wetland type) but randomly deleting 5% of the samples used. Quality coefficients k_i were then obtained by the weighted average of the quality coefficients of these 100 PCCA analyses, rescaled to a 0–10 value and rounded to the nearest integer. Figure 3 shows the results of the variability in coefficient estimation using this procedure. Results indicate a high robustness of quality coefficients in those species that show a narrow range of quality coefficients variability (see *Megacyclops viridis* or *Cypria ophtalmica* in Fig. 3) and a lower robustness in those species with wider quality coefficient variability (see *Simocephalus exspinosus* or *Eucyclops serrulatus* in Fig. 3).

The *RIC* value is used as a non-biased estimation of crustacean (micro- and macrocrustaceans) and insect richness (presence–absence data). *RIC* is calculated as the sum of the number of crustacean genera, the number of families of immature stages of insects (nymphs, pupae and larvae) and the number of genera of adult Coleoptera and Heteroptera. The resulting $QAELS_{2010}$ index is the combination of $ACCO_{2010}$ and *RIC* values, which differ depending on wetland types:

$$\text{Thalassohaline wetlands}: \quad QAELS_{2010} = (1 + ACCO_{2010}), \tag{3}$$

$$\text{Permanent freshwater wetlands}: \quad QAELS_{2010} = (1 + ACCO_{2010}) + \log_{10}(RIC + 1), \tag{4}$$

$$\text{Temporary freshwater wetlands}: \quad QAELS_{2010} = (1 + ACCO_{2010}) + \log_{10}(RIC + 1). \tag{5}$$

RIC is not used for $QAELS_{2010}$ computation in thalassohaline wetlands because *RIC* inclusion reduces correlation between $QAELS_{2010}$ and the variables related to water quality (Table 1). In thalassohaline ecosystems freshwater inputs also imply nutrient inputs and can be considered as disturbances that affect community structure

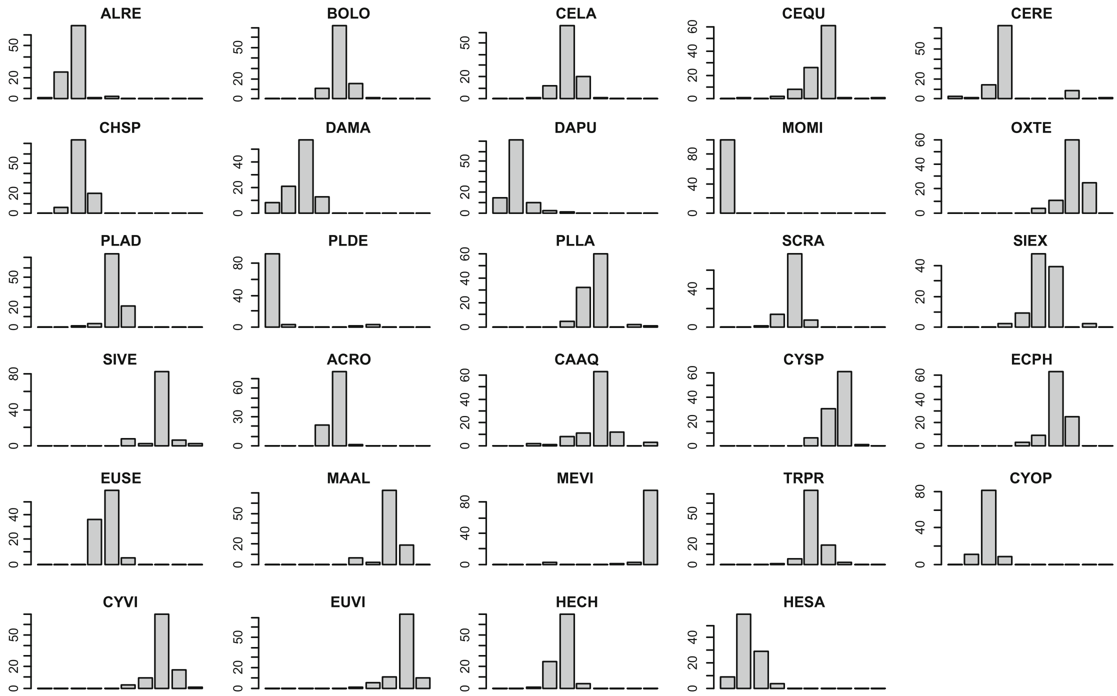

Fig. 3 Variation of quality coefficients (k_i in Eq. 1) in permanent freshwater wetlands after 100 iterations of the PCCA analysis, where randomly 5% of samples used was deleted. *Columns* represent the standardised value of k_i, from 1 (*left column*) to 10 (*right column*). *Column height* indicates the number of PCCAs where the species achieved a determinate k_i score. Species codes: CLADOCERANS—ALRE, *Coronatella rectangula*; BOLO, *Bosmina longirostris*; CELA, *Ceriodaphnia laticaudata*; CEQU, *C. quadrangula*; CERE, *C. reticulata*; CHSP, *Chydorus sphaericus*; DAMA, *Daphnia magna*; DAPU, *D. pulicaria*; MOMI, *Moina micrura*; OXTE, *Oxyurella tenuicaudis*; PLAD, *Pleuroxus aduncus*; PLDE, *P. denticulatus*; PLLA, *P. laevis*; SCRA, *Scapholeberis rammneri*; SIEX, *Simocephalus exspinosus*; SIVE, *S. vetulus*. COPEPODS—ACRO, *Acanthocyclops* gr. *robustus-vernalis*; CAAQ, *Calanipeda aquaedulcis*; CYSP, *Cyclops* sp.; ECPH, *Ectocyclops phaleratus*; EUSE, *Eucyclops serrulatus*; MAAL, *Macrocyclops albidus*; MEVI, *Megacyclops viridis*; TRPR, *Tropocyclops prasinus*. OSTRACODS—CYOP, *Cypria ophtalmica*; CYVI, *Cypridopsis vidua*; EUVI, *Eucypris virens*; HECH, *Herpetocypris chevreuxi*; HESA, *Heterocypris salina*

[53–55]. Freshwater inputs usually increase the number of species in those thalassohaline waters [43, 56]. Thus, an increase in species richness in these ecosystems may indicate a higher degree of disturbance related to higher nutrient concentrations coming with freshwater inputs.

