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6 **Characteristics of the ichthyofauna of a temperate microtidal estuary with a reverse salinity**

7 **gradient, including inter-decadal comparisons**

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9 L. Veale[a], J. R. Tweedley[a], K. R. Clarke[a,b,]

10 C. S. Hallett[a] and I. C. Potter[a*].

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12

13 [a] Centre for Fish and Fisheries Research

14 Murdoch University, South Street, Murdoch,

15 Western Australia, 6150

16

17 [b] Plymouth Marine Laboratory, Prospect Place, West Hoe,

18 Plymouth PL1 3DH, United Kingdom

19

20

21

22 *Author to whom corresponding author should be addressed. Tel.: +61 (08) 9239 8801;

23 Fax: +61 (08) 9360 6303; email: i.potter@murdoch.edu.au.

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25 Running headline: Ichthyofauna of a reverse salinity estuary

Abstract

Data on the fish fauna of the Leschenault Estuary on the lower west coast of Australia were collected and used as a model to elucidate the characteristics of permanently-open estuaries with a reverse salinity gradient that undergo similar seasonal changes to many estuaries with Mediterranean climates. Focus was placed on determining 1) the relationships of the number of species, density, life cycle category and species composition of fishes with region (within estuary), season and year and salinity, 2) whether species are partitioned along the lengths of such systems and 3) the extent and significance of any inter-decadal changes in species composition. The analyses and interpretation involved using multi-factorial PERMANOVA and ANOSIM designs, and three new or recently published visualisation tools, *i.e.* modified nMDS plots, coherent species curves and segmented bubble plots. The base, lower, upper and apex regions of the Leschenault Estuary, along which the salinity increased in each season except winter when most rainfall occurs, were sampled seasonally for the two years between winter 2008 and autumn 2010. Estuarine residents contributed twice as many individuals, but less than half the number of species as marine taxa. While the numbers of marine species and estuarine residents declined between the basal/lower and apex regions, the individuals of marine species dominated the catches in the basal region and estuarine residents the other three regions. Ichthyofaunal composition in each region underwent conspicuous annual cyclical changes, due to time-staggered differences in recruitment among species, and changed sequentially along the estuary, both paralleling salinity trends. Different groups of species characterised the fauna in the different regions and seasons, thereby partitioning resources among species. The ichthyofauna of the apex region, in which salinities reached 54 and temperatures 36ºC, recorded the highest maximum density and, in terms of abundance was dominated (90%) by three atherinid species, emphasising the ability of this family to tolerate extreme conditions. Comparisons between the data for 2008-10 and 1994 demonstrate that the spotted hardyhead *Craterocephalus mugiloides* and the common hardyhead *Atherinomorus*

51   *vaigiensis* had colonised and become very abundant in the Leschenault Estuary in the intervening

52   period. This represents a southwards extension of the distribution of these essentially tropical

53   species during a period of increasing coastal water temperatures as a result of climate change. The

54   abundance of weed-associated species, *e.g.* the western gobbleguts *Ostorhinchus rueppellii* and the

55   soldier *Gymnapistes marmoratus*, increased, whereas that of the longfinned goby *Favonigobius*

56   *lateralis* decreased, probably reflecting increases in eutrophication and siltation, respectively.

57

58   **Key words:** Fish composition, hypersalinity, partitioning, environmental factors, climate change,

59   Atherinidae.

Introduction

It is becoming increasingly evident that estuaries with low inflow are as common as those with a persistent and significant influx of fresh water and which produce the classical low-salinity density driven circulations described for many estuaries (Largier, 2010). When the evaporative loss of fresh water in low-flow estuaries exceeds fresh water input from precipitation and run off, the salinity in the basins of such systems becomes greater than in the ocean (Largier *et al.*, 1997; Largier, 2010). These types of estuaries are therefore typically found in microtidal regions subject to seasonal or prolonged aridity and where the residence time in the estuary during dry periods is thus long (Largier *et al.*, 1997; Wolanski, 2007; Largier, 2010). In many parts of the world with a Mediterranean climate, the basins of permanently-open estuaries are hypersaline during the dry summer months (net evaporation), but constitute a classical estuary (net dilution) during the wet winter months, leading Largier *et al.* (1997) to suggest that they represented a major class of estuary, which they termed "Mediterranean estuaries". Furthermore, in many of these estuaries, the extent of hypersalinity during the warm dry period increases markedly in an upstream direction from the estuary mouth, thus producing a reverse salinity gradient within these systems. Such reverse trends are also found in permanently-open estuaries in tropical regions that have been subjected to prolonged periods of low rainfall, but with the waters throughout most of these systems remaining hypersaline in all seasons (Simier *et al.*, 2004; Kantoussan *et al.*, 2012).

The permanently-open Leschenault Estuary in microtidal south-western Australia, the subject of the current study, contains the short and narrow entrance channel and large basin typical of many estuaries in southern Australia and southern Africa (Potter *et al.*, 1990). It differs, however, from most other large estuaries in these regions in that its tributaries discharge into its basal region and therefore opposite the entrance channel (Semeniuk *et al.*, 2000; Fig. 1). Thus, as the apex of the basin of this permanently-open estuary receives no riverine input, its salinity exceeds that of seawater during the warm dry months, thereby producing a reverse salinity gradient, a condition

85   that persists "for most seasons" (Semeniuk *et al.*, 2000). While the large Coorong Estuary further

86   east in South Australia likewise has a reverse salinity gradient and receives its riverine input at the

87   lower end of its basin and thus near its entrance channel, the salinity regime in this system has

88   changed over time through abstraction and reductions in rainfall and is modified by the use of

89   barrages to regulate freshwater input (Webster, 2010; Zampatti *et al.*, 2010; Ferguson *et al.*, 2013).

90   Permanently-open estuaries are among the most productive of all aquatic ecosystems

91   (Schelske & Odum, 1961; Whittaker & Likens, 1975; Elliott & Whitfield, 2011) and thus,

92   irrespective of geographical location or whether the salinity gradient is normal or reverse, provide a

93   rich source of food for the numerous marine fish species that use these systems, especially as a

94   nursery area (Blaber & Blaber, 1980; Elliott & Hemmingway, 2002; Able & Fahay, 2010; Potter *et*

95   *al.*, 1990; 2013). This productive environment is also exploited by species that complete their life

96   cycle in these systems and which are particularly abundant in the microtidal estuaries of south-

97   western Australia, a region which has a Mediterranean climate (Potter & Hyndes, 1999; Potter *et*

98   *al.*, 2013). These species typically reproduce between the warm and dry spring to early autumn

99   months, when water movement due to tidal action and fresh water discharge is limited and salinities

100 and temperatures remain relatively stable, thus providing conditions ideal for spawning and

101 recruitment success (*e.g.* Prince & Potter, 1983; Chrystal *et al.*, 1985; Potter & Hyndes, 1999; Sarre

102 & Potter, 1999).

103   The collective data for the relatively few studies of the fish faunas of permanently-open

104 estuaries with a conspicuous reverse salinity gradient indicate that increases in salinity from the

105 lowermost to uppermost regions of the estuary are accompanied by certain changes in the

106 ichthyofauna. These include declines in the number of species, overall density and density of

107 marine species and changes in species composition (*e.g.* Simier *et al.*, 2004; Ter Morshuizen &

108 Whitfield, 1994; Brookes *et al.*, 2009; Kantoussan *et al.*, 2012). There is a need, however, to

109 produce an account that integrates the characteristics of the fish faunas of the above type of estuary,

110   using data collected seasonally for more than a year and which applies statistical approaches to

111   tease out the ways and relative extents to which the number of species, density, species composition

112   and partitioning change along the salinity gradient and during the year.

113   While the present study focuses on permanently-open estuaries with a reverse salinity

114   gradient, the results of extensive studies in the St Lucia Estuary in southern Africa provide

115   invaluable information on how the characteristics of the fish faunas can be influenced by salinity

116   (Whitfield *et al.*, 2006). This system falls, however, in a different category to the Leschenault and

117   similar estuaries in that it sometimes closes for protracted periods and that this feature, in

118   conjunction with very marked alterations in freshwater input due to extreme periodic differences in

119   rainfall, results in vast changes in salinity of 1 to 200 (Whitfield *et al.*, 2006; Cyrus, 2013).

120   The first overall aim of this study was to use the Leschenault Estuary as a model to increase

121   our knowledge and understanding of the characteristics of the fish faunas of permanently-open

122   estuaries in which there is a reverse salinity gradient. This involved, *inter alia*, determining the

123   ways and relative extents to which number of species, density, species compositions and

124   contributions of different life cycle categories and guilds *sensu* Potter *et al.* (2013) vary spatially,

125   seasonally and between successive years. Particular attention was also focussed on testing the

126   hypotheses that any spatial or temporal trends in species composition would be associated with

127   changes in salinity and that the various species would form groups along the estuary and thus

128   facilitate partitioning of the resources of the estuary among the members of the estuarine fish

129   community. For this purpose, fishes throughout the Leschenault Estuary were sampled seasonally

130   for two years, *i.e.* between winter 2008 and autumn 2010. The resultant data were subjected to a

131   range of analyses, including multi-factorial PERMANOVA and ANOSIM designs, and

132   visualisation tools, *i.e.* modified nMDS plots, coherent species curves and segmented bubble plots,

133   which collectively enhanced the ability to interpret the ecological implications of the results for the

134   Leschenault Estuary and other permanently-open systems with a reverse salinity gradient.

135   The second overall aim was to compare the characteristics of the fish fauna at sites in the

136   lower half of the estuary in 2008-10 with those recorded previously at the same sites in 1994 (Potter

137   *et al.*, 1997, 2000) to test the following hypotheses that are based on changes known to have

138   occurred to the environment between the two periods. 1) An increase in macroalgae (Hugues-dit-

139   Ciles *et al*., 2012) and in local coastal water temperatures due to climate change (Lough *et al.*,

140   2012) have been accompanied by increases in the abundance of fish species typically associated

141   with plant material and/or which lie at the southern (warmer) end of their distribution. 2) Increased

142   sedimentation (Hugues-dit-Ciles *et al.*, 2012) has been accompanied by a decline in the abundance

143   of the southern longfin goby *Favonigobius lateralis* Macleay 1881, a species that is very abundant

144   in south-western Australian estuaries (Potter & Hyndes, 1999) and known to be detrimentally

145   influenced by increased siltation (Gill & Potter, 1993). 3) The above changes led to a conspicuous

146   shift in the species compositions between the two periods.

