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## Correspondence between zooplankton assemblages and the Estuary Environment Classification system

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## **Abstract**

We tested whether variability in zooplankton assemblages was consistent with the categories of estuarine environments proposed by the 'Estuary Environment Classification' system (EEC) (Hume et al., 2007) across a variety of North Island, New Zealand, estuaries. The EEC classifies estuaries into eight categories (A to F) based primarily on a combination of three abiotic controlling factors: ocean forcing, river forcing and basin morphometry. Additionally, we tested whether Remane's curve, which predicts higher diversities of benthic macrofauna and high and low salinities, can be applied to zooplankton assemblages. We focused on three of the eight EEC categories (B, D and F), which covered the range of estuaries with river inputs dominating (B) to ocean influence dominating (F). Additionally, we included samples from river (FW) and sea (MW) to encompass the entire salinity range. Zooplankton assemblages varied across the categories examined in accordance with a salinity gradient predicted by the EEC. Three groups of zooplankton were distinguishable: the first formed by the most freshwater categories, FW and B, and dominated by rotifers (primarily Bdelloidea) and estuarine copepods (*Gladioferans pectinatus*), a second group formed by categories D and F, of intermediate salinity, dominated by copepods (*Euterpina acutifrons*), and a final group including the purely marine category MW and dominated also by *E. acutifrons* along with other marine taxa. Zooplankton diversity responded to the salinity gradient in a manner expected from Remane's curve. The results of this study support others which have shown salinity to be the main factor driving zooplankton community composition and diversity.

**Keywords:** transitional ecosystems, estuary environment classification (EEC), Remane's curve, zooplankton, salinity, New Zealand

## 1. Introduction

‘Transitional waters’ is a miscellaneous term that covers a wide range of aquatic ecosystems including estuaries, fjords, lentic lagoons, river mouths, tidal creeks, deltas and similar coastal environments (e.g., McLusky and Elliot, 2007; Tagliapietra et al., 2009; Basset et al., 2012). The boundary between one type of transitional water and another is not always clear, with each of these ecosystems differing from the others in their hydrology, morphology, geology and biology. In the present study, we focus on estuaries, which can in general be defined as “*semi-enclosed coastal bodies of water which have a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage*” (Pritchard, 1967). A number of attempts to classify estuaries has been carried out, taking into account abiotic factors (e.g., geomorphology; Hume et al., 1988; Reddering, 1998), the origin and evolution of estuaries (Roy, 1984), hydrology and salinity (Hansen and Rattray 1966; Scott, 1993), or combinations of the above (Engle et al., 2007). However, such classifications are human impositions, and do not always correspond well with the biotic nature of the systems. Nevertheless, such classifications are not trivial, as they can influence political, management and conservational resources actions (Bowker et al., 2000).

Hume et al. (2007) developed a classification system for estuarine environments in New Zealand, referred to as the ‘Estuary Environment Classification’ (EEC). This classification system, in short, is based on a hierarchical view of the abiotic components that comprise estuarine environments. The EEC postulates that climatic, oceanic, riverine and catchment factors ‘control’ a hierarchy of processes, which broadly determine the physical and biological characteristics of estuaries. Since its development, the classification system has been applied in New Zealand and other areas of the world, although with modifications (e.g., South Korea) (Jang and Hwang, 2013). Based on the EEC, it is expected that the

hydrodynamic processes, including riverine and oceanic inputs, should determine the characteristics of the estuaries, such as water clarity, stratification, proportion of intertidal area, and salinity. Simultaneously, it is expected that these features will shape the biological characteristics of the estuaries. However, the correspondence between the EEC (based exclusively on abiotic factors) and biological characteristics has not yet been tested.

Within estuarine environments, salinity values typically acquire the shape of a gradient, which is directly linked to the hydrogeomorphology (i.e., the content of freshwater is greater in upper than in lower estuary areas), which is in line with the provisions of EEC system. This salinity gradient has been demonstrated by numerous studies to be one of the most important factors driving the heterogeneity of habitats and biodiversity in both terrestrial and aquatic ecosystems (e.g., Dobson and Frid, 1998; Wagner, 1999; Briggs et al., 2003; Silvestri, 2005). With this salinity gradient can be expected changes in community composition and species richness. Richness is expected to be higher in systems with salinity values close to purely fresh- or fully marine-waters than in brackish (transitional) waters. This is an expression of the biodiversity pattern ‘Remane’s curve’ (Remane, 1934), which although sometimes questioned (e.g., Barnes, 1989; Attrill and Rundle, 2002; Telesh et al., 2011a), is still typically considered as the model that best describes the general pattern of diversity in aquatic systems.

Given the differences in physical conditions among types of estuaries defined by the EEC, and the salinity gradient expected because of them, a correspondence between hydrogeomorphological types of estuarine environments based on the EEC, and biological assemblages determined by the salinity gradient, could be expected. In the present study, we tested whether three categories of estuaries assessed by the EEC show a correspondence with the distribution of the zooplankton assemblages. We hypothesize that: 1) the taxonomic composition of zooplankton will vary concomitant with the EEC categories, and composition

will change across a gradient from greater freshwater influence to greater marine influence; and that 2) a pattern of zooplankton diversity will be observed consistent with Remane's curve. The expected patterns in taxonomic composition and diversity were analysed for zooplankton assemblages, which are commonly disregarded in studies of transitional environments in favour to macro-communities (e.g., benthic macroinvertebrates or fish). However, zooplankton play a prominent role in the functioning of aquatic ecosystems as, for example, key links in food-chains between primary producers and fish (Capriulo et al., 2002; Turner, 2004).

## **2. Material and Methods**

### *2.1. Estuary Environment Classification (EEC)*

The purpose of EEC is to categorize estuaries according to their externally influenced physical characteristics (Hume et al., 2007). EEC is composed of four levels according to the spatial scales and processes, with Level 1 being the broadest scale (regional level ranging  $10^6$ - $10^4$  km<sup>2</sup>) and Level 4 the finest (sub-estuary level ranging 1-0.1 km<sup>2</sup>) (see Figure 1 in Hume et al., 2007). Within the large-scale variation described at Level 1, the variation in characteristics among individual whole estuaries are dominated first by estuary-scale 'hydrodynamic' processes (Level 2) and then by 'catchment' processes (catchment geology and catchment land cover) (Level 3). For this study, we examined estuaries within a single region, and thus considered the estuary types recognised at Level 2 (i.e., estuary-scale). Level 2 discriminates estuaries based on basin morphometry, and the degree of river and oceanic forcing. We also chose Level 2 because this level will have the greatest influence on biological characteristics among estuaries in a given area, as the biota ultimately will be directly affected by the hydrodynamic processes occurring within estuaries, due to their circulation, mixing, stratification, flushing and sedimentation.