Because maximum values of $QAELS_{2010}$ index differ in the different wetland types, each $QAELS_{2010}$ index was standardised with the division by the maximum $QAELS_{2010}$ value reached for a specific wetland type:

$$\text{Thalassohaline wetlands}: \quad QAELS^{e}_{2010} = \frac{QAELS_{2010}}{10.97}, \tag{6}$$

Table 1 Spearman correlation coefficients between variables related to trophic state and the *ACCO* or the *ACCO* + *RIC* indexes in permanent freshwater (PF), temporary freshwater (TF) and thalassohaline (TA) wetlands

		TN	TP	SRP	DIN	Chla	*TRIX*
PF	*ACCO*	n.s.	−0.27***	−0.20*	n.s.	−0.32***	−0.38***
	ACCO + *RIC*	n.s.	−0.35***	−0.31***	n.s.	−0.34***	−0.36***
TF	*ACCO*	−0.42**	−0.27*	−0.26*	−0.42**	−0.28*	−0.57***
	ACCO + *RIC*	−0.40*	n.s.	−0.32*	−0.39*	−0.34*	−0.57***
TA	*ACCO*	−0.48***	n.s.	−0.43***	n.s.	−0.22*	−0.42***
	ACCO + *RIC*	−0.49***	n.s.	−0.41***	n.s.	−0.24*	−0.43***

Note that the addition of the *RIC* value does not increase the correlation in TA wetlands. All trophic variables, except the *TRIX* index, were log transformed

TN total nitrogen, TP total phosphorus, SRP soluble reactive phosphate, DIN dissolved inorganic nitrogen, Chla chlorophyll-*a*

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *n.s.* not significant ($p > 0.05$)

$$\text{Permanent freshwater wetlands}: QAELS^{e}_{2010} = \frac{QAELS_{2010}}{12.44}, \tag{7}$$

$$\text{Temporary freshwater wetlands}: QAELS^{e}_{2010} = \frac{QAELS_{2010}}{11.08}, \tag{8}$$

where the divisor number corresponds to the maximum $QAELS_{2010}$ value obtained in each wetland type.

3.4 Required Taxonomic Resolution

Boix et al. [23], in their previous version of the *QAELS* index ($QAELS_{2004}$), showed that low levels of resolution in microcrustacean taxa determination were not acceptable, since correlations between the index obtained at species level and the index computed using taxonomic determination at main group or at family level gave low correlations (even not significant in some cases). When using the resolution at genus level, correlation values oscillated between 0.667 and 0.986, depending on wetland types. According to this, we correlated the $ACCO_{2010}$ values using taxonomic resolutions at species and genus level (Fig. 4) and found a high correlation for thalassohaline and permanent freshwater wetlands, but a low one for temporary freshwater wetlands. Thus, results suggest that a resolution at genus level is suitable for thalassohaline and permanent freshwater wetlands, but for temporary freshwater wetlands, the highest level of resolution is needed. Thus, in order to simplify the computation of $ACCO_{2010}$ index, we propose a taxonomic resolution at genera level for thalassohaline and permanent freshwater waterbodies, but at

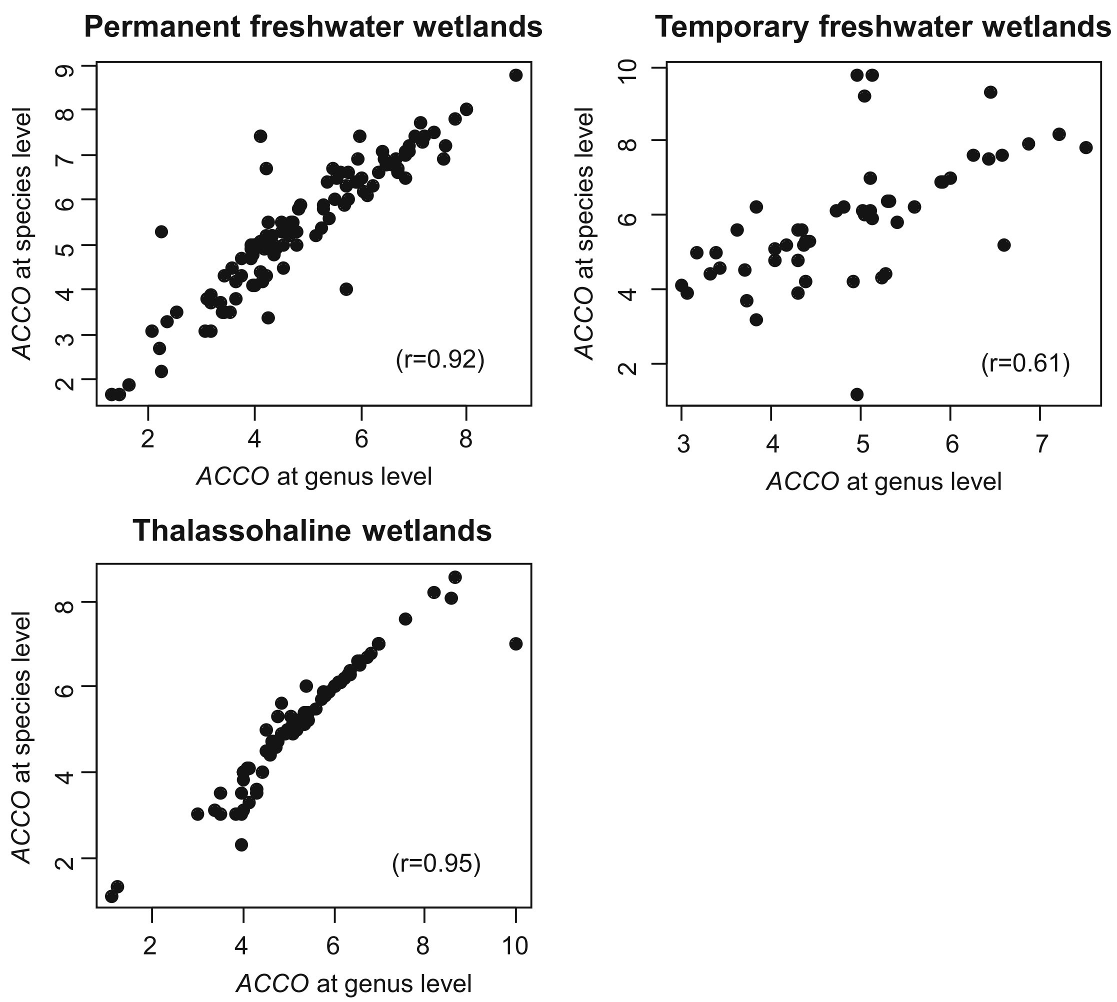

Fig. 4 Correlations between the *ACCO* indexes estimated with different taxonomic resolutions