147

148   **Materials and methods**

149   *Sampling in 2008-10*

150   Fishes at four regularly-spaced sites throughout the lengths of each of the base, lower, upper

151   and apex regions of the Leschenault Estuary (Fig. 1) were sampled using a 21.5 m seine net in each

152   season between the Austral winter (July) of 2008 and the Austral autumn (April) of 2010. The seine

153   net, which comprised two 10 m long wings (6 m of 9 mm mesh and 4 m of 3 mm mesh) and a

154   1.5 m long bunt (3 mm mesh), swept an area of *c.* 116 m^2 and fished to a maximum depth of 1.5 m.

155   Three of the four sites in both the base and lower regions of the estuary were the same as those

156   sampled at six weekly intervals (*i.e.* twice seasonally) between the summer and spring of 1994

157   using a seine net with the same dimensions (Potter *et al.*, 1997). On each sampling occasion, the

158   salinity and water temperature at each site were measured in the middle of the water column using a

159   Yellow Springs International Model 85. Note that the estuary is shallow, generally < 1.5 m in depth,

160   and vertically well mixed (Semeniuk *et al.*, 2000) and that, following Brauner *et al.* (2013), the term

161   hypersalinity is, for convenience, used to refer to salinities greater than that of seawater, *i.e.* 35.

162   Note also that the Leschenault Estuary fulfils the criteria for an estuary as defined by Potter *et al.*

163   (2010), *e.g.* it receives periodic discharge from rivers and can become hypersaline.

164   The fishes were immediately euthanized in an ice slurry and transported to the laboratory

165   where they were each identified to species. The total number of individuals of each species in each

166   sample was recorded and converted to a density (number of fish 100 m^{-2}), which was then used to

167   calculate the mean density of each species in the whole estuary and in each of its four regions. The

168   total length of each fish was measured to the nearest 1 mm, except when a large number of a species

169   was caught, when the total lengths of a random sample of 50 individuals of that species were

170   recorded.

171

172   *Numbers of species and densities of fishes*

173   The number of species and density (number 100 m^{-2}) of fishes recorded at each of the four

174   sites sampled in the four regions of the Leschenault Estuary in the eight consecutive seasons

175   between winter 2008 and autumn 2010 were separately input to univariate Analysis of Variance, but

176   using the permutation form in which the statistical tests do not make traditional normality

177   assumptions (Anderson, 2001). This is effected as a special case of multivariate analysis in

178   PRIMER v6 software (Clarke & Gorley, 2006) by constructing a Euclidean distance matrix among

179   values of the single variable and entering those into the PERMANOVA routine (Anderson *et al.*,

180   2008). The resulting tests determined whether the number of species and density of fishes differed

181   significantly between the two successive years (*i.e.* winter 2008 to autumn 2009 and winter 2009 to

182   autumn 2010 and subsequently referred to as 2008/09 and 2009/10, respectively), seasons, regions

183   and sites, and the extent of any interactions among these factors. All factors were considered fixed

184   and crossed apart from the sites, which were treated as a random factor nested within regions,

185 though crossed with years and seasons, the same four sites being revisited on each sampling

186 occasion. The three-way interaction term year × season × site was dropped from the model and thus

187 utilised as the residual spatio-temporal variability for some of the tests, a robust and conservative

188 procedure. Examination of the values for these variables demonstrated that, prior to

189 PERMANOVA, the number of species required a square-root transformation and density a

190 $\log_e(x+1)$ transformation to meet the test assumption of homogeneous dispersions among *a priori*

191 groups (see Anderson, 2001).

192

193 *Life cycle categories and guilds*

194 Each species was assigned to a life-cycle guild within a category according to the way in

195 which it uses estuaries (Potter *et al.*, 2013), based on numerous studies of the biology of fish species

196 in south-western Australia as reported in the review of Potter & Hyndes (1999). Definition of the

197 two categories and five guilds relevant to the current study are as follows. Marine category, *i.e.*

198 species that spawn at sea. Marine straggler (MS) guild, *i.e.* species that spawn at sea and typically

199 enter estuaries sporadically and in low numbers and are most common in the lower reaches, where

200 salinities typically do not decline far below ~35. Marine estuarine-opportunist (MEO) guild,

201 *i.e.* species that spawn at sea and regularly enter estuaries in substantial numbers, particularly as

202 juveniles, but also use, to varying degrees, coastal marine waters as alternative nursery areas.

203 Estuarine category, *i.e.* species with populations in which the individuals complete their life cycles

204 within the estuary. Solely estuarine (E) guild, *i.e.* species typically found only in estuaries.

205 Estuarine and marine (E&M) guild, *i.e.* species represented by populations whose individuals

206 complete their life cycle either in estuaries or coastal marine waters. Estuarine and freshwater

207 (E&F) guild, *i.e.* species represented by populations whose individuals complete their life cycle

208 either in estuaries or fresh water. Thus, as no species belonging to the other two categories in Potter

209 *et al.* (2013) were caught in the Leschenault Estuary, *i.e.* diadromous and freshwater, all species

210 represented either the marine or estuarine categories, the latter subsequently being referred to as

211 estuarine residents. The numbers of marine species and estuarine resident species and the

212 proportional contributions of the individuals of those two categories to the total number of all fish in

213 each region of the estuary were then calculated for each season and for the whole year collectively.

214

215 *Multivariate analyses of species compositions*

216 The numbers of each fish species recorded at each of the four sites in each region in each

217 season in 2008/09 and 2009/10 were subject to a pre-treatment shown to be effective for fish data of

218 this type (Clarke *et al.*, 2014). This involved a) dispersion weighting to down-weight the effects of

219 those species whose numbers exhibited erratic differences among replicate samples due to

220 schooling (Clarke *et al.*, 2006), followed by b) square-root transformation to down-weight the

221 contributions of species with consistently high values (across replicates within a group) in relation

222 to those with consistently low values. The resultant data were then used to construct a Bray-Curtis

223 similarity matrix, which was subjected to the same four-way PERMANOVA design as described

224 above, though now with genuinely multivariate data, with the focus being on determining whether

225 there were significant interactions between year, season and region.

226 Separate two-way crossed Analysis of Similarity tests (ANOSIM, Clarke & Green, 1988;

227 Clarke, 1993) were used to assess the relative magnitudes of overall year, season and region factors

228 (subsuming both main and interaction effects), via the universally-scaled ANOSIM $\bar{R}$ statistic. This

229 was computed, in turn, for each factor (region, year or season) *vs* the other two factors combined,

230 thereby removing the combined effects of those other factors (Lek *et al.*, 2011; French *et al.*, 2012).

231 The ANOSIM $\bar{R}$ values typically range from *c.* 0, when the average similarities among and within

232 groups of the target factor do not differ (in all strata of the excised combined factor), up to 1, when

233 the compositions of all samples within each group are more similar to each other than to those of

234 any sample from other groups, again in all strata of the second factor (Clarke & Warwick, 2001).

235   Non-metric Multidimensional Scaling (nMDS) ordination was used to interpret interactions

236   (or lack of interactions) between factors that resulted from the PERMANOVA tests. These

237   configurations ordinate Bray-Curtis dissimilarities from the pre-treated data, which have been

238   averaged over the four sites and one of the three factors (year, season, region) in turn, resulting in

239   'interaction plots' for season × region, year × region and season × year respectively. Such

240   ordinations of mean values can sometimes involve few points (only eight here for the latter two

241   combinations) and the standard algorithm for rank-based nMDS can be susceptible to finding

242   degenerate solutions which have zero stress, but in which the highest-similarity samples collapse

243   onto single points even though not 100% similar. This typically results from the paucity of

244   information in a small number of ranks to constrain the ordination technique adequately, and occurs

245   here for the year × region interaction plot. An effective solution is detailed for this type of problem

246   in Hallett *et al*. (submitted). It utilises a modified nMDS algorithm (by R.N. Gorley, within a

247   development version of PRIMER v7), in which optimisation is of a stress function mixing mainly

248   nMDS with a minor component of metric MDS (mMDS, here 95% and 5% though the proportions

249   of the mix are not critical). This retains the flexibility of nMDS to describe complex structures in

250   low-dimensional space but the small amount of measurement information is sufficient to 'fix the

251   collapse' of a subset of points in the nMDS.

252   Bubble plots, in which circles whose sizes represent the magnitude of the mean salinity in

253   appropriate combinations of region, season and year (averaging over the omitted factor, as before)

254   were then overlaid on the points for samples on the ordination plots, to visualise the extent to which

255   ichthyofaunal composition was related to salinity (Clarke *et al.*, 2008).

256

257   *Seasonal and regional partitioning of the main fish species*

258   When there was a significant difference between the ichthyofaunal compositions of *a priori*

259   groups, Similarity Percentages analysis (SIMPER; Clarke & Gorley, 2006) was used to identify the

260   species which typified the ichthyofaunal composition of each *a priori* group and which were

261   responsible for distinguishing between the fish compositions in each pair of groups. The pattern of

262   change in the relative counts for individual species across both season and region (averaging over

263   the two years and the four sites in each region) is further examined by the new technique of

264   'coherent species curves' (Somerfield & Clarke, 2013). This uses a variant of the similarity profiles

265   (Type 1 SIMPROF) of Clarke *et al.* (2008), applied to species rather than to samples. It identifies,

266   via a cluster analysis of species similarities, the groups of species whose patterns of relative

267   abundance over the samples are indistinguishable within a group but statistically significant

268   between groups, when tested by the appropriate permutation procedure (Type 3 SIMPROF).

269   Species similarities are defined by a species-standardised form of Bray-Curtis, namely Whittaker's

270   index of association (Whittaker, 1952), calculated on the original abundance scales. The index

271   'relativises' each set of (averaged) species counts to 100 across the samples, so it is wise first to

272   exclude species found only sporadically and in small numbers, since they will only add random

273   noise to the species similarities (Clarke & Warwick, 2001). Visualisation of the resulting 'coherent

274   species groups' is by simple line plots of the relative abundances in each sequential season in each

275   region.