## *2.2. Selection of the sites*

Fifteen sites around the North Island, New Zealand (Figure 1), were surveyed in October and November 2011 (austral spring). The sites were selected based on the EEC system developed by Hume et al. (2007). However, not all categories within Level 2 could be sampled for a variety of reasons; for example, logistic limitations prevented us from reaching some sites, while some categories were represented by a limited number of estuaries. Therefore, in our selection of the EEC categories used, we considered the following criteria: estuaries in each category were well represented in the North Island, they were logistically feasible to reach, categories were not very similar to each other and they were geographically well distributed (i.e., whenever possible, for the same category, say B, we chose three estuaries from this category, one in the north, one in the east, and one in the west of the island). Eventually, we chose three (B, D and F) of the eight categories (A to H), as these were well represented in the North Island, provided a contrast in types of estuary across the full gradient of categories, and also enabled for sampling to be undertaken of estuaries of the same category at geographically separated sites (Figure 2). Additionally, we also collected samples from three rivers (freshwater, FW) and three ocean sites (marine water, MW). Thus, the final design consisted of five categories (FW, B, D, F and MW), with three sites per category, and three sampling stations per site (replicates) separated by 500 m each, which yielded a total of 45 samples (Table 1). For further description of the selected estuaries including land use and land cover, refer to Appendix 1.

## *2.3. Sampling procedure*

At each sampling station, 40 L of water was filtered through a plankton net (40  $\mu\text{m}$  mesh). Sampling was undertaken by wading, with samples collected at a depth of 0.5 m to 1.5 m.

Zooplankton were preserved in ca. 75% ethanol (final concentration). In the laboratory the contents of each vial were washed through a 40 µm mesh to remove ethanol, and samples made up to a known volume ranging from 10 mL to 200 mL (depending on the amount of sediment or zooplankton within the samples). Samples were enumerated in 5-ml aliquots in a Perspex counting tray until at least 300 individuals were encountered (13 of 45 samples), or until the entire sample was examined if less than 300 individuals were found (the remaining 32 samples). All zooplankton samples were collected approximately 3 hours before or after low and high tide providing an influence of both fresh- and marine water. Additionally, at each sampling station standard water-chemistry variables were measured *in situ* using field electrodes (Yellow Springs Instruments, Ohio, USA, and Orion, England), including surface water temperature (°C), salinity, dissolved oxygen concentration (mg/L), and pH.

#### 2.4. Data Analysis

Changes in salinity and the other water-chemistry variables among categories were analysed using one-way ANOVA for parametric data (pH and water temperature) and Kruskal-Wallis test for non-parametric data (salinity and dissolved oxygen), followed by post-hoc tests (HSD-Tukey and Mann-Whitney U-test, respectively). Category of estuary was considered a fixed factor with five levels (FW, B, D, F and MW). Univariate tests were carried out with R software 3.2.2 (R Development Core Team 2014).

We estimated species richness (and 95% confidence intervals) for each of the five categories using the nonparametric estimator Chao 2, calculated based on species present in the three sites from each category, providing a single number for each estuary type (Gotelli and Colwell, 2001). This estimator is considered one of the most reliable predictors of total species richness, including for zooplankton, when a similar number of samples is used (Dumont & Segers 1996; Hortal et al., 2006; Muirhead et al. 2006). Following Colwell et al.

(2004), the criterion used to determine if the results obtained by the Chao2 estimator were significantly different among categories ( $p < 0.05$ ) was the absence of overlap in their 95% confidence intervals. Chao 2 estimations were carried out with EstimateS software (Colwell, 2005).

Finally, to examine whether zooplankton composition differed among categories (FW, B, D, F and MW), non-metric multidimensional scaling (MDS), based on a Bray–Curtis similarity matrix, was used. Zooplankton densities were  $\log(x+1)$  transformed prior to analysis to downweigh the importance of highly abundant species, and to take in to consideration less abundant species. To test for differences in zooplankton assemblages among categories, one way-Analysis of Similarity (ANOSIM) was applied. ANOSIM is a non-parametric permutation method undertaken on the similarity matrix underlying the MDS ordination. The calculated statistic ‘R’ provides a measure of the dissimilarity among groups of samples, selected *a priori*, and ranges from -1 to +1. An R-value close to 1 indicates that the species composition among the selected groups is dissimilar, while an R-value close to 0 indicates that species composition among groups is similar. Additionally, a Similarity Percentage (SIMPER, Clarke 1993) analysis was used to examine the contribution of each zooplankton taxon to the average dissimilarity between possible pairs of categories compared (e.g., FW vs. B; FW vs. D, and so on), and the average similarity within a group (within each category). Consequently, information about which zooplankton taxa best characterize each category can be obtained. The MDS, ANOSIM and SIMPER analyses were performed using PRIMER 6 (Clarke and Warwick, 2001). For additional information regarding density data, see Appendix 2.

### **3. Results**

#### ***3.1. Water-chemistry***



Salinity values significantly differed among categories (H (4, n=45)=39.0,  $p < 0.01$ ). FW and B showed marginally non-significant differences between them (post-hoc U-test  $p = 0.059$ ; FW=0.10±0.0 and B=0.24±0.36) and both had significantly lower salinity values than the rest of the categories ( $p < 0.01$ ) (Figure 3a). The F and MW categories had the highest salinity values ( $p < 0.01$ ; F=30.34±0.76 and MW=30.26±0.71) (Figure 3a). Category D was significantly different ( $p < 0.01$ ) from all of the other four categories, having intermediate salinities (27.50±1.27) (Figure 3a). Significant differences in pH were observed among categories ( $F_{4, 40} = 43.24$ ,  $p < 0.01$ ). Two groups were well-differentiated: one group comprised the FW and B categories and had significantly lower pH values ( $p < 0.01$ ) than a second group comprised of category D, F and MW sites (Figure 3b). Oxygen values were significantly different among categories (H (4, n=45)=17.02,  $p < 0.01$ ); in particular, higher oxygen concentrations were observed in the F and MW categories ( $p < 0.01$ ) than in the other categories (Figure 3c). Water temperature was significantly higher in categories D and F than in the rest of the categories ( $F_{4, 40} = 4.84$ ,  $p < 0.01$  and post-hoc  $p < 0.01$ ), likely because these categories were also the shallowest (Figure 3d).

### **3.2. Taxon richness (Chao2 estimator)**

The total number of taxa estimated by the Chao2 estimator differed significantly among categories. The most freshwater categories (FW: 32 CI [28-51] and B: 29 [23-55]) and the purely marine category (MW: 34 [32-46]) had significantly higher (CIs did not overlap) zooplankton diversity estimates than the sites with intermediate salinity (D: 18 [18-22] and F: 22 [22-23]) (Figure 4a). Thus, we observed zooplankton richness to reflect the pattern described by Remane's curve (Figure 4b).