species level for temporary freshwaters. Quality coefficients (k_i) obtained for each microcrustacean taxa are listed in Tables 2 and 3.

3.5 *Water Quality Thresholds*

To define the $QAELS^{e}_{2010}$ boundaries that separate the five categories proposed by the WFD (high, good, moderate, poor or bad), we follow the recommendations of the REFCOND document [57]. Five different Wallin et al. [57] proposals were tested, listed in Table 4. To select which of the five methods was the most suitable, we performed Spearman correlations between the water quality classes and variables dealing with trophic state (nutrients, chlorophyll-*a*, *TRIX* value). Results obtained in the five different proposals gave significant relationships between the water quality classes and the trophic-related variables. We chose proposal 5, which gave the highest correlation values (Fig. 5). The resulting category boundaries for each wetland type are listed in Table 5.

Table 2 Quality coefficients (k_i in Eq. 1) of each indicator genus for the computation of the $ACCO_{2010}$ value in each wetland type

	TA	PF
CLADOCERA		
Alona	–	8
Bosmina	–	5
Ceriodaphnia	–	4
Chydorus	5	3
Coronatella	–	8
Daphnia	1	2
Moina	–	1
Oxyurella	–	8
Pleuroxus	3	5
Scapholeberis	–	8
Simocephalus	4	7
COPEPODA		
Acanthocyclops	4	4
Calanipeda	6	6
Canuella	4	–
Cletocamptus	4	–
Cyclops	7	8
Diacyclops	7	–
Ectocyclops	–	7
Eucyclops	3	4
Eurytemora	7	–
Halicyclops	5	–
Harpacticus	7	–
Macrocyclops	–	8
Megacyclops	–	10
Mesochra	10	–
Nitokra	7	–
Paracyclops	–	1
Pseudonychocamptus	5	–
Tisbe	3	–
Tropocyclops	9	6
OSTRACODA		
Cypria	–	3
Cyprideis	5	–
Cypridopsis	7	8
Eucypris	6	8
Herpetocypris	–	4
Heterocypris	4	1
Loxoconcha	5	–
Sarscypridopsis	1	–
Xestoleberis	6	–

(–) Genera with no indicator value in this wetland type
TA thalassohaline wetlands, *PF* permanent freshwater wetlands

Table 3 Quality coefficients (k_i in Eq. 1) of each indicator species for the computation of the $ACCO_{2010}$ value in temporary freshwater wetlands, where a taxonomical resolution to species level is required

CLADOCERA	
Coronatella rectangula	3
Ceriodaphnia quadrangula	5
Ceriodaphnia reticulata	3
Chydorus sphaericus	6
Daphnia curvirostris	10
Daphnia magna	3
Daphnia obtusa	1
Daphnia pulicaria	7
Moina brachiata	5
Simocephalus exspinosus	6
Simocephalus vetulus	7
COPEPODA	
Acanthocyclops gr. *robustus-vernalis*	5
Canthocamptus staphylinus	9
Cyclops sp.	5
Diacyclops bicuspidatus	8
Diacyclops bisetosus	4
Diaptomus cyaneus	10
Megacyclops viridis	5
Metacyclops minutus	7
Mixodiaptomus incrassatus	7
Mixodiaptomus kupelwieseri	6
Neolovenula alluaudi	4
OSTRACODA	
Cyclocypris ovum	4
Cypridopsis vidua	8
Eucypris virens	5
Herpetocypris chevreuxi	7
Heterocypris barbara	4
Heterocypris incongruens	5
Plesiocypridopsis newtoni	4

4 The Use of Chironomidae as a Bioindicator: The *EQAT* Index

4.1 Background

Within the aquatic insects' assemblages found in the Mediterranean lagoons and wetlands of Spain, Chironomidae tend to be the most abundant and rich in species [58–61]. Chironomidae larvae are present in all habitats and have a great variety of biological traits; for example, *Chironomus* burrows in the sediment collecting organic matter that is being accumulated as fine sediment, while *Psectrocladius* tends to live attached to the helophytes and the submerged vegetation, feeding on

Table 4 Proposals for category boundaries tested

Proposal 1	All the locations of a given type were considered together
	High: $QAELS^{e}_{2010} > \mathrm{P90}$
	Good: $\mathrm{P75} < QAELS^{e}_{2010} < \mathrm{P90}$
	Moderate: $\mathrm{P50} < QAELS^{e}_{2010} < \mathrm{P75}$
	Poor: $\mathrm{P25} < QAELS^{e}_{2010} < \mathrm{P50}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P25}$
Proposal 2	Only locations under reference conditions (ref) were considered, from which the standard deviation (SD) is calculated
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P90ref} - \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Moderate: $\mathrm{P90ref} - 2 \cdot \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref} - \mathrm{SD}$
	Poor: $\mathrm{P90ref} - 3 \cdot \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref} - 2 \cdot \mathrm{SD}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P90ref} - 3 \cdot \mathrm{SD}$
Proposal 3	Only locations under reference conditions (ref) were considered, from which the standard deviation (SD) is calculated
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P90ref} - \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Moderate to bad categories, obtained by dividing the remaining values of the index in equal parts
Proposal 4	Only locations under reference conditions (ref) were considered, from which the standard deviation (SD) is calculated
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P90ref} - \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Percentiles of no reference locations (no_ref) were used for the remaining categories.
	Moderate: $\mathrm{P50no\_ref} < QAELS^{e}_{2010} < \mathrm{P90ref} - \mathrm{SD}$
	Poor: $\mathrm{P25no\_ref} < QAELS^{e}_{2010} < \mathrm{P50no\_ref}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P25no\_ref}$
Proposal 5	Only locations under reference conditions (ref) were considered for the high category. Percentiles of no reference locations (no_ref) were used for the remaining categories
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P75no\_ref} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Moderate: $\mathrm{P50no\_ref} < QAELS^{e}_{2010} < \mathrm{P75no\_ref}$
	Poor: $\mathrm{P25no\_ref} < QAELS^{e}_{2010} < \mathrm{P50no\_ref}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P25no\_ref}$