276

277   *Comparisons between the fish faunas in 2008-10 and 1994*

278   For comparing the species compositions of fishes in the two years sampled in 2008-10 *vs* the

279   single year of 1994, the analyses have focused on determining whether the ichthyofaunas were

280   more similar in 2008/09 and 2009/10 than either was to that in 1994.

281   The total number of individuals of the various fish species caught at three sites in the base

282   and lower regions of the estuary twice in each season between summer and spring in 1994 and those

283   caught subsequently at the same sites once in each season between winter 2008 and autumn 2010

284   (two years) were calculated. These data were used to determine and compare, in tabular form, the

mean densities (numbers $100 \, \text{m}^{-2}$) of each fish species and their percentage contributions to the total catch of fishes in 1994 and 2008-10. Note that, as the fish faunas were sampled twice seasonally in 1994, the data for that year were derived from the same number of samples (48) as in 2008-10 when sampling was conducted once seasonally over two years.

The multivariate data on fish composition from the above sample structure were used to construct a Bray-Curtis similarity matrix, input to a four-way PERMANOVA design, comprising three years (1994, 2008/09, 2009/10), four seasons, two regions (base and lower) and three sites in each region, with replicate seasonal observations only for the samples in 1994. The permutation in the PERMANOVA+ software (Anderson *et al.*, 2008) can accommodate this imbalance at the replicate level or, as previously, the residual variability can be conservatively estimated from the year × season × site interaction (these mean squares turn out to be effectively identical). Note that type III sums of squares were used in the PERMANOVA test, but the robustness of the results in the unbalanced case was checked using a number of sequential (type I) sum of squares calculations, with model terms entered in different sequences. The matrix was also subjected to two-way crossed ANOSIM tests for one factor *vs* the other factors combined (see earlier) to determine the relative influence of each factor. The pairwise $\bar{R}$ statistics computed from the two-way crossed test for year *vs* season and region combined were used to assess the extent of differences between the three years, *i.e.* 2008/09, 2009/10 and 1994. SIMPER was used to identify the species that contributed to the distinction between years.

nMDS ordination on appropriately averaged data then explored visually the basis for any interactions between factors, as detected by PERMANOVA. There was no necessity in this case (now with 12 points on the plot) to constrain the nMDS with a small percentage of a mMDS solution, but a new display method was adopted here, also utilising an alpha development version of PRIMER v7 software. Segmented bubble plots, which overlay multiple variables on a single ordination, were used to visualise the changes in major species identified in corresponding SIMPER

13

310   analyses, *e.g.* as being important in distinguishing the composition in the more recent years from the

311   earlier year. Here, segment size represents (pre-treated) abundance, as input to the nMDS, on a

312   common scale for all species of dispersion weighted, square-rooted, then averaged abundance for

313   the 12 year × season combinations.

314

315   **Results**

316   *Salinity and water temperature*

317   In all seasons except winter, the mean salinities throughout 2008-10 followed a gradient,

318   being lowest in the base region and highest in the apex (Fig. 2a). The minimum mean salinity in any

319   season was the *c.* 23 recorded in the former region in winter 2008/09, while the maximum mean

320   was the *c.* 50 recorded in the apex region in the summer of 2009/10. Furthermore, in both years, the

321   salinities in each region were higher during summer and autumn than in winter and spring.

322   Mean seasonal water temperatures in each region underwent pronounced changes, typically

323   rising from their minima in winter to their maxima in summer and then declining in autumn

324   (Fig. 2b). The highest mean water temperatures in both years were recorded in the apex and upper

325   regions. The minimum mean seasonal temperature was the *c.* 15ºC recorded in each region in the

326   winter of 2008/09 and 2009/10, whereas the maximum was the *c.* 36ºC recorded in the upper region

327   in the summer of 2009/10.

328

329   *Characteristics of the fish fauna of the Leschenault Estuary in 2008-10*

330   *Numbers of species and densities of fishes*

331   Four-way PERMANOVA for the number of species showed that the two-way interactions

332   for year × season and season × region and the three-way year × season × region interaction were

333   highly significant (Appendix 1a). This somewhat complex picture was apparent from the means

334   plots (Fig. 2c), with the three-way interaction seen as a decline in species numbers in the spring and

14

335 summer in the upper and apex regions, only in 2009/10, not matched in the base and lower regions.

336 Interpretation of the two-way interactions and main effects (Appendix 1a) is therefore compromised

337 by the presence of this substantial three-way interaction, though the numbers of species were

338 consistently highest in the base and lower regions (Fig. 2c) and lowest in the apex region. ANOVA

339 for the mean densities of fishes (Appendix 1b) also resulted in a significant and substantial three-

340 way interaction, reflecting less consistent trends across regions, with the mean values being greatest

341 in the lower region in five of the eight seasons but in the apex in two others (Fig. 2d).

342

343 *Contributions of fish species and life cycle categories*

344   A total of 27044 fish, representing 43 species, was caught in the Leschenault Estuary

345 between winter 2008 and autumn 2010 (Table I). The most abundant species were the solely

346 estuarine species, the elongate hardyhead *Atherinosoma elongata* Klunzinger 1879 and the spotted

347 hardyhead *Craterocephalus mugiloides* (McCulloch 1912)*,* the marine estuarine-opportunists the

348 sandy sprat *Hyperlophus vittatus* Castelnau 1865 and the yelloweye mullet *Aldrichetta forsteri*

349 Valenciennes 1836 and the estuarine & marine species the silver fish *Leptatherina presbyteroides*

350 (Richardson 1843)*,* which collectively contributed *c.* 75% to the total catch. The total number of

351 fish caught in the base, upper and apex regions ranged only from *c.* 5400 to 5800 per region, and

352 thus each was only just over half of that recorded in the lower estuary, *i.e. c.* 10400 (Table I). The

353 total number of species declined markedly from 32 and 35 in the base and lower regions,

354 respectively, to 22 in the upper region and only 12 in the apex.

355   The fauna in the base of the estuary was dominated by *A. forsteri* and *H. vittatus*, with

356 densities of *c.* 40 and 46 fish 100 m^{-2}, respectively, and collectively contributed 56% to the total

357 catch in this region (Table I). *Hyperlophus vittatus* was also abundant in the lower region, *i.e. c.* 45

358 fish 100 m^{-2}, where it contributed *c.* 16%. The atherinids *A. elongata*, *C. mugiloides* and

359 *L. presbyteroides* constituted the other three most abundant species in the lower region, with

360 densities ranging from *c.* 33 to 68 fish 100 m^{-2} and, together with *H. vittatus*, comprised 64% of the

361 total catch. *Atherinosoma elongata* was so abundant in the upper and apex regions that its densities

362 in these regions were *c.* 75 and 107 fish 100 m^{-2}, respectively, and its percentage contribution as

363 high as *c.* 50 and 67 %, respectively. While the densities of *C. mugiloides* in the upper and apex

364 regions were far less than those of *A. elongata*, they were still substantial and greater than those of

365 any other species, with their numbers contributing *c.* 18 and 16 %, respectively, to the total catches

366 in those regions.

367 Twenty one of the species caught throughout the estuary were marine estuarine-

368 opportunists, while nine were marine stragglers, seven were estuarine & marine, four were solely

369 estuarine and two were estuarine & freshwater (Table I). Overall, the number of species that

370 represented the marine category (*i.e.* marine estuarine-opportunists and marine stragglers) declined

371 markedly from 23 in both the base and lower regions to 14 in the upper region and seven in the apex

372 (Fig. 3a). The number of species in the estuarine category (*i.e.* solely estuarine, estuarine & marine

373 and estuarine & freshwater) declined from a maximum of 12 in the lower region to a minimum of

374 five in the apex. The overall trends exhibited throughout the estuary by the number of species in

375 both the marine and estuarine categories essentially replicate those in each season, demonstrating

376 that they remain similar throughout the year (Fig. 3a-e).

377 In terms of number of individuals, the overall contributions by marine species was far

378 greater in the base region (70%) than lower region (28%), which, in turn, was greater than the 22%

379 in the upper region and 15% in the apex (Fig. 3a). The estuarine category thus exhibited the

380 converse trend. The contribution to the abundances by marine species in the base region was

381 broadly consistent over seasons, but was far higher in spring and summer than autumn and winter in

382 the lower region (Fig. 3b-e). The estuarine category dominated the total catch of fish in both the

383 upper and apex regions in summer and autumn (Fig. 3d).

384

Multivariate analyses of species compositions

386   On the basis of a four-way PERMANOVA using data on ichthyofaunal composition for

387   2008-10, each of the two- and three-way interactions were significant (Appendix 2a). However,

388   several of the interactions have pseudo-F values close to 1, and are only marginally significant on

389   sometimes very powerful tests, *i.e.* high numerator and denominator degrees of freedom, *e.g.* all

390   those involving site terms. The only conspicuous interactions are season × region and year × season

391   (borne out by later plots), with all main effects appearing to be substantial and dominating the

392   interactions. Subsuming main effects and interactions, the $\bar{R}$ values for the two-way crossed

393   ANOSIM analyses for each factor *vs* the other two factors combined were greater for season (0.51)

394   and region (0.46) than for year (0.35).

395   The results of the tests were explored visually by nMDS ordinations on (pre-treated) data

396   matrices averaged over sites and each factor in turn, to obtain 2-way 'interaction plots' for pairs of

397   factors (Fig. 4), the second of which (Fig. 4c) needed to modify a degenerate nMDS solution by

398   adding a trace component of mMDS (see Methods). For the season × region ordination, the samples

399   for spring and summer lie above those for winter and autumn in each region. Furthermore, the

400   points for each successive season in all four regions change in a clockwise cyclical manner and the

401   points for each region progress sequentially from left to right along the horizontal axis, in the order

402   of base, lower, upper and apex (Fig. 4a). The season × region interaction is evident in the slightly

403   modified seasonal pattern for the lower region. The bubble plot of mean salinity at the times of

404   sampling, superimposed on this ordination, shows that, in each region, the seasonal changes in

405   ichthyofaunal composition from spring to summer coincided with substantial increases in salinity,

406   whereas changes from autumn to winter coincided with declines in salinity (Fig. 4b).