### **3.3. Zooplankton assemblages**

A total of 64 taxa were recognised in this study (Figure 5). Some taxa were exclusively present in only one of the categories (e.g., *Dicranophorus epicharis* in FW, *Gladioferens pectinatus* in B, *Miscegenus heretaunga* in MW), reflecting the stenohaline character of some species. Other zooplankton taxa were present in several categories (e.g., *Tenagomysis macropsis* and *Euterpina acutifrons*, both in B, D, F and MW), indicating their euryhaline character (Figure 5). These differences and similarities in composition among categories were also reinforced by the results obtained from the ANOSIM and SIMPER analyses. ANOSIM results showed non-significant differences between the categories FW and B (albeit marginally,  $p=0.059$ ) (Table 2). On one hand, both categories shared a high abundance of Bdelloid rotifers (Figure 6a); conversely, the taxa that most contributed to the average dissimilarities between these categories were the copepod *Acanthocyclops robustus* and the cladoceran *Bosmina meridionalis*, both present in category FW, and the copepod *Gladioferens pectinatus* and rotifer *Dicranophorus* sp., both present in category B (Table 3). Categories D and F had zooplankton communities that were not significantly different (R-value close to 0;  $p$ -value  $>0.05$ ) difference (Table 2). Both categories shared a high abundance of the copepod *Euterpina acutifrons* (Figure 6b); however, the taxa that most contributed to the average dissimilarities between these categories were the copepod *Corycaeus* sp. and larvae of bivalve and polychaete. Although these species were present in both categories, the first two taxa were more abundant in category F than D, and polychaetes in category D than F. Finally, the category MW was significantly different from all other categories (ANOSIM;  $p < 0.01$  for all pair-comparisons which included this category) (Table 3), although shared ~58% of contributing taxa with category F (Table 2).

Based on zooplankton assemblages, a group formed by the FW and B categories could be differentiated. This FW-B group differs from a group containing the D and F categories, which were similar in composition to each other. Finally, MW differed from all

the other categories, although in terms of taxonomic composition was closer to D-F than to FW-B (Figure 6).

#### **4. Discussion**

Our study aimed to determine whether the already well-established categories of estuarine environments based on abiotic components ('Estuary Environment Classification'; EEC) (Hume et al., 2007) had a biotic consistency with zooplankton assemblages. Additionally, the biodiversity pattern related to the salinity gradient for aquatic systems proposed by Remane (1934) was tested in connection with the salinity gradient expected from the EEC categories.

Our results supported the hypothesis that abiotic variability is a major factor influencing the distribution and species diversity of zooplankton among estuarine environments (Laprise and Dodson, 1994). In our study, zooplankton assemblages were distributed into three well defined groups that correlated primarily with the salinity gradient, as expected among estuarine environments. As expressed by other authors (David et al., 2016) the response of zooplankton to the salinity gradients is not a simple matter of the osmoregulation abilities of species, but that different salinities are associated with differences in environmental characteristics and hydrodynamic features (as captured by the EEC), which also have strong ecological consequences on organisms. Salinity gradients are generated by the mixing of fresh- and marine-waters entering the estuaries, and its strength is strongly regulated by the intensity of both factors, as predicted by the EEC scheme (Hume et al., 2007). In our study, three zooplankton assemblages could be defined as: (1) a tidal-freshwater group including FW and B categories and characterized by the dominance of Bdelloidea, rotifers and cladocerans (*Dicranophorus* sp., *Bosmina meridionalis*, *Keratella cochlearis*)

and the calanoid copepod (*Gladioferans pectinatus*), (2) a true-estuarine group including D and F categories and characterized by the copepods *Euterpina acutifrons*, *Corycaeus* sp. and Polychaete larvae, and (3) a marine group comprised of only the MW category and characterized also by the copepods *Euterpina acutifrons*, in addition to other marine taxa (e.g., larvae of bivalves and barnacle cypris larvae). Similar variation in zooplankton composition relating to salinity gradients has been previously observed in other estuarine systems both in New Zealand and elsewhere e.g., the Avon-Heathcote Estuary, New Zealand (Roper et al., 1983); the St. Lawrence estuary, North America (Laprise and Dodson, 1994); Seine estuary, eastern English Channel, France (Mouny and Dauvin, 2002); the Coleroon, south-east India (Rajkuma et al., 2014); Guadalquivir estuary, Spain (Taglialatela et al., 2014). However, in these works the compositional changes were observed along longitudinal gradients only, from upstream to downstream within the same estuary, rather than across distinct estuary systems, such as conducted in the present study. Indeed, most published studies on estuarine zooplankton communities, not only in New Zealand but globally, have frequently focused on single estuaries (or at most include two types, usually “open vs. closed” estuaries; e.g., Froneman, 2004; Naumenko, 2009; Vieira et al., 2016), while comprehensive studies including a wide typology of estuaries and covering a broad geographical space are rare (Duggan et al., 2008).

The zooplankton assemblages of New Zealand estuaries were dominated by calanoid copepods (e.g., *Gladioferans pectinatus*, *Temora turbinata*) and cosmopolitan harpacticoid copepods (e.g., *Euterpina acutifrons*), consistent with many other estuarine and coastal areas elsewhere, in particular on Pacific coastlines (Mouny and Dauvin, 2002; Schallenberg et al., 2003; Conway et al., 2003; Marques et al., 2006; Duggan et al., 2008). The named copepods are widespread, principally inshore marine species, that can tolerate a wide range of salinities and so, it was consistent with its presence across categories (B, D, F, MW). Although, *G.*

*pectinatus* is commonly found in New Zealand at salinities between 4 and 20 (e.g., Roper et al., 1983; Duggan and White, 2010), and has the peculiarity of being an estuarine copepod that can maintain populations within estuaries under flood conditions, probably as individuals can cling to littoral vegetation (Newton, 1994). Because of this ability, *G. pectinatus* can recolonise estuaries following floods, and so their importance in category B is consistent with the physical characteristics of this category. Furthermore, the present study also includes rotifers, which were typically associated with freshwaters (category FW) (e.g., *Keratella cochlearis*, *Polyarthra dolichoptera*), though they were also present in the category B (Bdelloid, *Dicranophorus* sp.), in agreement with other studies of estuaries in New Zealand and elsewhere (Schallenberg and Burns, 2003; Fontaneto et al., 2006; Duggan and White et al., 2010).

We observed zooplankton assemblages to have distributions associated with the effects of hydrological factors (i.e., oceanic and river influences), which are directly related to salinity gradients, more so than to basin morphometry. For instance, there were no significant differences in zooplankton composition between categories D and F, both of which are characterized by little or no river influence, but differ morphometrically (category D does not have arms in the basin, whereas category F does; Figure 2). If we consider these similarities and differences among categories in a broader context than the purely ecological, such as in the field of management, it could open an interesting debate. From a manager's viewpoint, the categories (or "management units") defined by EEC may be managed differently as they respond to well-differentiated physical processes, as proposed by Hume et al. (2007). However, these categories may display similar biotic components and biological behaviour (as for categories D and F). Accordingly, we need to highlight the importance of defining "ecological units" for management, which may not always agree with physical classifications. Ideally, and whenever possible, a combination of physical classification

factors, as proposed by the EEC, complemented by a biological characterization, should be carried out for better management actions. In particular, we call for the inclusion of zooplankton as bioindicators in environmental monitoring, particularly in estuarine environments. As shown in other studies (e.g., Li, 2000, Beaugrand et al., 2002, Bonnet and Frid, 2004), zooplankton have been demonstrated to respond to abiotic (e.g., salinity, pH, pollutants), biotic (e.g., food limitation, predation), as well as hydrogeodynamic parameters (e.g., winds, currents, water exchange), which along its ubiquity and its key role in the transmission of material and energy in the food web, among other attributes, can make of zooplankton a valuable component for environmental and management purposes such as water quality indexes (Boix et al., 2005).