P percentiles, *SD* standard deviation

fresh algae [62]. Moreover, they are present over wide environmental ranges (including salinity), with some species being very sensitive to pollution, whereas others can survive in anoxic and polluted environments. Therefore, they have been successfully used as indicators of water quality in rivers [63–65] and lakes [66–68].

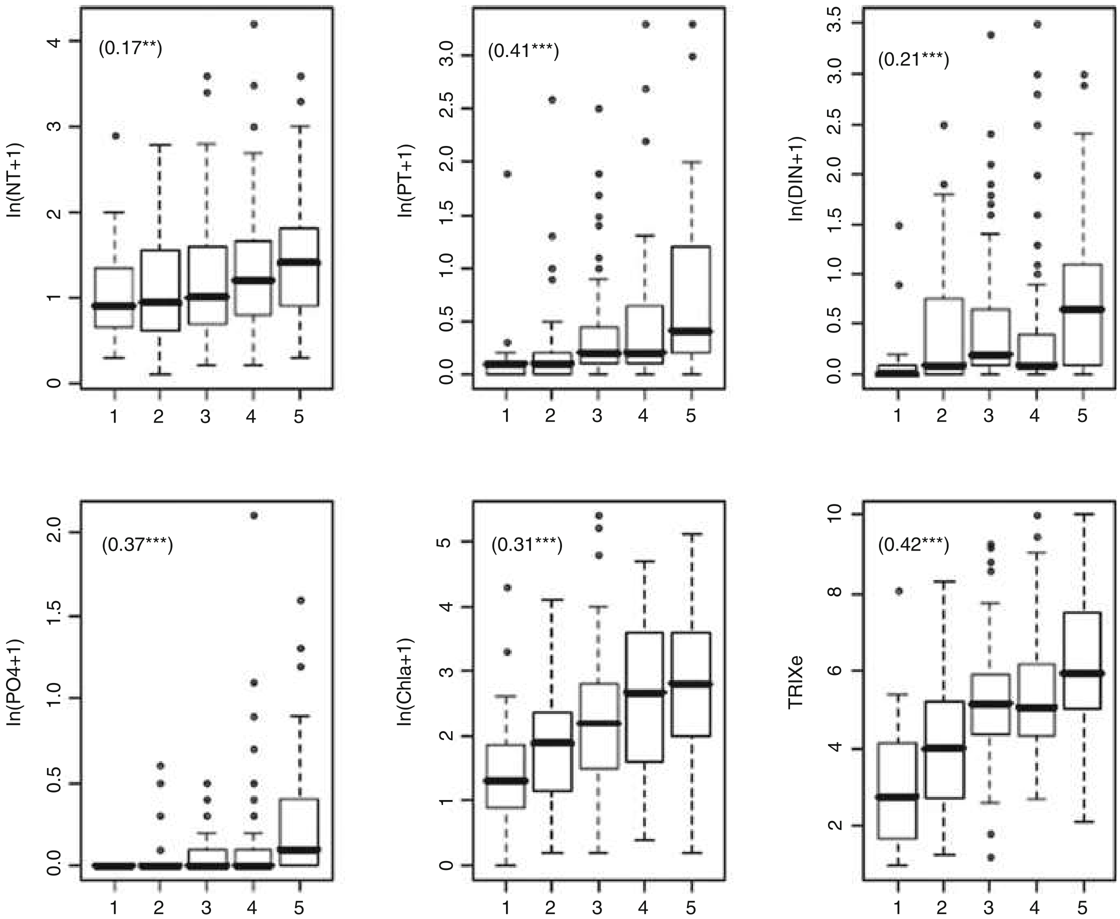

Fig. 5 *Boxplots* showing the variability of the different environmental variables related to trophic state for each $QAELS^{e}_{2010}$ quality class (*1*, high; *2*, good; *3*, moderate; *4*, poor; *5*, bad). $QAELS^{e}_{2010}$ quality classes were obtained as described in Table 4 proposal 5. The overall Spearman correlation coefficient is also included (**, $p < 0.01$; ***, $p < 0.001$). Codes of environmental variables as in Fig. 2

The *EQAT* biomonitoring tool is based on Chironomidae and has the advantage that it is cost-effective (cheap, involving low time consumption in the field and the laboratory and easy to use) and that it integrates all the habitats within the ecosystem. The tool can be easily used by trained technicians to assess water quality status on a regular basis and to help identify those waterbodies being at risk of failing to meet their environmental objectives according to the WFD.

4.2 *How to Use* EQAT?