407   On the year × region plot, averaged over seasons (Fig. 4c), the samples in both years follow

408   very similar trajectories over the sequence of base to apex regions, indicating little interaction. In

409   fact, the borderline year × region interaction detected by PERMANOVA (Appendix 2a) is only

410   evident in marginal differences in the size of compositional changes between adjacent regions

411   (*i.e.* smaller changes from base to lower regions in 2008/9 than 2009/10 and larger changes from

412   upper to apex regions). The trend in ichthyofaunal composition from the base to apex regions is

413   associated with a sequential increase in mean salinities in both years (Fig. 4d). On the final

414   ordination plot, of season × year means, the samples for both years changed in an essentially

415   cyclical manner over successive seasons (Fig. 4e), though anticlockwise for 2008/9 and clockwise

416   for 2009/10. The important distinction here, explaining the significant season × year interaction, is

417   the difference in composition between the two winter samples, a disparity not shared by pairs of

418   samples for the other seasons.

419   As region and season were more influential than year, the interaction between the first two

420   factors was explored by pooling the data for the two years and undertaking a series of one-way

421   ANOSIM tests for region using separate resemblance matrices constructed from the fish

422   abundances in each season (Table II). Significant differences among regions were detected in each

423   season, being greatest in spring ($R = 0.56$) and lowest in winter ($R = 0.12$). In all seasons except

424   winter, the regional difference in composition was greatest for the apex and upper regions *vs* the

425   base and lower regions ($R = 0.34$-0.94). In each season, *F. lateralis* was always among the species

426   characterising the ichthyofaunas in the base and lower regions, while *A. elongata* was always

427   among the species typifying those in the upper and apex regions (Appendix 3).

428

429   *Seasonal and regional partitioning of the main fish species*

430   The 'coherent curves' technique, identifying cohorts of species with common patterns of

431   mean abundance across the 16 conditions (4 seasons by 4 regions), results in simple line plots of

432   relative abundance, grouped according to the results from sequences of Type 3 SIMPROF tests, in

433   which most species are notable for the relative concentration of their numbers only in certain

434   regions of the estuary and/or certain times of the year (Fig. 5). Thus, for example, in terms of region

435 and most abundant species overall, the species in groups I and II, *e.g. F. lateralis, H. vittatus,*

436 *A. forsteri* and *L. presyteroides,* were caught mainly in the base and lower regions (Fig. 5a, b),

437 whereas those in groups III and IV *e.g.* the western hardyhead *Leptatherina wallacei* (Prince,

438 Ivantsoff & Potter 1982) and the western gobbleguts *Ostorhinchus rueppellii* (Günther 1859) were

439 obtained very largely from the lower region (Fig. 5c, d), and those in groups V and VI, *e.g.* the sea

440 mullet *Mugil cephalus* L. 1758, *A. elongata* and *C. mugiloides* came predominantly from the upper

441 and apex regions (Fig. 5e, f). In the context of season, the abundance of certain groups of species

442 produced particularly sharp peaks at certain times of the year, *i.e.* groups III and IV in spring and

443 summer, respectively, in the lower estuary, group V (a single species) in winter in the upper and

444 apex regions and group VI in autumn in the upper estuary.

445

446 *Comparisons between fish faunas in 1994 and 2008-10*

447 *Contributions of fish species and life-cycle guilds*

448 A total of 13483 fishes was recorded in seasonal samples collected between winter 2008 and

449 autumn 2010 at three sites in each of the base and lower regions of the Leschenault Estuary

450 (Table III). This total approaches the 14601 fish recorded twice seasonally between summer and

451 spring in 1994 and thus for the same total number of samples. It was particularly striking that two

452 tropical atherinids, *C. mugiloides* and the common hardyhead *Atherinomorus vaigiensis* (Quoy &

453 Gaimard 1825), ranked as high as fifth and ninth, respectively, in 2008-10, and yet were not even

454 caught in 1994. However, eight of the 11 most abundant species caught in 2008-10 did also rank

455 among the 11 most abundant species in 1994 and the four most abundant species in 2008-10 ranked

456 among the most numerous species in the earlier period (Table III). Yet, among those species,

457 *A. elongata* was far more abundant in 2008/09, whereas the reverse was true for *A. forsteri* and

458 *H. vittatus*. While *F. lateralis* ranked only sixth and contributed 8.1% to the catch in 2008-10 it

459 ranked first and contributed 36.5% to the catches in 1994.

460   The percentage contributions made by each life-cycle guild to the number of species were

461 relatively similar in 1994 and 2008-10, *i.e.* solely estuarine, 12 *vs* 14 %; estuarine & marine, 18 *vs*

462 19 % and marine estuarine-opportunist, 48 *vs* 56, respectively (Table III). Furthermore, each of

463 these life cycle guilds made similar contributions to the total number of individuals in each period,

464 with the exception of estuarine residents, which were relatively more numerous in 2008-10 (32%)

465 than in 1994 (8%), due, in particular, to far larger numbers of *A. elongata* and to *C. mugiloides*

466 being caught only in the more recent period.

467

468 *Comparisons of ichthyofaunal compositions in 1994 vs 2008-10*

469   In terms of ichthyofaunal compositions, the year $\times$ season, season $\times$ region and year $\times$

470 season $\times$ region terms were significant, but clearly at a minor level, with the three-way interaction

471 being particularly borderline and effectively negligible (Appendix 2b). The $\bar{R}$ values for the two-

472 way crossed ANOSIM tests for each factor *vs* the other two factors combined were greater for year

473 (0.30) and season (0.28) than for region (0.12). In the case of year *vs* season and region combined,

474 pairwise comparisons demonstrated that, while the compositions in 2008/09 and 2009/10 were not

475 significantly different ($R = 0.10$, $P > 0.05$), they both differed from that in 1994 ($P = 0.001$), with R

476 statistic values of 0.29 and 0.42, respectively.

477   The year $\times$ season interaction can be visualised, in a nMDS ordination derived from mean

478 abundance of each species in each region (Fig. 6a). On this plot, the seasonal samples in each of the

479 three years change in a common anti-clockwise cyclical direction, with those for 1994 lying clearly

480 to the left of those for the 2008/09 and 2009/10 years, in which the samples for the two springs and

481 the two summers are virtually coincident. The trajectory of the seasonal samples in 1994 differs

482 however, from that in both 2008/09 and 2009/10, thus defining the year $\times$ season interaction. A

483 two-way crossed SIMPER analysis, contrasting 1994 with the combined 2008/09 and 2009/10

484 samples, removing the effect of season by comparing years only within seasons, demonstrated that

the species which most heavily and consistently discriminated the fish compositions in the earlier

and later years were *F. lateralis, C. mugiloides*, *A. forsteri*, *A. vaigiensis, A. elongata,* and the

Weeping Toado *Torquigener pleurogramma* Regan 1903, with all but the first species being

consistently more abundant in 2008-10.

The abundances of the above six species (on a common scale of pre-treated then averaged

values, as input to the multivariate analysis) are shown on the segmented bubble plot for each

season and year in Fig. 6b. They thus provide a concise description for the derivation of much of the

difference observed between the ichthyofaunal communities in the early and later years in the

nMDS. Although *F. lateralis* was always present in substantial numbers in all seasons and years, it

is clear that the abundances of this gobiid were much higher in 1994 than in 2008-10. In contrast,

the abundances of *A. elongata, A. forsteri* and *T. pleurogramma C. mugiloides, A. vaigiensis* were

found in larger numbers in the later years, particularly in the summer periods for *A. forsteri* and

A. elongata, and in all but the autumn periods for *T. pleurogramma*. It was noted previously that

C. mugiloides and *A. vaigiensis* were absent altogether in 1994 and this differentiation is seen to

provide a clear contribution to the community assessment. Also notable from the plot are the closely

matching values of all displayed species for the two spring and summer samples in the later years,

which gives some diagnostic reassurance that the (approximate) 2-d nMDS configuration genuinely

reflects the higher-dimensional information.

The converse component of the 2-way SIMPER analysis, comparing seasons having

removed the year effects, will identify some contributions from species already displayed in Fig. 6b

but, by definition, they are less likely to be the most consistent discriminators of the seasonal cycle

across all years, because of their differentiation of the years. Instead, a second set of species are

displayed in Fig. 6c, which highlight the seasonal differences. Most of these are seen to be species

with higher numbers in the spring or summer periods, being absent or less abundant in the autumn

or winter: *H. vittatus* is a major contributor of this type. Other species have somewhat different

510 phasing: the King George whiting, *Sillaginodes punctatus* (Cuvier 1829) has substantial

511 (transformed) abundances throughout the spring, summer and autumn (peaking in summer) but is

512 largely absent in winter; the soldier *Gymnapistes marmoratus* Cuvier 1829 is more restricted to

513 spring and summer, *O. rueppellii* is characteristically only a summer species and the spotted

514 pipefish *Stigmatopora argus* Richardson 1840, only a spring species. In contrast, numbers of the

515 bluespot goby, *Pseudogobius olorum* Sauvage 1880, tend to peak in the winter and spring and

516 decline or are absent altogether in the summer and autumn. One notable absence from both Figs 6b

517 and 6c is *L. presbyteroides*. In spite of its large numbers, being ranked third and fourth in terms of

518 total abundance for the early and later years respectively (Table III), it is largely present across all

519 years and seasons and plays little role in distinguishing between years, although, as earlier

520 observed, it is one of the species characterising the differences between the base/lower and

521 upper/apex regions of the estuary in 2008-10. The remainder of the 'top ten' ranked species listed in

522 Table III for both periods, with the exception of *L. wallacei,* are represented in Fig. 6 and their

523 temporal patterns, in so far as these influence the community assessment from a multivariate

524 analysis, are readily discernible from this novel segmented bubble plot.

525

526 **Discussion**

527 *Salinities and water temperatures*

528 The seasonal trends exhibited by salinity throughout the Leschenault Estuary in 2008-10

529 demonstrate that, during the warm dry summer months of two successive years, there was a

530 pronounced reverse salinity gradient, with salinities increasing from the base region to the apex,

531 where they were far greater than sea water, *i.e.* 35, and reached 56 at one sampling site. Although

532 that gradient was also present in spring and autumn, it was less pronounced and disappeared in

533 winter, when salinities in all regions declined to less than 35. The exceptionally high salinities in the

534 apex region in summer were due to a combination of very high evaporative loss from the shallow

535   waters of this region (typically <1.5 m), the absence of tributary rivers in the upper three-quarters of

536   the estuary and limited rainfall during this dry period. The particularly pronounced decline in

537   salinity between autumn and winter in the shallow apex and upper regions is produced mainly by

538   freshwater, which, during that period of heavy precipitation, enters the apex region directly and via

539   surface run off and from a large drain. The concomitant decline in salinity in the deeper base and

540   lower regions was due largely to freshwater discharge into the bottom end of the estuary from the

541   two tributaries that supply this system (Fig. 1).