The zooplankton assemblages of the systems studied matched the diversity patterns predicted by the classic Remane's curve (Remane, 1934). Despite the limitations in this study (i.e., no replication in time, and the salinity gradient not fully reflected), it is still possible to observe the highest total estimated richness' (Chao2) in the most freshwater (FW-B) and marine (MW) systems, and the lowest values in the intermediate systems (D-F). Therefore, this classical diversity pattern described originally for macrozoobenthos might also be applied to zooplankton assemblages, although this conclusion has to be considered with caution because of those aforementioned limitations. Nevertheless, these results are consistent with those reported by other authors for estuarine zooplankton assemblages (Modéran et al., 2010; Tagliatella et al., 2014). However, other studies based on zooplankton assemblages in the Baltic Sea have shown the opposite pattern to Remane's curve (see Telesh et al., 2011a). Different reasons may explain the disagreement between estuarine studies and the Baltic study of Telesh et al., such as the salinity range considered, the stability of salinity gradients through time, how biota are taxonomically grouped, or the

manner in which diversity was estimated (Telesh et al., 2010a; Ptacnik et al., 2011; Telesh et al., 2011b).

We considered three of the eight categories of estuaries proposed by EEC, B, D and F (Hume et al., 2007). Despite missing some categories, our selection provides a good representation of the variety of estuaries present in North Island of New Zealand, and compasses the range of those in the EEC scheme. To select categories we considered, among other criteria, that they were well represented in the North Island (e.g., categories G and H were limited to the South Island) and were not very similar to each other (e.g., category B was very similar to category C). Thus, we consider that the results obtained here can be extrapolated to other estuaries from New Zealand and other countries. We have shown that the distribution of zooplankton assemblages responded reasonably well to the categories proposed by the EEC. Overall, our results reinforce the validity and usefulness of this classification scheme.

Finally, the ecological importance of the zooplankton component in estuarine studies must be stressed. Estuaries are complex systems, highly controlled by a changeable physical environment and where the ecological interactions are difficult to disentangle. Moreover, and in addition to the natural stress conditions, estuaries are very vulnerable to human pressures, in particular pollution. As such, both the highly variable physical environment and anthropogenic alterations make the estuarine environment a challenging habitat for biota (Rosenberg, 1976; Elliott and Whitfield, 2011; Almeida et al., 2012; Biancalana et al., 2012). In this context, the tidal estuarine zooplankton community and its ecological role has been scarcely explored globally, and in particular in New Zealand estuaries, where many more publications have been focused on fish (Francis et al., 2011), mangroves (Lovelock et al., 2006) or seagrass (Alfaro, 2006) communities. Nevertheless, zooplankton play a unique ecological role in estuaries as major food resource for many pelagic fishes and other higher

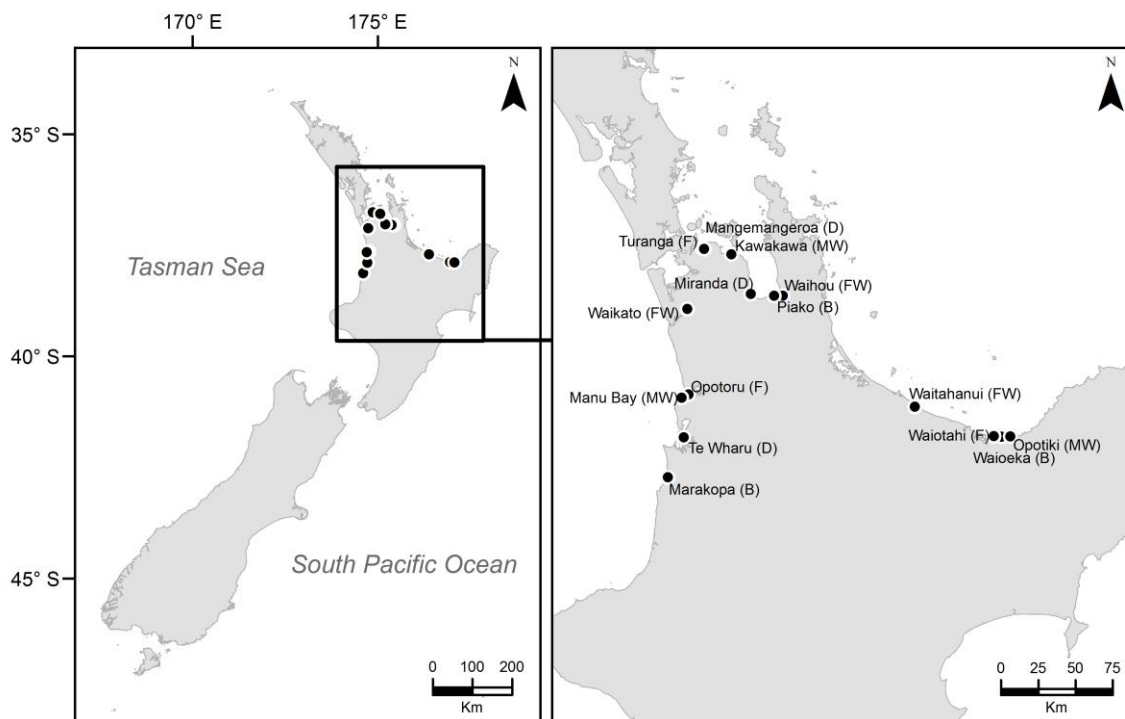
trophic levels (Newton, 1996). We have demonstrated that zooplankton assemblages can match the abiotic classification of estuarine systems, although morphometric factors likely have less influence on zooplankton than the riverine and oceanic fluxes. This will be largely due to the more direct influence of salinity on the osmoregulatory abilities (salinity tolerance) of different zooplankton species, as has been demonstrated in estuaries in Europe and America (Tackx et al., 2004; Roman et al., 2005; Marques et al., 2006; Marques et al., 2007). Additionally, similar patterns of diversity described for benthic fauna (traditionally a better studied estuarine component) can be also applied to zooplankton assemblages. Further research is needed to assess the ecological role of zooplankton communities in New Zealand estuaries, since no previous comprehensive studies on these taxa have included more than one estuary. Moreover, this research was conducted without considering the temporal scale; as such, we did not consider zooplankton dynamics between tidal cycles and among seasons (Marques et al., 2006; Tagliatela et al., 2014; Rajkumar et al., 2015; Menéndez et al., 2015). Further, we included only three of the eight categories of estuaries proposed by the EEC, so this research cannot be considered an endpoint. Research and monitoring of estuaries should continue to document the relationship between zooplankton communities and classification systems for estuaries, exploring the match between their biological and physical characteristics, as well as to predict whether and how the seasonality of zooplankton assemblages will change under temporal and spatial conditions.