The Chironomidae (Diptera) are holometabolous insects with four life stages (egg, larva, pupa and adult). Larvae grow in the water associated to the different available habitats. Eventually the late fourth instar larvae develop wing pads, moult to pupae and then swim to the water surface where adults cast their pupal skin (exuviae) and emerge to mate [69]. Since all the chironomid larvae inhabiting a given waterbody

Table 5 Boundaries of the different $QAELS^e_{2010}$ quality classes in permanent freshwater (PF), temporary freshwater (TF) and thalassohaline (TA) wetlands

Quality class	PF	TF	TA
High	$QAELS^e_{2010} \geq 0.86$	$QAELS^e_{2010} \geq 0.89$	$QAELS^e_{2010} \geq 0.72$
Good	$0.58 \leq QAELS^e_{2010} < 0.86$	$0.68 \leq QAELS^e_{2010} < 0.89$	$0.62 \leq QAELS^e_{2010} < 0.72$
Moderate	$0.51 \leq QAELS^e_{2010} < 0.58$	$0.56 \leq QAELS^e_{2010} < 0.68$	$0.55 \leq QAELS^e_{2010} < 0.62$
Poor	$0.39 \leq QAELS^e_{2010} < 0.51$	$0.45 \leq QAELS^e_{2010} < 0.56$	$0.46 \leq QAELS^e_{2010} < 0.55$
Bad	$QAELS^e_{2010} < 0.39$	$QAELS^e_{2010} < 0.45$	$QAELS^e_{2010} < 0.46$

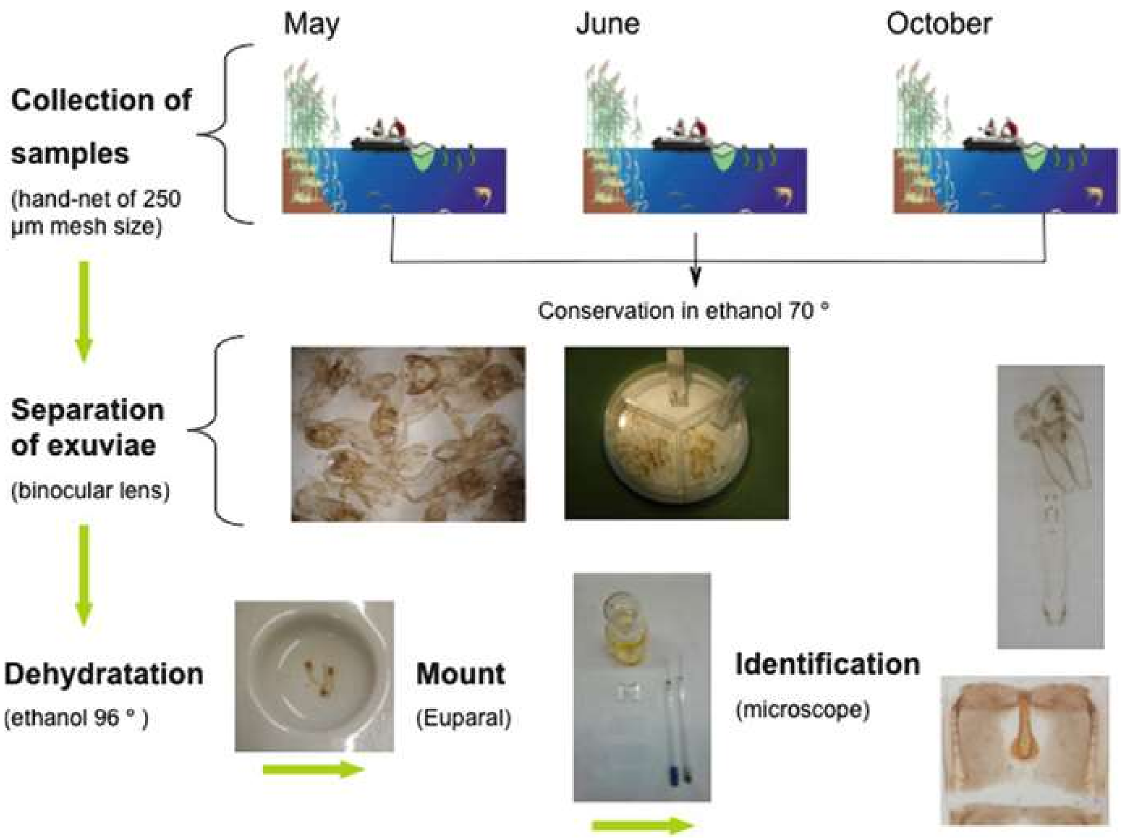

Fig. 6 Methodological scheme for the collection and processing of Chironomidae exuviae needed to apply the *EQAT* index. The first step is the collection of samples (ideally performed three times a year, coinciding with the periods of maximum emergence of Chironomidae), and *green arrows* indicate each next methodological step

will eventually undergo this metamorphosis, pupal exuviae collection has a great potential for characterising the whole chironomid community. Collection of samples is easy and fast. First the areas of accumulation of organic matter (characterised sometimes by the presence of white foam) must be identified, and then chironomid exuviae can be collected there by sweeping a 250 μm mesh size hand net along the shore. Ideally the samples should be collected on three different occasions (May, June and October), which are likely to comprise the maximum emergence periods of chironomids in Mediterranean lagoons [70]. The three samples can be merged and considered as one. Once collected, the samples must be preserved in ethanol 70° and taken to the laboratory. In the laboratory the samples must be sieved through a 250 μm mesh and placed in a Petri dish. Chironomid pupal exuviae must be removed from debris and identified to family level using binocular magnifying lens. Then they must be dehydrated using ethanol 96°, mounted permanently in Euparal on a microscope slide and identified to genus [71] using a high-magnification (400×) microscope (Fig. 6). We use genus level instead of species level identification because it is considerably less time-consuming and the genus-level index is equally robust for detecting changes in the environment [31].

Each genus has a score according to its sensitivity to pollution. When assessing the status of a given wetland, the index value is a simple function of the relative abundance of each chironomid genus and its indicator score:

$$EQAT = \sum_{i=1}^{s} n_i \cdot k_i, \tag{9}$$

where S is the number of genera, n_i the relative abundance of the genus i and k_i its quality coefficient.