542   High levels of solar radiation, limited precipitation and the shallowness of the water also

543   account for the very high water temperatures in the upper and apex regions of the Leschenault

544   Estuary during summer. As with salinity, the maximum recorded water temperature of 36ºC is

545   almost certainly an under-estimate of the true maximum as temperatures were measured only at the

546   time of sampling. The extreme salinities and temperatures in the upper and apex regions in summer

547   would pose a strong physiological challenge to many of the fish species typically found in estuaries

548   on the lower west coast of Australia.

549

550   *Main characteristics of the fish fauna in the Leschenault Estuary*

551   Analyses of the data, derived from the approximately 27000 fishes, representing 43 species

552   caught during seasonal sampling of the four regions of the Leschenault Estuary over two years,

553   provided an integrated picture of the characteristics of the ichthyofauna of this permanently-open

554   estuary with a reverse salinity gradient. The overall number of species was shown to decline from

555   the base and lower regions to the upper and then apex regions during spring and summer, when the

556   reverse salinity gradient was most pronounced. This presumably reflects, in part, a progressive

557   decline along that gradient, in the number of species capable of osmoregulating effectively in

558   hypersalinities. This decline is more pronounced with marine species than with estuarine residents,

559   which was expected as the latter contain some euryhaline species that are particularly well adapted

560 to living in the highly variable environments found in estuaries (see later). The trend towards a

561 reduced number of species along the reverse salinity gradient parallels that recorded for other

562 estuaries with such a gradient, such as the permanently-open and microtidal Sine Saloum and

563 Casamance estuaries in west Africa (Simier *et al.*, 2004; Kantoussan *et al.*, 2012) and the

564 periodically-closed Lake St Lucia in Africa (Cyrus *et al.*, 2011).

565   It was striking that, while densities in the base and/or lower regions were greater than in the

566 upper and apex regions in most seasons, very high densities were recorded in the apex region in the

567 autumn and winter of 2008/09 and in the upper region in the autumn of both 2008/09 and 2009/10.

568 This was due to the recruitment of large numbers of 0+ individuals of three atherinid species,

569 *A. elongata*, *C. mugiloides* and *A. vaigiensis,* following their spring to summer spawning periods

570 (Prince & Potter, 1983). The presence, in most seasons, of greater densities of fishes in the lower

571 region than other regions (see also Table I) probably reflects the greater heterogeneity of habitat in

572 this region, through containing substantial areas of seagrass and macroalage as well as unvegetated

573 substrata (Wurm & Semeniuk, 2000), and does not undergo the extreme changes in salinity

574 experienced by the upper and apex regions.

575   Our data also demonstrate that the ichthyofauna of the Leschenault Estuary consisted

576 entirely of marine species or estuarine residents. However, while the overall number of marine

577 species was well over twice that of estuarine residents, the reverse was true for overall relative

578 abundance. This reflected the fact that, whereas marine species were far more numerous than

579 estuarine residents in the base region, the exact opposite was the case in each of the other three

580 regions and especially so in the upper and apex regions in summer and autumn. These trends imply,

581 firstly, that the essentially marine environment created in the base region by the direct intrusion of

582 marine waters through the entrance channel, which joins this region with the Indian Ocean, provides

583 an ideal environment for the marine species that enter the Leschenault Estuary. The trends also

584 imply, however, that further up the estuary, and particularly in the upper and apex regions in

585 summer and autumn, possess features that make those regions far more conducive to occupation by

586 estuarine residents than marine species. It is thus relevant that tidal water movements are relatively

587 weak in this estuary and do not extend into the upper and apex regions (Charteris & Deeley, 2000;

588 Gillibrand *et al.,* 2012) and that the waters in those regions in summer and autumn become

589 hypersaline and particularly warm (see next section for significance and particularly for certain

590 atherinids).

591   The above regional trends helps account for the finding that the percentage contributions

592 made by estuarine residents to the abundance of fishes in samples from the Leschenault Estuary

593 were greater than those in the two large permanently-open estuaries to the north, *i.e.* Swan-Canning

594 and Peel-Harvey estuaries, recognising, however, that those contributions were still substantial

595 (Loneragan *et al*., 1989; Young & Potter, 2003). The large abundances of a number of estuarine

596 residents in the microtidal estuaries of Western Australian has been attributed to these species

597 typically spawning in these systems during the warm and dry months when environmental

598 conditions are relatively benign and thus conducive to spawning success and recruitment (Potter &

599 Hyndes, 1999).

600

601 *The importance of atherinids*

602   The small number of species that are apparently well adapted to living in the high salinities

603 and water temperatures that characterise the upper and apex regions of the Leschenault Estuary in

604 summer and autumn would benefit from an inevitable reduction in interspecific competition for

605 spatial and food resources. This parallels the situation in other estuaries with reverse salinity

606 gradients, or at least high salinities in their upper reaches, such as the Casamance Estuary in western

607 Africa and Kariega Estuary in southern Africa, where the ichthyofauna of the upper reaches were

608 dominated by estuarine residents (Ter Morshuizen & Whitfield, 1994; Kantoussen *et al.*, 2012).

The domination of the fish faunas of the upper and apex regions of the Leschenault Estuary by estuarine residents was largely due to the overwhelming prevalence of atherinids in these two regions (68 and 83 %) and, in particular, of *Atherinosoma elongata* and *Craterocephalus mugiloides*. These species are known to be able to tolerate a wide range of salinities (Thompson & Withers, 1992; Young & Potter, 2002). *Atherinosoma elongata* was so abundant in the upper and apex regions that it contributed as much as one half and two thirds to the total catch in these regions, respectively. These data emphasize that certain species of the Atherinidae are particularly well adapted to living in high salinities and thus able to exploit the opportunities provided by a reduction in the competition from other species that are less tolerant of such conditions. In this context, it is relevant that *A. elongata* was the only fish species to survive in the Wellstead Estuary on the lower west coast of Australia, when salinities in this normally-closed estuary had risen to 122 (Young & Potter, 2002) and that a closely-related species, the smallmouth hardyhead *Atherinosoma microstoma* Günther 1861, was the only species caught in the very high salinities at the uppermost site in the Coorong Estuary, which has a reverse salinity gradient (Brookes *et al*., 2009; Zampatti *et al*., 2010). Experimental studies have shown that the latter species can osmoregulate in salinities up to at least 80 (Wedderburn *et al.*, 2008). Furthermore, two atherinid species in the Mondego Estuary in Portugal were more abundant in drought than in non-drought years when salinities were less (Baptista *et al.*, 2010). It is also noteworthy that the only other abundant species in the upper and apex regions was another atherinid, *Atherinomorus vaigiensis*, and that this species has also been found in the hypersaline regions of a large embayment (Bayly, 1972), recognising, however, that this species spawns in marine waters and recruits into estuaries as juveniles (Prince & Potter, 1983). As this atherinid, and also *C. mugiloides*, are tropical species, they would be well adapted to tolerating the high temperatures present in the shallow waters of the upper estuary during summer.

633   In contrast, in particular to *A. elongata*, the atherinid *Leptatherina presbyteroides*, which is

634   represented by both marine and estuarine populations (Prince & Potter, 1983), is more abundant in

635   the base and lower than upper and apex regions of the estuary and thus exhibits a 'preference' for

636   that part of the estuary which is subject to appreciable tidal influence and thus never becomes

637   conspicuously hypersaline. The numbers of this species and all other atherinids collectively account

638   for as much as 60% of the total catch taken throughout the Leschenault Estuary, thus emphasizing

639   the importance of this versatile family in this system. In terms of overall abundance, however, two

640   marine species *Hyperlophus vittatus* and *Aldrichetta forsteri* ranked third and fourth and the

641   estuarine residents *Favonigobius lateralis* and *Ostorhinchus rueppellii* ranked sixth and eighth,

642   respectively.

643   The low numerical rankings of the atherinid *Leptatherina wallacei* (12) and the gobiid

644   *P. olorum* (32) in the Leschenault Estuary contrast markedly with those in the permanently-open

645   Swan-Canning (3 and 13) and Blackwood River (1 and 8) estuaries to the north and south of the

646   Leschenault, respectively (Loneragan *et al.*, 1989; Valesini *et al.*, 1997). In the latter estuaries, these

647   two species were most abundant by far in the upper regions, where, due to riverine discharge and

648   freshwater run-off, and thus, in contrast to the Leschenault Estuary, salinities rarely approach that of

649   seawater. Similarly, in the Sine Saloum system in west Africa where hypersalinities are observed all

650   year round, several species that are common in brackish waters of other estuaries in this region are

651   absent or present in low numbers (Simier *et al.*, 2004).

652

653   *Seasonal and regional trends in species composition*

654   The trends shown on the nMDs ordination plots in Figs 4a and b emphasise that the

655   composition of the ichthyofauna changed in a cyclical manner in each region of the estuary and that

656   these sequential changes were associated with the trends exhibited by salinity. The seasonal changes

657   in the lower and middle areas of the estuary were strongly related to the seasonal patterns of

658   immigration and emigration of marine species, such as *H. vittatus, Gymnapistes marmoratus*,

659   *S. punctatus*, *A. forsteri* and *Stigmatopora argus,* which were represented by greater numbers in

660   spring and/or summer. In contrast, the seasonal changes in the apex region reflected, *inter alia,* the

661   presence of only three species (mainly the estuarine resident atherinids *A. elongata* and

662   *C. mugiloides*) in summer, when salinities became markedly elevated (see earlier), and a wider

663   range of species during other times of the year when salinities declined. Cyclical changes in

664   composition have been shown to occur in other microtidal estuaries (*e.g.* Young & Potter, 2003;

665   Hoeksema & Potter, 2006) and also macrotidal estuaries, where they reflected predominantly time-

666   staggered immigrations and emigrations of the juveniles of marine species and migrating

667   diadromous species (*e.g.* Potter *et al.*, 1986; Thiel & Potter, 2001; Maes *et al.*, 2005).