### **Acknowledgements**

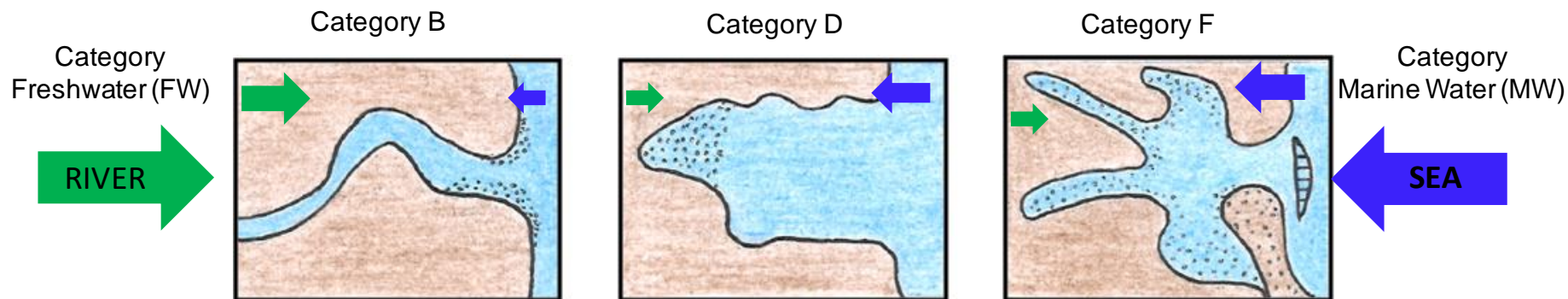
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information about estuary catchment parameters. Information of estuaries obtained from NIWA's Estuarine Classification database.



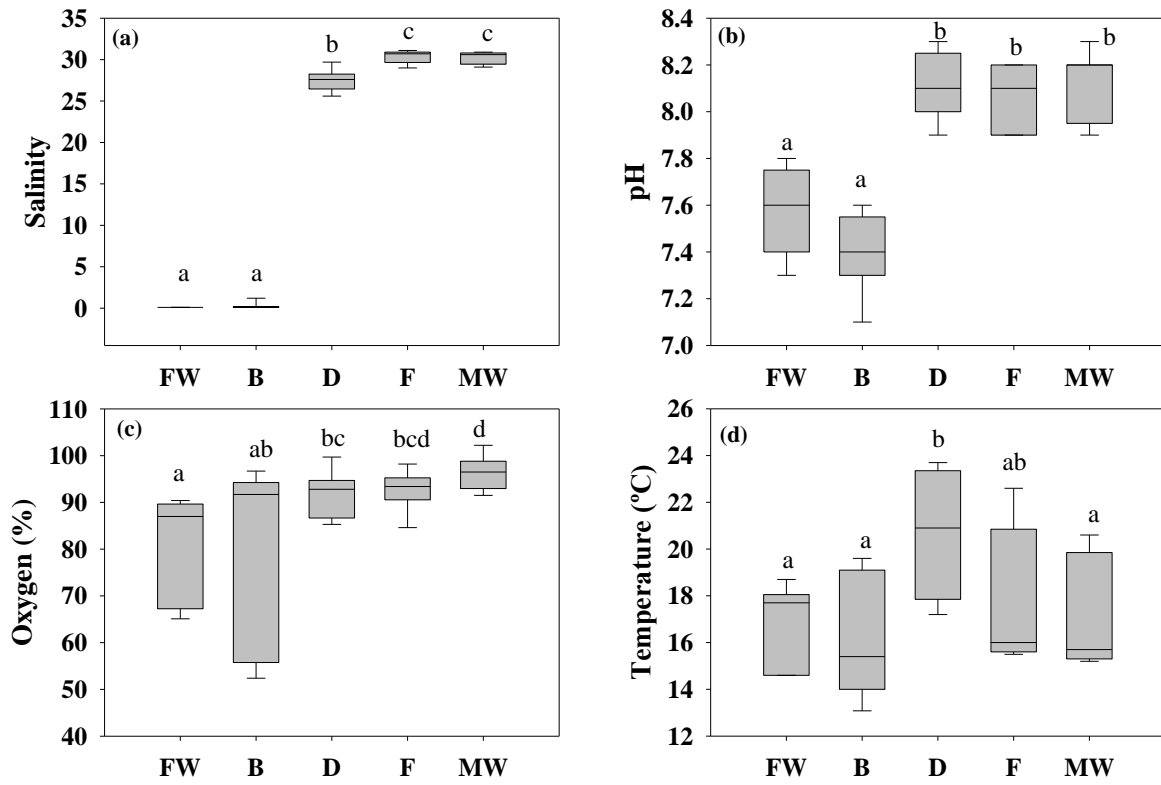
**Figure 1.** Map of the study area showing the location of the 15 sampling sites.



**Figure 2.** Schematic drawings of the three hydrodynamic categories of estuary (B, D and F) selected from Hume et al. (2007) for this study. Additionally, freshwater (FW) and marine water (MW) categories were included. The relative levels of tidal- versus river-forcing scales are shown in the size of the blue and green arrows, respectively. The categories are diagnosed by particular combinations of the three controlling factors (ocean forcing, river forcing and basin morphometry). **Category B.** Elongated basins of simple shape and several to ten metres depth. Largely subtidal. Volume of river flow delivered during a tidal cycle is a significant proportion of the total volume of the basin, and is greater than the tidal volume entering the basin. Hydrodynamic processes are dominated by river flows. Sand bars at the entrance on littoral drift shores. In deeper systems a circulation pattern and a salt wedge develop. Well flushed and floods expel much of the ocean water from the estuary. Wind mixing and wave driven resuspension are minor. Generally muddy substrate with sand in main channels. Commonly termed “tidal river mouths”. **Category D.** Shallow, circular to slightly elongated basins with simple shorelines and wide entrances that are open to the ocean. Mostly sub-tidal with small intertidal areas restricted to the upper reaches. Little river influence and circulation is weak and ocean forced. Swell enters the bay and resuspends seabed sediments. Hydrodynamic processes are dominated by the ocean. Wind generated mixing and wave driven estuary-scale sedimentation occurs. Substrate is sandy, except in areas where wave resuspension of the substrate is limited by depth. Commonly termed “coastal embayments”. **Category F.** Shallow basins and narrow mouths, usually formed by a spit of sand barrier. Complex shorelines with numerous arms leading off a main basin. Extensive intertidal area cut by channels. Sand bodies (ebb and flood tidal deltas) occur at the mouth on littoral drift shores. Tidal prism makes up a large proportion of the tidal volume. River inputs over the tidal cycle are very small compared to the total volume of the estuary. Hydrodynamic processes dominated by the tides. Little wind generated circulation, mixing and wave resuspension of the substrate because of the restricted fetch. Substrate sandy in main body and muddy in the arms. Poor flushing. Commonly termed “drowned valleys”. This is an adapted and reduced version of the full conceptual model described by Hume et al. (2007). For a more detailed description for the categories included in this study and remaining categories refer to the original manuscript Hume et al. (2007).