4.3 How Was EQAT Designed?

Chironomidae exuviae were collected in 37 permanent shallow waterbodies associated to several wetland areas in Catalonia, especially in the coastal area. Then genus scores were derived from the indicator species analysis (INDVAL), proposed by Dufrêne and Legendre [72]. The INVDAL analysis can be considered as a statistically robust alternative to the expert judgement, since it is based on the taxa abundance and frequency in a given group of sites (e.g. polluted versus non-polluted sites). The aim of the analysis was to obtain a score that reflected the indicator potential of each genus along the pollution gradient. For this purpose a 5-step procedure was followed:

1. To obtain the species scores based on their tolerance to pollution, the *TRIX* index (Eq. 1) was used as a pressure indicator gradient. All the sites were classified in one of the five trophic categories proposed by Vollenwieder [49]: high, good, moderate, poor or bad.
2. The INDVAL analysis assigned each genus to a most probable group of sites (high, good, moderate, poor or bad) according to the relative abundance and frequency of the genus in each of the groups. The indicator (IV) and the p values (resulting from INDVAL) indicate how strongly the genus is linked to each group (the higher the IV value and the lower the p-value, the stronger is the link between the species and the group).
3. A scaled indicator value (SIV) was obtained for each genus taking into account its IV and its p values and the IV and p values of the rest of the genera assigned to the same group of sites.
4. Once a score (SIV) was obtained for each genus, it was rescaled from 0 to 1. First the importance of each group was weighted by dividing the number of genera assigned to it by the INDVAL analysis by the total number of genera. Then the boundaries between the five groups of sites (each of them enclosing a variable number of associated indicator genera) were settled according to the weight of each group.
5. The *EQAT* can be finally calculated as a function of the relative abundance of each genus and its indicator score.

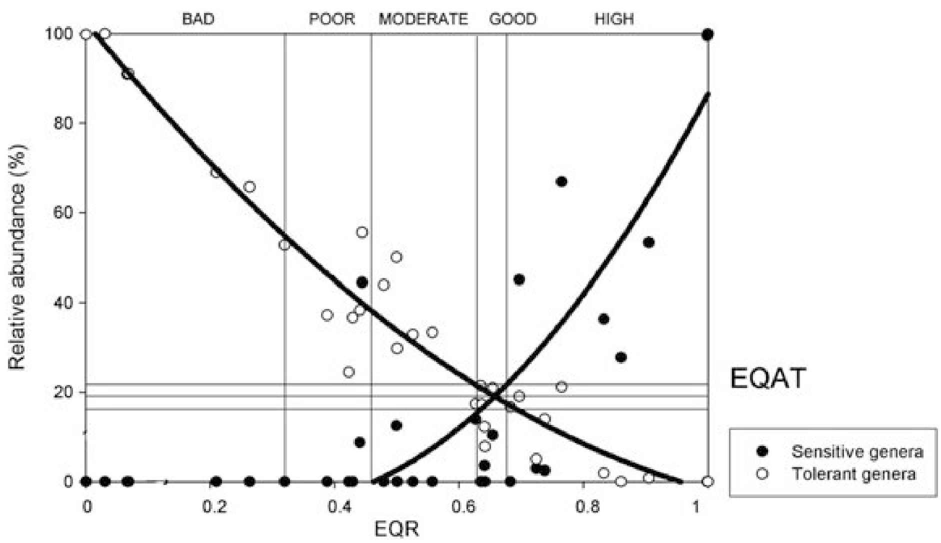

Fig. 7 Establishment of the quality class boundaries for the *EQAT* index, following Ruse [68]. On the *X*-axis the Ecological Quality Ratio (EQR), on the *Y*-axis the percentage of sensitive and tolerant genera. *Vertical lines* mark the boundaries between the quality classes: high, good, moderate, poor and bad

4.4 Establishing the Quality Class Boundaries

The final goal of the index was to assign each site to an ecological status category (high, good, moderate, poor and bad). In order to do this, the boundaries between the five categories had to be established. The class boundaries were derived from a plot of the relative frequency (%) of sensitive (genus score > 80th percentile of all the genus scores) and tolerant (genus score <20th percentile of all the genus scores) genera versus the Ecological Quality Ratio (EQR) (Fig. 7), as proposed by Ruse [68]. Since no truly reference sites could be found, the EQR was calculated taking as reference sites those that registered a maximum value of the *EQAT* index. The class boundaries were set as follows: high/good = the EQR corresponding to the relative frequency of the crossover point plus the SD; good/moderate = the EQR corresponding to the relative frequency of the crossover point minus the SD; moderate/poor = the fitted 0% of sensitive genera; poor/bad = no sensitive genera occurred and all the observed scores were well below reference (expert criteria).

4.5 Applicability

EQAT is a promising tool for monitoring the status of Mediterranean wetlands, as requested by the WFD. The index can be confidently applied in Mediterranean coastal lagoons and wetlands, but it would probably need to be adjusted in order to be used in another systems and/or geographical regions. The tool is particularly well suited for wetlands and small confined lagoons with a wide range of salinities and natural or artificial freshwater inputs, which are very common along the Mediterranean coast. These ecosystems are of great value (e.g. as a resting place for migratory birds), and at the same time, they are subjected to strong human pressures [31]. As being the last stop between the river and the sea, they receive large wastewater discharges [73] that can have great impacts on the biological communities [56]. Moreover human development tends to concentrate on the coast (e.g. Barcelona), causing problems like the hydrological alteration of the lagoons and habitat fragmentation. Therefore, the status of these coastal lagoons and wetlands needs to be continuously monitored to detect any anthropogenic impacts. In this regard, cost-effective (cheap, easy and fast) tools like *EQAT* can be very useful, since they allow the assessment of water quality of big geographic areas by nonexpert personnel within a short time period.

5 Assessing Habitat Condition: The *ECELS* Index

5.1 Background

$QAELS^{e}_{2010}$ and *EQAT* indexes reveal water quality in wetlands by means of the relationship between taxonomic composition and water nutrient charges. However, there are other aspects of wetland ecological status that are not necessarily related to water quality. This is the case of the habitat condition, which includes wetland hydromorphological aspects, human pressures or vegetation conservation status. Thus, artificial ponds built for irrigation purposes, with a very poor natural value, may have high water quality (e.g. if they are filled with pumped groundwater). On the other hand, valuable natural waterbodies may be stressed by agricultural or livestock pollution, resulting in poor water quality. Moreover, some wetlands with high water quality may be degraded in their littoral morphology or have been subjected to a strong human pressure, such as surrounding urbanisation or other human impacts. To address this question, we proposed an in situ rapid assessment method to define wetland habitat condition adapted for Mediterranean wetlands, following the rationale of other rapid assessments developed for wetlands [74] and lotic environments, such as *RCE* [75] or *QBR* [76]. This is the *ECELS* index, fully described in Sala et al. [32] and ACA [47].