668   The use in Figs 4c and d of bubble plots to denote salinity differences, show that

669   ichthyofaunal composition changed progressively along the estuary and in conjunction with

670   increasing salinity. These changes reflect the shift from domination by marine species of

671   ichthyofauna in the base region to the extreme dominance in the apex region of *A. elongata*,

672   *C. mugiloides* and *A. vaigiensis*, which each belong to a family known to be able to tolerate high

673   salinities (see earlier). The ichthyofaunas of two estuaries in west Africa with a reverse salinity

674   gradient have also been shown to change in composition and undergo a reduction in number of

675   species along that gradient (Simier *et al.*, 2004; Kantoussan *et al.*, 2012). $\bar{R}$ values demonstrated

676   that inter-annual variations in ichthyofaunal composition in the Leschenault Estuary were less than

677   those due to seasonal and regional changes, reflecting the consistency of the pronounced seasonal

678   and regional trends exhibited by salinity and temperature in the two years.

679   The use of SIMPROF, in conjunction with coherent species curve analyses, demonstrated

680   that the spatial and temporal resources in the Leschenault Estuary are largely partitioned among

681   separate groups of fish species. The species in groups I and II, *i.e.* those mainly confined to the base

682   and lower regions of the estuary, all belonged to the marine estuarine-opportunist guild, except for

683 *F. lateralis* and *L. presbyteroides*, which belong to the estuarine & marine guild and are thus also

684 represented by discrete populations in marine waters. These species are also typically found in the

685 lower regions of other south-western Australian estuaries (Gill & Potter, 1993; Prince *et al.*, 1982).

686 In contrast, three of the six species (the yellowtail grunter *Amniataba caudavittata* Richardson

687 1845, *A. elongata* and *C. mugiloides*) in group VI, *i.e.* which occurred mainly in the upper and apex

688 regions, are, confined to estuaries in south-western Australia (Prince & Potter, 1983; Wise *et al.*,

689 1994; Potter & Hyndes, 1999). Two of the other species are marine estuarine opportunists (the

690 yellowfin whiting *Sillago schomburgkii* Peters 1864 and *A. vaigiensis*) are known to be able to

691 tolerate high salinities (Bayly, 1972; Lenanton, 1977).

692 The species which were largely restricted to the lower region of the estuary were mainly

693 caught in either spring (group III) or summer (group IV). Thus, *c.* 50-70 % of the individuals of

694 *G. marmoratus*, *L. wallacei*, *S. argus* and the hairy pipefish *Urocampus carinirostris* Castelnau

695 1872 were caught in this region during spring and *c.* 50-100 % of individuals of *O. rueppellii*, the

696 blue weed whiting *Haletta semifasciata* Valenciennes 1840, the western striped grunter *Pelates*

697 *octolineatus* Jenyns 1840 and the tarwhine *Rhabdosargus sarba* Forsskål 1775 were obtained from

698 this region in summer. It is thus relevant that the above species are associated with aquatic

699 vegetation (Travers & Potter, 2002; Young & Potter, 2003; Hesp *et al.*, 2004; Valesini *et al.*, 2004)

700 and that macroalgal growths are prolific in these seasons (Hugues-dit-Ciles *et al.*, 2012).

701 The partitioning of spatial and seasonal resources among groups of species in the

702 Leschenault Estuary would reduce the potential for competition among the most abundant marine

703 species and estuarine residents in this system.

704

705 *Comparisons of the fish faunas in 1994 and 2008-10*

706 It is particularly pertinent that the species compositions in 2008/9 and 2009/10 were not

707 significantly different, but that the composition in each of these years was significantly different

708   from that in 1994, a feature emphasised by a comparison of the distributions of the samples for the

709   three years in the ordination plots shown in Fig. 6. This trend is consistent with the hypothesis that

710   changes in the environment between those two periods were accompanied by a change in

711   ichthyofaunal composition. The most important contributors to these ichthyofaunal differences were

712   unexpected, however, involving a contrast between the presence in 2008-10 of substantial numbers

713   of *A. vaigiensis,* and more particularly *C. mugiloides*, and the absence of these species in 1994

714   (Table III; Potter *et al*., 1997, 2000). As only *A. vaigiensis,* of these two species, has been recorded

715   in an estuary further south and then only in very low numbers (Valesini *et al*., 1997), there has been

716   a southwards extension in the range of in which these designated tropical species (Hoese *et al*.,

717   2006) are abundant. This presumably reflects the influence of the conspicuous increases in water

718   temperature, which, as a result of climate change, have occurred along the south-western coast of

719   Australia during particularly the last 20 years (Pearce & Feng, 2007; Pearce *et al*., 2011; Lough, *et*

720   *al*., 2012). Such a conclusion is consistent with the fact that, during the last 30 years, the abundance

721   of *C. mugiloides* has increased in both the Swan-Canning (*cf.* Loneragan *et al*., 1989; Valesini *et al*.,

722   2009, 2011, 2013) and Peel-Harvey estuaries (*cf.* Potter *et al.*, 1983; Loneragan *et al*., 1986; Veale,

723   2013), which are located *c.* 140 and 85 km to the north of the Leschenault Estuary, respectively.

724   The differences in composition between 2008-10 and 1994 also reflect increases in the

725   densities and contributions to the total catch by *O. rueppellii* and *G. marmoratus*. It is thus relevant

726   that these species are strongly associated with aquatic vegetation (Travers & Potter, 2002; Young &

727   Potter, 2003; Valesini *et al.*, 2004), and that the estuary became more eutrophic between the two

728   periods, which is reflected in an increase in macroalgae (Hugues-dit-Ciles *et al*., 2012). As the

729   distribution of *O. rueppellii* includes the northernmost and thus tropical regions of Australia (Allen

730   *et al.*, 2006), the increased abundance of this apogonid may also reflect the beneficial effects of

731   higher water temperatures for such species. In contrast to the above two species, the overall density,

732   contribution to the total catch and ranking by abundance of *F. lateralis* was far less in 2008-10 than

733 in 1994. Since this gobiid suffered a greater mortality when housed in aquaria containing silt rather

734 than sand (Gill & Potter, 1993), the reduced abundance of this species is probably related to the

735 effects of an increase in siltation in this estuary (McKenna, 2007; Kilminster, 2010; Hugues-dit-

736 Ciles *et al.*, 2012).

737 In summary, the results of this integrated study, in combination with those on a range of

738 largely comparable estuaries, have elucidated the features that characterise permanently-open

739 estuaries with a reverse salinity gradient. The results demonstrate that the number of species,

740 including those of both the marine and estuarine category, decline along the salinity gradient.

741 However, very high densities were recorded in the apex and upper regions in autumn, due

742 predominantly to large numbers of three species of atherinid, a family that contains certain species

743 capable of tolerating extreme salinities. Coherent species curves demonstrated that species tend to

744 form groups along the estuary and according to season, thereby resulting in partitioning of resources

745 among the members of the ichthyofauna and thus reducing the potential for interspecific

746 competition. Ichthyofaunal composition was shown to undergo pronounced and progressive

747 changes along the estuary and with season in association with changes in salinity. Inter-decadal

748 comparisons emphasised that the ichthyofaunal composition of estuaries can undergo conspicuous

749 changes due to the introduction of new species as coastal water temperature increase as a result of

750 climate change and through changes in the relative abundance of some species in association with

751 other changes in the environment, such as increased siltation and eutrophication.

752

757 Development Commission and Murdoch University. This study was carried out in accordance with

758 Murdoch University Animal Ethics Permit R2170/08.

759

760 **References**

761

762 Able, K. & Fahay, M. (2010). *Ecology of estuarine fishes: temperate waters of the western North*

763 *Atlantic*. Baltimore: The Johns Hopkins University Press.

764 Allen, G. R., Cross, N. J. & Hoese, D. F. (2006). Apogonidae. In *Zoological Catalogue of*

765 *Australia*, Vol. 35, Part 2 (Beesley, P.L. & Wells, A., eds), pp. 1081-1112. Victoria:

766 Australian Biological Resources Study and Commonwealth Scientific and Industrial Research

767 Organisation Publishing.

768 Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance.

769 *Austral Ecology* **26**, 32–46.doi: 10.1111/j.1442-9993.2001.01070.pp.x

770 Anderson, M. J., Gorley, R. N. & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to*

771 *Software and Statistical Methods*. Plymouth: PRIMER-E.

772 Baptista, J., Martinho, F., Dolbeth, M., Viegas, I., Cabral, H. & Pardal, M. (2010). Effects of

773 freshwater flow on the fish assemblage of the Mondego estuary (Portugal): comparison

774 between drought and non-drought years. *Marine and Freshwater Research* **61**, 490–

775 501.doi:10.1071/MF09174.

776 Bayly, I. A. E. (1972). Salinity tolerance and osmotic behavior of animals in athalassic saline and

777 marine hypersaline waters. *Annual Review of Ecology and Systematics* **3**, 233–

778 268.doi:10.1146/annurev.es.03.110172.001313.

779 Blaber, S. J. M. & Blaber, T. G. (1980). Factors affecting the distribution of juvenile estuarine and

780 inshore fish. *Journal of Fish Biology* **17**, 143–162. doi:10.1111/j.1095-8649.1980.tb02749.x.

781 Brauner, C. J., Gonzalez, R. J. & Wilson, J. M. (2013). Extreme environments: hypersaline,

782   alkaline, and ion-poor waters. *Fish Physiology* **32**, 435–476.

783 Charteris, A. & Deeley, D.M. (2000). Hydrodynamics of Leschenault Inlet, Western Australia.

784   *Journal of the Royal Society of Western Australia* **83**(4), 251-254.

785 Chrystal, P. J., Potter, I. C., Loneragan, N. R. & Holt, P. C. (1985). Age structure, growth rates,

786   movement patterns and feeding in an estuarine population of the cardinalfish *Apogon*

787   *rueppellii*. *Marine Biology* **85**, 185–197.doi:10.1007/BF00397437.

788 Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure.

789   *Australian Journal of Ecology* **18**, 117–143.doi:10.1111/j.1442-9993.1993.tb00438.x.

790 Clarke, K. R. & Gorley, R. N. (2006). *PRIMER v6: User Manual/Tutorial*. Plymouth: PRIMER-E.

791 Clarke, K. R. & Green, R. H. (1988). Statistical design and analysis for a" biological effects" study.

792   *Marine Ecology Progress Series* **46**, 213–226.