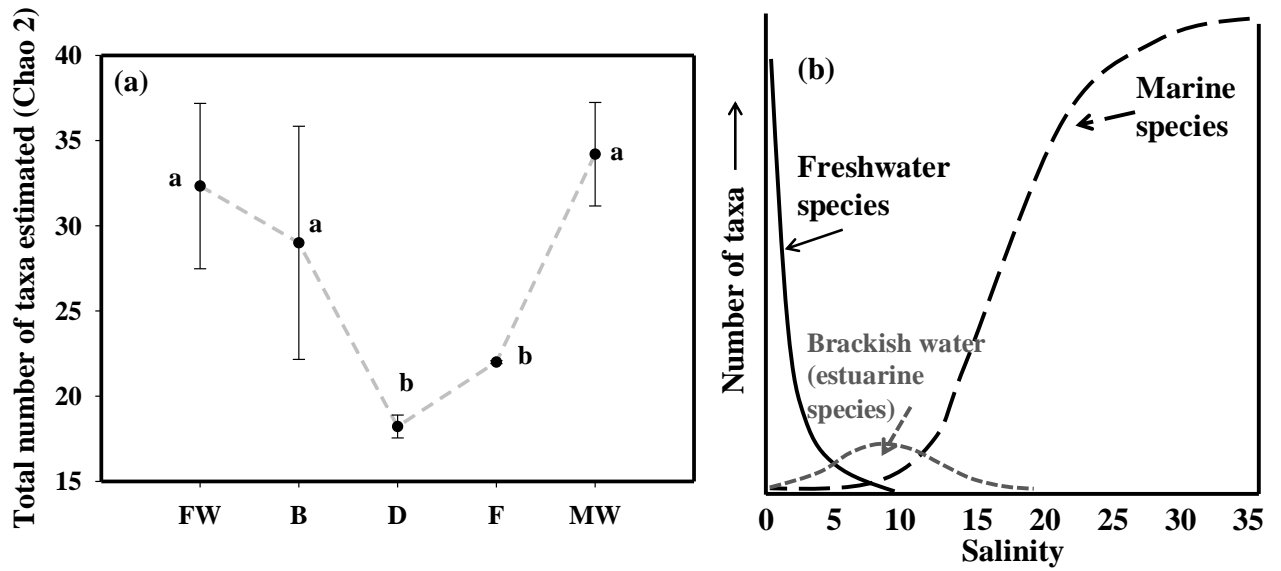
**Table 1.** Summary of the characteristics of the 15 sites selected in this study (see Figure 1 for locations in the map). (Source NIWA, coastal explorer <http://wrenz.niwa.co.nz/webmodel/coastal>). A more comprehensive table including land cover and natural vegetation is available in Appendix 1.

Name	Spatial location	Category	Latitude	Longitude	Intertidal area (% of HW area)	Mean depth (m)	Mean annual river discharge (cumecs)	Width of mouth (m)	Catchment area (km <sup>2</sup> )
Waitahanui Stream	east	FW	-37.834746	176.595518	0.00	2.70	6.73	40.19	3023.00
Waihou River	north	FW	-37.189883	175.564892	6.69	1.66	97.65	1000.00	1980.07
Waikato River	west	FW	-37.283175	174.843359	7.70	6.44	695.92	727.67	14481.08
Marakopa River	west	B	-38.302395	174.719747	13.86	4.51	25.28	230.06	366.85
Waioeke River	east	B	-37.993456	177.267864	13.95	3.30	84.30	306.40	1172.52
Piako River	north	B	-37.192190	175.498613	25.60	4.37	54.21	862.00	1461.21
Miranda Stream	north	D	-37.185399	175.321800	95.26	1.77	0.02	159.12	0.63
Te Wharu Bay	west	D	-38.060550	174.834930	99.99	1.48	0.16	1159.93	3.84
Mangemangeroa Estuary	north	D	-36.915426	174.946546	86.92	1.53	0.38	694.48	10.01
Waiotahi River	east	F	-37.991173	177.206890	68.32	1.75	8.02	376.25	147.97
Turanga Creek	north	F	-36.919129	174.962429	73.64	1.48	1.09	291.06	27.97
Opotoru River (Raglan)	west	F	-37.800482	174.866390	83.88	1.70	2.86	107.73	58.74
Opotiki	east	MW	-37.988303	177.334060	not applicable	1.00	not applicable	not applicable	not applicable
Manu Bay	west	MW	-37.821976	174.814076	not applicable	1.30	not applicable	not applicable	not applicable
Kawakawa Bay	north	MW	-36.948140	175.168240	not applicable	1.00	not applicable	not applicable	not applicable