5.1.1 *ECELS* Components

As described in Sala et al. [32], *ECELS* index is based on 5 components: littoral morphology, human activity, water aspect, emergent vegetation and hydrophytic vegetation. These components consider the attributes that a well-preserved wetland should have, according to a revision of widely used attributes in conservation assessments [74, 77–82], together with additional criteria that were derived from an exhaustive survey conducted by the authors.

The basin littoral morphology component (score 0–20) evaluates the slope of the wetland littoral, assuming that smooth slopes facilitate expansion of water during flooding events and allow the existence of different habitats that may increase the overall biodiversity. Anthropogenic alteration usually limits potential expansion of flooded areas with the alteration of littoral morphology and the presence of structures or activities, such as levees or burials. The human activity component (score 0–20) considers the human uses around and inside the wetland basin and in its neighbouring catchments. This includes agriculture and livestock activities, hydraulic equipment affecting water volume and turnover, transport and building facilities in the surroundings or presence of allochthonous or domestic fauna. Other aspects of human presence, such as frequency or presence of rubbish, and even those with a positive effect, such as protection and management, are considered as well. The water aspect component (score 0–10) does not try to evaluate its water quality. It only takes into account some water characteristics, such as transparency and odour, which can reflect intense anthropogenic influence. The emergent vegetation component (score 0–30) assesses the abundance and zonation of the vegetation belt, using a rough, semi-quantitative abundance approach. Species dominance, the presence of exotic plants and the presence or absence of trees around the wetland are also considered. Finally, the hydrophytic vegetation component (score 0–20) analyses the submerged and floating macrophytes using a very similar rough abundance approach. Thus, the maximum score for a wetland is 100.

By means of the analyses of these five components, *ECELS* index tries to highlight how far is the wetland from the structure, composition and zonation of a reference wetland [83–85]. A wetland with an *ECELS* score of 100 would be this with absence of human uses or structures, a gradual slope of its littoral that favours water expansion during flooding and the existence of a well-developed littoral community, a complete belt of emergent vegetation and a dense recover of submerged macrophytes. On the other hand, a wetland with an *ECELS* score of 0 might be a bad-operating aeration tank of a waste water treatment plant, with a constant surface of the flooded area, man-made control of water turnover, high turbidity, strong odour and absence of emergent and submerged vegetation. The *ECELS* scores obtained are categorised following the guidelines of the WFD as follows: high $\geq$90; good 70–89; moderate 50–69; poor 30–49; bad $<$30.

5.2 Applicability

ECELS index has been used elsewhere for wetland characterisation and for habitat conservation assessment [47, 86, 87]. The components of *ECELS* index are independent among themselves, each one informing about a complementary aspect. This structure makes it easy to identify the degradation problems of a particular wetland, which is useful for management purposes in order to determine the specific problems in conservation status or to identify which aspects of a managed wetland can be enhanced to reach a higher habitat condition.

Further its use in ecological status characterisation, one of the main advantages of the *ECELS* index, is that it gives a numerical value for an attribute that usually is categorical, as is the case of habitat condition. This facilitates the use of habitat condition in further numerical analyses dealing with wetland ecological functioning. In this sense, *ECELS* index has been included in environmental data matrix in the analysis of the effects of anthropogenic pressures on diatoms and macroinvertebrate species composition [88], on wetland species biodiversity patterns [89] and on dispersal ability patterns of passive dispersers in aquatic invertebrate assemblages [90].

6 Evaluating Ecological Status in Mediterranean Wetlands of Catalonia

The evaluation of the ecological status in a wetland can be obtained by means of a double entry table, combining a water quality index and a habitat condition index. Table 6 summarises the ecological status evaluation using $QAELS^{e}_{2010}$ and *ECELS* indexes.

According to estimation of ecological status in Table 6, the percentage of sampled wetlands in Catalonia that achieved the standards of high or good ecological status required by the WFD was low in permanent freshwater (14%) and thalassohaline (18.4%) wetlands (Table 7). This percentage is higher in temporary freshwater wetlands (30.8%). In the case of permanent freshwater wetlands, we did not find any wetland with high ecological status, while no temporary freshwater wetlands fall into the bad class. Having a look to the $QAELS^{e}_{2010}$ and *ECELS* percentages, we can assume that high ecological status in thalassohaline and temporary freshwater wetlands was mainly not achieved due to a low water quality (lower $QAELS^{e}_{2010}$ values), a low habitat condition being the main cause of the impairment to achieve good ecological status in permanent freshwater wetlands. These differences may be the consequence of the different human pressures that these ecosystems have suffered. Historically, humans have reduced the extension of

Table 6 Estimation of the ecological status of a wetland by means of the combination of the $QAELS^{e}_{2010}$ (water quality) and the *ECELS* (habitat condition) indexes

		$QAELS^{e}_{2010}$ quality classes				
		I	II	III	IV	V
ECELS quality classes	I	High	Good	Good	Moderate	Poor
	II	Good	Good	Moderate	Moderate	Poor
	III	Good	Moderate	Moderate	Poor	Bad
	IV	Moderate	Moderate	Poor	Poor	Bad
	V	Poor	Poor	Bad	Bad	Bad

Table 7 Percentage of wetlands for each wetland type falling in each quality class (I, high; II, good; III, moderate; IV, poor; V, bad) of water quality ($QAELS^{e}_{2010}$ index), habitat condition (*ECELS* index) and the resulting ecological status in a selection of 105 wetlands located throughout Catalonia