793 Clarke, K. R. & Warwick, R. M. (2001). *Change in marine communities: an approach to statistical*

794   *analysis and interpretation, 2nd ed*. Plymouth: PRIMER-E.

795 Clarke, K. R., Chapman, M. G., Somerfield, P. J. & Needham, H. R. (2006). Dispersion-based

796   weighting of species counts in assemblage analyses. *Marine Ecology Progress Series*

797   **320**, 11–27.

798 Clarke, K. R., Somerfield, P. J. & Gorley, R. N. (2008). Testing of null hypotheses in exploratory

799   community analyses: similarity profiles and biota-environment linkage. *Journal of*

800   *Experimental Marine Biology and Ecology* **366**, 56–69.doi:10.1016/j.jembe.2008.07.009.

801 Clarke, K. R., Tweedley, J. R. & Valesini, F. J. (2014). Simple shade plots aid better long-term

802   choices of data pre-treatment in multivariate assemblage studies. *Journal of the Marine*

803   *Biological Association of the UK* **94**, 1–16.doi:10.1017/S0025315413001227.

804 Cyrus, D. P. (2013). Fish and Fisheries, In *Ecology and Conservation of Estuarine Ecosystems:*
805 *Lake St Lucia as a Global Model*, (Perissinotto, R., Stretch, D. D., Taylor, R. H. & Taylor, R.
806 H., eds), pp. 291-315. Cambridge: Cambridge University Press.

807 Cyrus, D., Jerling, H., MacKay, F. & Vivier, L. (2011). Lake St Lucia, Africa's largest estuarine
808 lake in crisis: combined effects of mouth closure, low levels and hypersalinity. *South African*
809 *Journal of Science* **107**, 01–13.doi:10.4102/sajs.v107i3/4.291.

810 Elliott, M. & Hemmingway, K. L. (2002). *Fishes in estuaries*. Oxford: Blackwell Science.

811 Elliott, M. & Whitfield, A. K. (2011). Challenging paradigms in estuarine ecology and
812 management. *Estuarine, Coastal and Shelf Science* **94**, 306–
813 314.doi:10.1016/j.ecss.2011.06.016.

814 Ferguson, G. J, Ward, T. M., Ye, Q., Geddes, M .C. & Gillanders, B. M. (2013). The impacts of
815 drought, flow regime, and fishing on the fish assemblage in Southern Australia's largest
816 temperate estuary. *Estuaries and Coasts* **36**, 737–753.doi:10.1007/s12237-012-9582-z.

817 French, B., Platell, M. E., Clarke, K. R. & Potter, I. C. (2012). Ranking of length-class, seasonal
818 and regional effects on dietary compositions of the co-occurring *Pagrus auratus* (Sparidae)
819 and *Pseudocaranx georgianus* (Carangidae). *Estuarine, Coastal and Shelf Science* **115**, 309–
820 325.doi:10.1016/j.ecss.2012.09.004.

821 Gill, H. S. & Potter, I. C. (1993). Spatial segregation amongst goby species within an Australian
822 estuary, with a comparison of the diets and salinity tolerance of the two most abundant
823 species. *Marine Biology* **117**, 515–526.doi:10.1007/BF00349327.

824 Hallett, C. S., Valesini, F. J., Clarke, K. R. & Hoeksema, S. D., submitted. Multiple lines of
825 evidence reveal complex impacts of a harmful algal bloom on estuarine fish communities.
826 *Marine Pollution Bulletin.*

827   Hesp, S. A., Hall, N. G. & Potter, I. C. (2004). Size-related movements of *Rhabdosargus sarba* in

828   three different environments and their influence on estimates of von Bertalanffy growth

829   parameters. *Marine Biology* **144**, 449–462.doi:10.1007/s00227-003-1217-2.

830   Hoeksema, S. D. & Potter, I. C. (2006). Diel, seasonal, regional and annual variations in the

831   characteristics of the ichthyofauna of the upper reaches of a large Australian microtidal

832   estuary. *Estuarine, Coastal and Shelf Science* **67**, 503–520.doi:10.1016/j.ecss.2005.12.003.

833   Hoese, D. F., Bray, D. J., Paxton, J. R. & Allen, G. R. (2006). Fishes. In *Zoological Catalogue of*

834   *Australia* (Beesley, P.L. & Wells, A., eds), Vol. 35, Part 2, pp. 671-1472. Victoria: Australian

835   Biological Resources Study and Commonwealth Scientific and Industrial Research

836   Organisation Publishing.

837   Kantoussan, J., Ecoutin, J. M., Simier, M., Tito de Morais, L. & Laë, R. (2012). Effects of salinity

838   on fish assemblage structure: An evaluation based on taxonomic and functional approaches in

839   the Casamance estuary (Senegal, West Africa). *Estuarine, Coastal and Shelf Science* **113**,

840   152–162.doi:10.1016/j.ecss.2012.07.018.

841   Largier, J. L. (2010). Low-inflow estuaries: hypersaline, inverse, and thermal scenarios. In

842   *Contemporary issues in estuarine physics,* (Valle-Levinson, A., ed), pp. 247-272. Cambridge:

843   Cambridge University Press.

844   Largier, J. L., Hollibaugh, J. T. & Smith, S. V. (1997). Seasonally hypersaline estuaries in

845   Mediterranean-climate regions. *Estuarine, Coastal and Shelf Science* **45**, 789–

846   797.doi:10.1006/ecss.1997.0279.

847   Lek, E., Fairclough, D. V., Platell, M. E., Clarke, K. R., Tweedley, J. R. & Potter, I. C. (2011). To

848   what extent are the dietary compositions of three abundant, co-occurring labrid species

849   different and related to latitude, habitat, body size and season?. *Journal of Fish Biology* **78**,

850   1913–1943.doi:10.1111/j.1095-8649.2011.02961.x.

851   Lenanton, R. C. (1977). Fishes from the hypersaline waters of the stromatolite zone of Shark Bay,

852   Western Australia. *Copeia* 387–390.

853   Loneragan, N. R., Potter, I. C., Lenanton, R. C. J. & Caputi, N. (1986). Spatial and seasonal

854   differences in the fish fauna in the shallows of a large Australian estuary. *Marine Biology* **92**,

855   575–586.doi:10.1007/BF00392517.

856   Loneragan, N. R., Potter, I. C. & Lenanton, R. C. J. (1989). Influence of site, season and year on

857   contributions made by marine, estuarine, diadromous and freshwater species to the fish fauna

858   of a temperate Australian estuary. *Marine Biology* **103**, 461–479.doi:10.1007/BF00399578.

859   Maes, J., Stevens, M. & Ollevier, F. (2005). The composition and community structure of the

860   ichthyofauna of the upper Scheldt Estuary: synthesis of a 10-year data collection (1991-2001).

861   *Journal of Applied Ichthyology* **21**, 86–93.doi:10.1111/j.1439-0426.2004.00628.x.

862   Pearce, A. & Feng, M. (2007). Observations of warming on the Western Australian continental

863   shelf. *Marine and Freshwater Research* **58**, 914–920.doi:10.1071/MF07082.

864   Potter, I. C. & Hyndes, G. A. (1999). Characteristics of the ichthyofaunas of southwestern

865   Australian estuaries, including comparisons with holarctic estuaries and estuaries elsewhere in

866   temperate   Australia: a   review.   *Australian   Journal   of   Ecology*   **24**,   395–

867   421.doi:10.1046/j.1442-9993.1999.00980.x

868   Potter, I. C., Beckley, L. E., Whitfield, A. K. & Lenanton, R. C. J. (1990). Comparisons between

869   the roles played by estuaries in the life cycles of fishes in temperate Western Australia and

870   southern Africa. *Environmental Biology of Fishes* **28**, 143–178.doi:10.1007/978-94-009-

871   2065-1_9.

872   Potter, I. C., Chalmer, P. N., Tiivel, D. J., Steckis, R. A., Platell, M. E. & Lenanton, R. C. J. (2000).

873   The fish fauna and finfish fishery of the Leschenault Estuary in south-western Australia.

874   *Journal of the Royal Society of Western Australia* **83**, 481–501.

875   Potter, I. C., Chuwen, B. M., Hoeksema, S. D. & Elliott, M. (2010). The concept of an estuary: a

876   definition that incorporates systems which can become closed to the ocean and hypersaline.

877   *Estuarine, Coastal and Shelf Science* **87**, 49–500.doi:10.1016/j.ecss.2010.01.021.

878   Potter, I. C., Claridge, P. N. & Warwick, R. M. (1986). Consistency of seasonal changes in an

879   estuarine fish assemblage. *Marine Ecology Progress Series* **32**, 217–228.

880   Potter, I. C., Loneragan, N. R., Lenanton, R. C. J., Chrystal, P. J. & Grant, C. G. (1983).

881   Abundance, distribution and age structure of fish populations in a Western Australia estuary.

882   *Journal of Zoology* **200**, 21–50.

883   Potter, I.C., Tivel, D., Valesini, F. J. & Hyndes, G. A. (1997). Comparisons between the

884   ichthyofaunas of a temperate lagoonal-like estuary and the embayment into which that estuary

885   discharges. *International Journal of Salt Lake Research* **5**, 337–

886   358.doi:10.1007/BF01995386.

887   Potter, I. C., Tweedley, J. R., Elliott, M. & Whitfield, A. K. (2013). The ways in which fish use

888   estuaries: a refinement and expansion of the guild approach. *Fish and Fisheries*

889   doi:10.1111/faf.12050.

890   Prince, J. D. & Potter, I. C. (1983). Life-cycle duration, growth and spawning times of five species

891   of Atherinidae (Teleostei) found in a Western Australian estuary. *Australian Journal of*

892   *Marine and Freshwater Research* **34**, 287–301.

893   Prince, J., Potter, I., Lenanton, R. & Loneragan, N. (1982). Segregation and feeding of atherinid

894   species (Teleostei) in south-western Australian estuaries. *Marine and Freshwater Research*

895   **33**, 865-880.

896   Sarre, G. A. & Potter, I. C. (1999). Comparisons between the reproductive biology of black bream

897   *Acanthopagrus butcheri* (Teleostei: Sparidae) in four estuaries with widely differing

898   characteristics. *International Journal of Salt Lake Research* **8**, 179–

899   210.doi:10.1007/BF02449931.