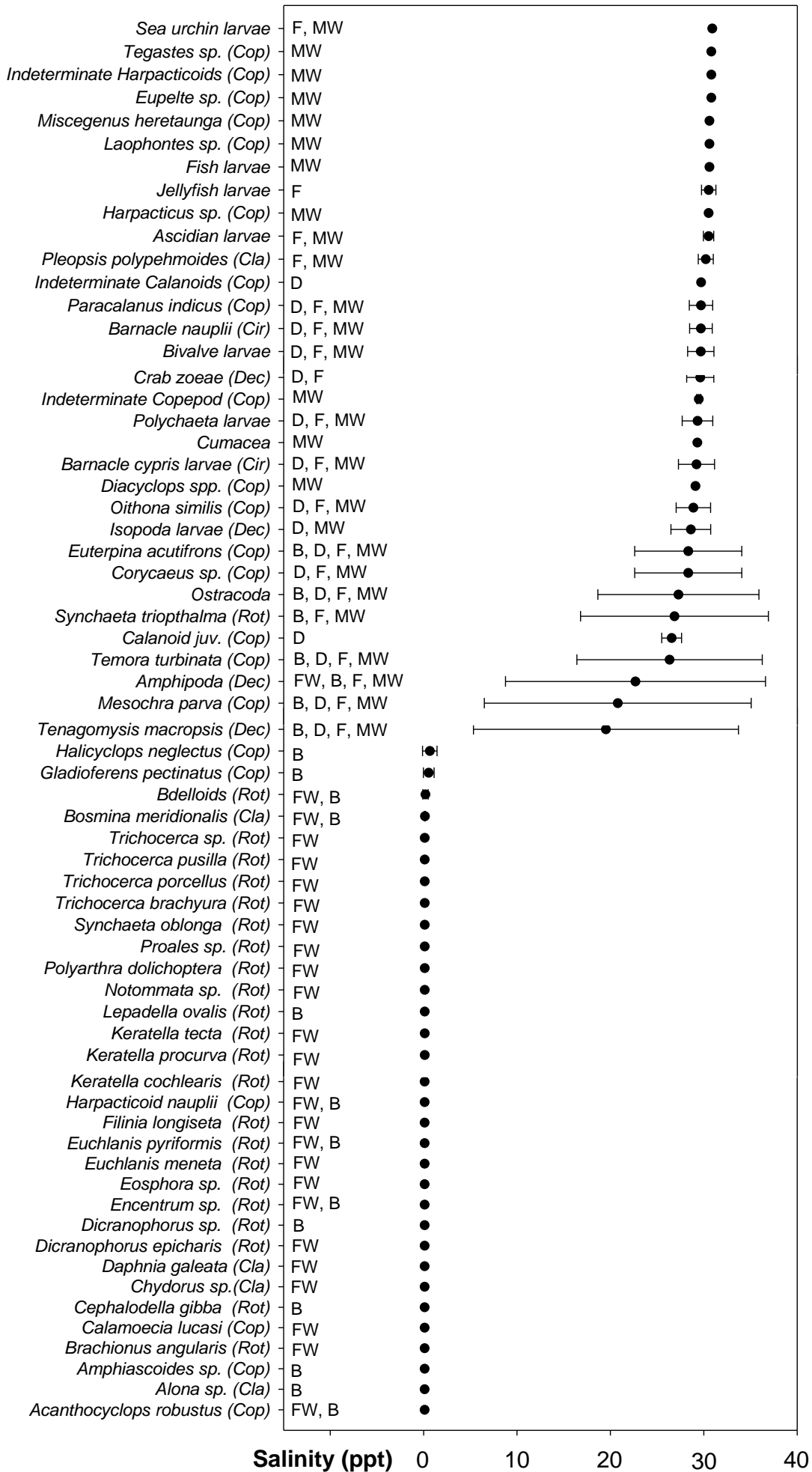


**Figure 3.** Water-chemistry variables in each category (FW (Freshwater), B, D, F and MW (Marine water)). Different letters indicate significant differences whereas similar letters indicate non-significant differences.

**Figure 4.** (a) Total richness estimator Chao 2 for each category. Mean and standard deviation are shown (b) Theoretical representation of Remane's curve (adapted from Dobson and Frid 1998).



**Figure 5.** Plot showing the range of distribution of the zooplankton taxa across salinity (black point is the mean and whiskers the standard deviation). The x-axis shows a salinity gradient, and the y-axis lists the zooplankton taxa, taxonomical groups in brackets (rot: rotifers, cla: cladocerans, cop: copepods, dec: decapods), and the category of estuary and FW and MW where the taxa were present (FW: Freshwater, D, B, F and MW: Marine water; according to Hume et al., 2007).



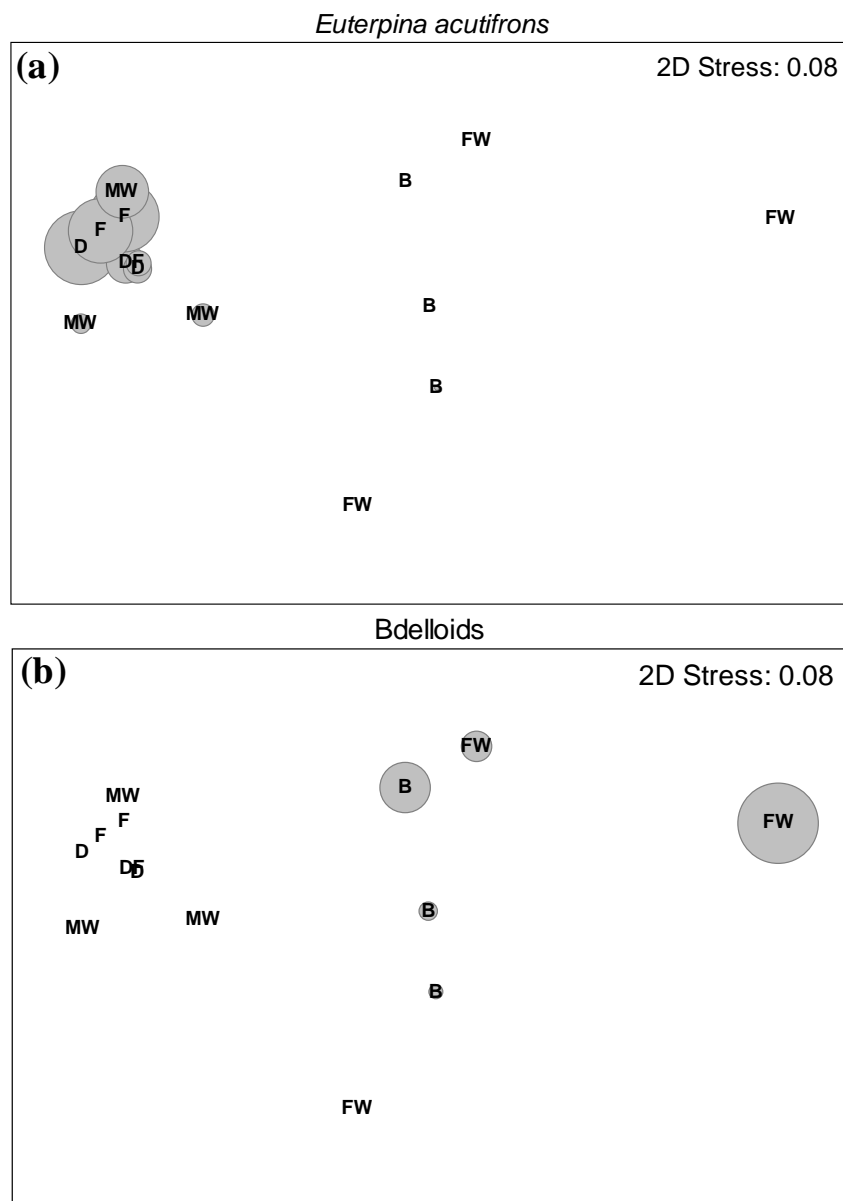
**Table 2.** Output from ANOSIM. The table shows the results for the pair-wise comparisons of zooplankton assemblages between categories that were considered in this study (FW: Freshwater, category D, category B, category F and MW: Marine water). R- and p-values obtained from ANOSIM are provided.

<b>Groups</b>	<b>R Statistic</b>	<b>p-value</b>
FW vs. B	0.145	0.059
FW vs. D	0.737	0.001
FW vs. MW	0.751	0.002
FW vs. F	0.737	0.001
B vs. D	0.881	0.001
B vs. F	0.902	0.001
B vs. MW	0.826	0.001
D vs. F	0.139	0.089
D vs. MW	0.302	0.001
F vs. MW	0.383	0.005



**Table 3.** Contribution of each taxon (listed in descending order of contribution) to the average similarity within each category (Contrib.%). Cumulative contributions (Cum.%) up to 90% are also shown. The taxonomic group of each zooplankton taxon is included in brackets.