	N	I	II	III	IV	V
$QAELS^{e}_{2010}$						
Thalassohaline wetlands	49	8.2	16.3	34.7	34.7	6.1
Permanent freshwater wetlands	43	4.7	34.9	20.9	23.3	16.3
Temporary freshwater wetlands	13	7.7	15.4	46.2	30.8	0.0
ECELS						
Thalassohaline wetlands	49	16.3	32.7	22.4	24.5	4.1
Permanent freshwater wetlands	43	2.3	16.3	37.2	32.6	11.6
Temporary freshwater wetlands	13	30.8	23.1	46.2	0.0	0.0
Ecological status						
Thalassohaline wetlands	49	4.1	14.3	49.0	26.5	6.1
Permanent freshwater wetlands	43	0.0	14.0	37.2	32.6	16.3
Temporary freshwater wetlands	13	7.7	23.1	46.2	23.1	0.0

permanent freshwater wetlands, using them for runoff and irrigation purposes and limiting their overflowing capacity. This affects water quality, but especially habitat condition. On the other hand, the historical fight of humans against temporary freshwater wetlands mainly consists on the burying of these temporary habitats and their substitution by farmlands. Thus, most of them disappear [91–93], but the remaining temporary freshwater wetlands still conserve high ecological standards.

Regarding *ECELS* results in Catalan wetlands, the decomposition of the *ECELS* index in five components allows to distinguish which part of habitat condition is more affected by human pressure (Fig. 8). Scores of the *ECELS* components 2 (human activity) and 5 (hydrophytic vegetation) are those that decrease faster with decreasing habitat condition, while components 3 (water characteristics) and 4 (emergent vegetation) remain unaltered even under intermediate habitat condition. Moreover, when comparing the results by waterbody type, it can be seen that permanent freshwater and thalassohaline wetlands show a gradual pattern of degradation that similarly affects *ECELS* components in both waterbody types. However, the pattern observed from temporary freshwater wetlands was different, and so TF the change from high to good habitat condition in those last wetlands is mainly due to the impoverishment of the hydrophytic vegetation.

$QAELS^{e}_{2010}$, *EQAT* and *ECELS* indexes are promising tools to evaluate the ecological status in Mediterranean wetlands and can help to provide criteria in the management of these endangered aquatic ecosystems. To recover their ecological functioning and to integrate them within responsible and sustainable human uses in their reception, basins must be a priority in order to ensure the welfare of future generations.

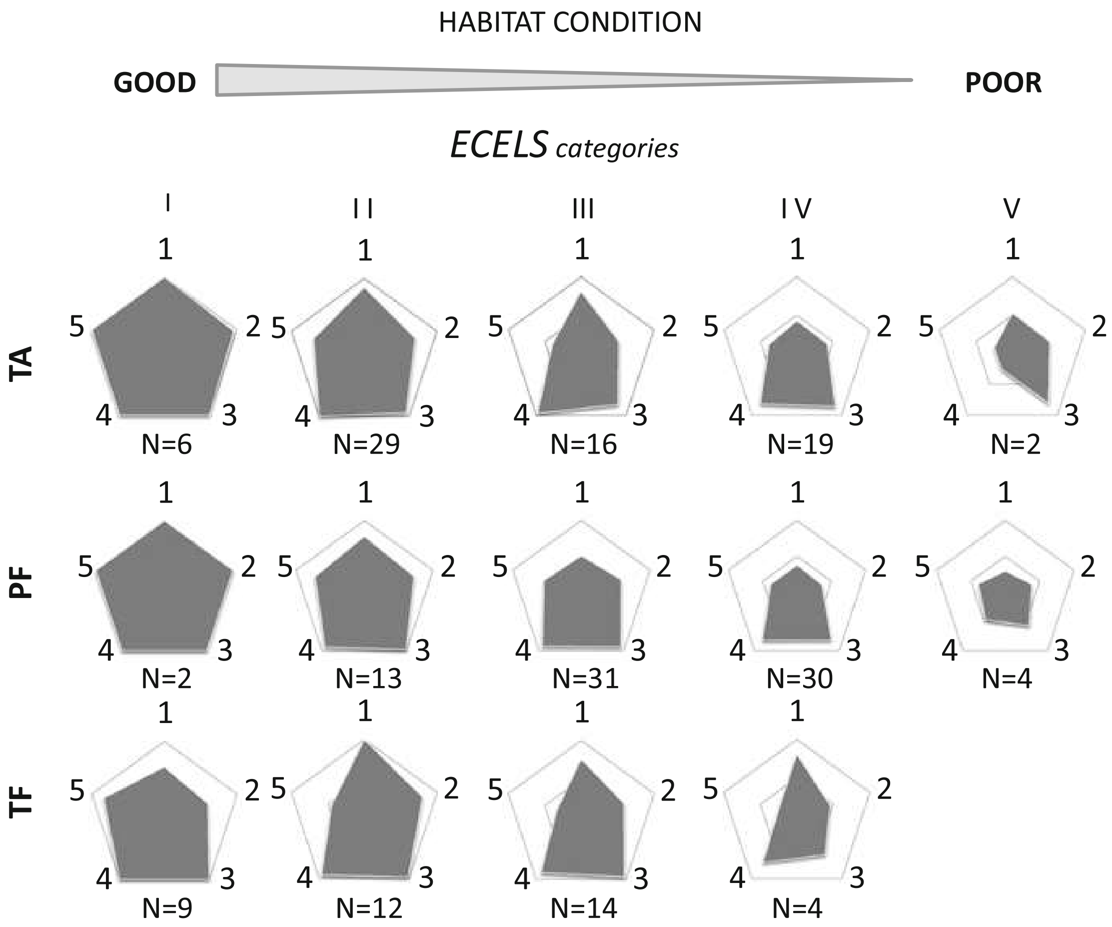

Fig. 8 Values of the *ECELS* components in Catalan wetlands sorted by wetland type (*TA* thalassohaline wetlands, *PF* permanent freshwater wetlands, *TF* temporary freshwater wetlands) and by *ECELS* quality categories (*I*, high; *II*, good; *III*, moderate; *IV*, poor; *V*, bad). Each *pentagon angle* represents a component of the *ECELS* index (*1*, littoral morphology; *2*, human activity; *3*, water characteristics; *4*, emergent vegetation; *5*, hydrophytic vegetation). The width of the *grey area* is proportional to the average value of the score of each component. *N* number of wetlands

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