<b>Category Freshwater (Avg. Sim. 11.77 %)</b>	<b>Contrib%</b>	<b>Cum.%</b>
Bdelloid rotifers	27.22	27.22
<i>Acanthocyclops robustus</i> (copepod)	14.39	41.61
<i>Bosmina meridionalis</i> (cladoceran)	12.17	53.78
<i>Keratella cochlearis</i> (rotifer)	11.36	65.14
<i>Polyarthra dolichoptera</i> (rotifer)	7.89	73.03
<i>Synchaeta oblonga</i> (rotifer)	7.26	80.29
<i>Trichocerca porcellus</i> (rotifer)	5.47	85.76
<i>Daphnia galeata</i> (cladoceran)	5.20	90.96
<b>Category B (Avg. Sim. 15.59 %)</b>		
Bdelloid rotifers	43.04	43.04
<i>Gladioferens pectinatus</i> (copepod)	20.10	63.14
<i>Dicranophorus</i> sp. (rotifer)	18.65	81.79
<i>Tenagomysis macropsis</i> (decapoda)	9.20	90.99
<b>Category D (Avg. Sim. 47.54 %)</b>		
<i>Euterpina acutifrons</i> (copepod)	37.70	37.70
Polychaete larvae	22.83	60.53
<i>Corycaeus</i> sp. (copepod)	21.47	82
<i>Oithona similis</i> (copepod)	11.55	93.55
<b>Category F (Avg. Sim. 44.71 %)</b>		
<i>Euterpina acutifrons</i> (copepod)	35.16	35.16
<i>Corycaeus</i> sp. (copepod)	27.45	62.6
Bivalve larvae	13.51	76.12
Polychaete larvae	8.59	84.71
<i>Temora turbinata</i> (copepod)	5.05	89.76
Jellyfish larvae	1.59	91.34
<b>Category MW (Avg. Sim. 27.16 %)</b>		
<i>Euterpina acutifrons</i> (copepod)	46.25	46.25
Bivalve larvae	11.04	57.29
Ostracoda	9.47	66.75
<i>Corycaeus</i> sp. (copepod)	9.46	76.21
<i>Temora turbinata</i> (copepod)	7.35	83.56
<i>Paracalanus indicus</i> (copepod)	5.58	89.14
Barnacle cypris larvae	2.52	91.66



**Figure 6.** (a) MDS ordination of sites based on zooplankton community composition. The five habitat categories are denoted (FW, B, D, F, MW) and sites are superimposed with “bubbles”, representing the characteristic zooplankton taxa of groups identified using SIMPER analysis (Table 2); (Bdelloid rotifers), most characteristic of categories FW and B, and *Euterpina acutifrons*, most characteristic of categories D-F and MW (b). The size of the bubbles increases with an increasing density of that zooplankton taxon (2-d stress = 0.08).

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Supplementary data

**Appendix 1.** Description of the selected estuaries using data from NIWA's Estuarine Classification database.

**Catchment hydrological properties:**

<b>Estuary name</b>	<b>Land catchment area (km<sup>2</sup>)</b>	<b>Largest stream order</b>	<b>Mean catchment rainfall (mm/yr)</b>	<b>Mean runoff (mm/yr)</b>	<b>Rain Power</b>
Mangamangaroa Estuary	10	1	1208	301	1153
Turanga Creek	28	3	1242	394	1773
Miranda Stream	1	1	1128	0	1205
Piako River	1461	4	1180	278	1166
Waihou River	1980	5	1569	564	4748
Waiotahi River	148	3	1724	669	8246
Waioeka River	1173	5	2287	1260	13865
Marakopa River	367	4	2193	1232	7190
Te Wharu Bay	4	1	1358	474	2455
Oporu River	59	2	1546	595	4371
Waikato River	14481	6	1529	585	3771
Waitahanui Stream	114	3	1881	653	3234



Supplementary data

**Natural vegetation (% of catchment area in native vegetation LRI\* classes)**

\*LRI: land resource information

<b>Estuary name</b>	<b>Native forest</b> (% catchment)	<b>Grassland</b> (% catchment)	<b>Scrub</b> (% catchment)	<b>Miscellaneous</b> (% catchment)	<b>Other</b> (% catchment)	<b>Total</b> (% catchment)
Mangamangaroa Estuary	0	0	37	0	63	100
Turanga Creek	0	0	2	0	98	100
Miranda Stream	0	0	0	0	100	100
Piako River	2	0	2	6	91	100
Waihou River	19	0	5	0	76	100
Waiotahi River	49	0	10	0	41	100
Waioeka River	70	0	1	0	29	100
Marakopa River	32	0	11	0	56	100
Te Wharu Bay	0	0	12	10	78	100
Opotoru River	5	0	0	0	95	100
Waikato River	10	1	7	2	80	100
Waitahanui Stream	1	0	6	0	93	100

Supplementary data

**LCDB Areas (%)**

\* LCDB: land cover database

<b>Estuary name</b>	<b>Urban</b> (% catchment)	<b>Urban_open</b> (% catchment)	<b>Prim_Pastoral</b> (% catchment)	<b>Indigenous_Forest</b> (% catchment)	<b>Planted_Forest</b> (% catchment)	<b>Scrub</b> (% catchment)	<b>Tussock</b> (% catchment)
Mangamangaroa Estuary	3	0	70	10	0	17	0
Turanga Creek	0	1	79	11	2	6	0
Miranda Stream	0	0	99	1	0	0	0
Piako River	1	0	87	3	1	0	0
Waihou River	1	0	60	25	10	4	0
Waiotahi River	0	0	26	56	14	3	0
Waioeke River	0	0	18	72	3	6	0
Marakopa River	0	0	50	43	3	4	0
Te Wharu Bay	0	0	65	17	0	13	0
Oporu River	2	1	81	14	0	3	0
Waikato River	1	0	54	13	17	6	1
Waitahanui Stream	0	0	27	24	46	2	0

Supplementary data

<b>Estuary name</b>	<b>Inland_Water (% catchment)</b>	<b>Inland_Wetland (% catchment)</b>	<b>Coastal_Wetlands (% catchment)</b>	<b>Bare_Ground (% catchment)</b>
Mangamangaroa Estuary	0	0	0	0
Turanga Creek	0	0	0	0
Miranda Stream	0	0	0	0
Piako River	0	8	0	0
Waihou River	0	0	0	0
Waiotahi River	0	0	0	0
Waioeka River	0	0	0	0
Marakopa River	0	0	0	0
Te Wharu Bay	0	2	2	0
Opotoru River	0	0	0	0
Waikato River	5	1	0	1
Waitahanui Stream	0	0	0	0

## Supplementary data

**Appendix 2.** Mean ( $\pm$ SD) densities of total zooplankton, and the main groups identified (rotifers, cladocerans, ostracods, copepods and meroplankton – the latter including fish larvae, polychaete larvae, crab larvae, barnacle nauplii, isopoda larvae, jellyfish larvae, ascidian larvae, sea urchin larvae and bivalve larvae), according to categories included in this study (FW: freshwater; B: estuary type B; D: estuary type D; F: estuary type F; MW: marine water).