900 Schelske, C. L. & Odum, E. P. (1961). Mechanisms maintaining high productivity in Georgia
901   estuaries. *Proceedings of the Gulf and Caribbean Fisheries Institute* **14**, 75–80.

902 Semeniuk, V., Semeniuk, T. A. & Unno, J. (2000). The Leschenault Inlet estuary: an overview.
903   *Journal of the Royal Society of Western Australia* **83**, 207–228.

904 Simier, M., Blanc, L., Aliaume, C., Diouf, P. S. & Albaret, J. J. (2004). Spatial and temporal
905   structure of fish assemblages in an "inverse estuary", the Sine Saloum system (Senegal).
906   *Estuarine, Coastal and Shelf Science* **59**, 69–86.doi:10.1016/j.ecss.2003.08.002.

907 Somerfield, P. J. & Clarke, K. R. (2013). Inverse analysis in non-parametric multivariate analyses:
908   distinguishing groups of associated species which covary coherently across samples. *Journal*
909   *of   Experimental   Marine   Biology   and   Ecology* **449**,   261–
910   273.doi:10.1016/j.jembe.2013.10.002.

911 Ter Morshuizen, L. D. & Whitfield, A. K. (1994). The distribution of littoral fish associated with
912   eelgrass *Zostera capensis* beds in the Kariega Estuary, a southern African system with a
913   reversed   salinity   gradient. *South   African   Journal   of   Marine   Science* **14**,   95–
914   105.doi:10.2989/025776194784287049.

915 Thiel, R. & Potter, I. C. (2001). The ichthyofauna composition of the Elbe Estuary: an analysis in
916   space and time. *Marine Biology* **138**, 603–616.doi:10.1007/s002270000491.

917 Thompson, G. G. & Withers, P. C. (1992). Osmoregulatory adjustments by three atherinids
918   (*Leptatherina presbyteroides*; *Craterocephalus mugiloides; Leptatherina wallacei*) to a range
919   of salinities. *Comparative Biochemistry and Physiology* **103**, 725–728.doi:10.1016/0300-
920   9629(92)90173-N.

921 Travers, M. J. & Potter, I. C. (2002). Factors influencing the characteristics of fish assemblages in a
922   large   subtropical   marine   embayment. *Journal   of   Fish   Biology* **61**,   764–784.doi:
923   10.1111/j.1095-8649.2002.tb00910.x.

924 Valesini, F. J., Potter, I. C. & Clarke, K. R. (2004). To what extent are the fish compositions at

925   nearshore sites along a heterogeneous coast related to habitat type? *Estuarine, Coastal and*

926   *Shelf Science* **60**, 737–754.doi:10.1016/j.ecss.2004.03.012.

927 Valesini, F. J., Potter, I. C., Platell, M. E. & Hyndes, G. A. (1997). Icthyofaunas of a temperate

928   estuary and adjacent marine embayment. Implications regarding choice of nursery area and

929   influence of environmental changes. *Marine Biology* **128**, 317–

930   328.doi:10.1007/s002270050097.

931 Valesini, F. J., Tweedley, J. R., Clarke, K. R. & Potter, I. C. (2013). The importance of local,

932   system-wide and regional spatial scales in structuring temperate estuarine fish communities.

933   *Estuaries and Coasts*.doi:10.1007/s12237-013-9720-2.

934 Webster, I. T. (2010). The hydrodynamics and salinity regime of a coastal lagoon–The Coorong,

935   Australia–Seasonal to multi-decadal timescales. *Estuarine, Coastal and Shelf Science* **90**,

936   264–274.doi:10.1016/j.ecss.2010.09.007.

937 Wedderburn, S. D., Walker, K. F. & Zampatti, B. P. (2008). Salinity may cause fragmentation of

938   hardyhead (Teleostei: Atherinidae) populations in the River Murray, Australia. *Marine and*

939   *Freshwater Research* **59**, 254–258.doi:10.1071/MF07205.

940 Whitfield, A. K., Taylor, R. H., Fox, C. & Cyrus, D. P. (2006). Fishes and salinities in the St Lucia

941   estuarine system—a review. *Reviews in Fish Biology and Fisheries* **16**, 1–

942   20.doi:10.1007/s11160-006-0003-x.

943 Whittaker, R. H. (1952). A study of summer foliage insect communities in the Great Smoky

944   Mountains. *Ecological Monographs* **22**, 1–44.

945 Whittaker, R. H. & Likens, G. E. (1975). *The biosphere and man*. In Primary Production of the

946   Biosphere (Lieth, H. & Whittaker, G. E., eds), pp. 305–328, New York: Springer-Verlag.

947 Wise, B., Potter, I. & Wallace, J. (1994). Growth, movements and diet of the terapontid *Amniataba*

948   *caudivittata* in an Australian estuary. *Journal of Fish Biology* **45**, 917–931.

949   Wolanski, E. (2007). *Estuarine Ecohydrology*. Netherlands: Elsevier.

950   Wurm, P. A. S. & Semeniuk, V. (2000). Leschenault Inlet estuary-its physical features and habitats

951   for benthic fauna. *Journal of the Royal Society of Western Australia* **83**, 229–250.

952   Young, G. C. & Potter, I. C. (2002). Influence of exceptionally high salinities, marked variations in

953   freshwater discharge and opening of estuary mouth on the 185 characteristics of the

954   ichthyofauna of a normally-closed estuary. *Estuarine Coastal and Shelf Science* **55**, 223–246.

955   Young, G. C. & Potter, I. C. (2003). Influence of an artificial entrance channel on the ichthyofauna

956   of a large estuary. *Marine Biology* **142**, 1181–1194.doi:10.1007/s00227-003-1012-0.

957   Zampatti, B. P., Bice, C. M. & Jennings, P. R. (2010). Temporal variability in fish assemblage

958   structure and recruitment in a freshwater-deprived estuary: The Coorong, Australia. *Marine*

959   *and Freshwater Research* **61**, 1298–1312.doi:10.1071/MF10024.

960

961   **Electronic References**

962

963   Brookes, J. D., Lamontagne, S., Aldridge, K. T., Benger. S., Bissett, A., Bucater, L., Cheshire, A.

964   C., Cook, P. L. M., Deegan, B. M., Dittmann, S., Fairweather, P. G., Fernandes, M. B., Ford,

965   P. W., Geddes, M. C., Gillanders, B. M. , Grigg, N.J., Haese, R. R., Krull, E., Langley, R. A.,

966   Lester, R. E., Loo, M., Munro, A. R., Noell, C. J., Nayar, S., Paton, D. C., Revill, A. T.,

967   Rogers, D. J., Rolston, A., Sharma. S. K., Short, D. A., Tanner, J. E., Webster, I. T., Wellman,

968   N. R. & Ye, Q. (2009). *An ecosystem assessment framework to guide management of the*

969   *Coorong*. Canberra: Commonwealth Scientific and Industrial Research Organisation

970   Publishing. Available at

971   http://www.clw.csiro.au/publications/waterforahealthycountry/cllamm/CLLAMM-Final-

972   Report-Ecosystem-Assessment.pdf

973   Gillibrand, P. A., Andrewartha, J. R. & Herzfeld, M. (2012). *Numerical hydrodynamic modelling of*

974   *the Leschenault Estuary,* Commonwealth Scientific and Industrial Research Organisation,

975   Australia.   October   2012.   Available   at

976   http://www.emg.cmar.csiro.au/www/dms/software.shoc/Leschenault_Final_Report_Oct2012.

977   pdf.

978   Hugues-dit-Ciles, J., Kelsey, P., Marillier, B., Robb, M., Forbes, V. & McKenna, M. (2012).

979   *Leschenault Estuary water quality improvement plan,* Department of Water, Western

980   Australia,   Australia.   October   2012.   Available   at

981   http://www.water.wa.gov.au/PublicationStore/first/103743.pdf.

982   Kilminster, K. L. (2010). *Sediment quality in three south-western Australian estuaries.* Water

983   Science technical series, Report No. 18, Department of Water, Western Australia, Australia.

984   January 2010. Available at http://www.water.wa.gov.au/PublicationStore/first/91777.pdf.

985   Lough J. M., Gutpa A. S. & Hobday A. J. (2012) *Temperature*. In Marine Climate Change in

986   Australia: Impacts and Adaptation Responses. 2012 Report Card. (Poloczanska, E. S. &

987   Hobday, A. J., eds), pp 402, Collingwood: Commonwealth Scientific and Industrial Research

988   Organisation   Publishing.   Available   at

989   http://www.oceanclimatechange.org.au/content/images/uploads/Marine_Report_Card_Austral

990   ia_2012.pdf.

991   McKenna, M. (2007). *The Leschenault estuarine system, south-western Australia- condition*

992   *statement and recommendations for management*. Department of Water, Government of

993   Western   Australia,   Australia.   June   2007.   Available   at

994   http://www.water.wa.gov.au/PublicationStore/first/76604.pdf.

995   Pearce, A., Lenanton, R., Jackson, G., Moore, J., Feng, M. & Gaughan, D. (2011). *The "marine*

996   *heat wave" off Western Australia during the summer of 2010/11*. Fisheries Research Report

997   No. 222, Department of Fisheries, Western Australia, Australia. Available at

998   http://www.fish.wa.gov.au/Documents/research_reports/frr222.pdf.

999 Valesini, F. J., Coen, N. J., Wildsmith, M. D., Hourston, M., Tweedley, J. R., Hallett, C. S., Linke,

1000   T. E., Potter, I. C. (2009). *Relationships between fish faunas and habitat type in south-western*

1001   *Australian estuaries. Fisheries Research and Development Corporation Final Report, Project*

1002   *2004/045.* Murdoch University, Perth, Western Australia, Australia. Available at

1003   http://frdc.com.au/research/Documents/Final_reports/2004-045-DLD.pdf.

1004 Valesini, F. J., Hallett, C. S., Cottingham, A., Hesp, S. A., Hoeksema, S. D., Hall, N. G., Linke, T.

1005   E. & Buckland, A. J. (2011). *Development of biotic indices for establishing and monitoring*

1006   *ecosystem health of the Swan-Canning Estuary. Final Technical Report.* Department of Water

1007   and Department of Fisheries, Perth, Western Australia, Australia. Available at

1008   http://www.swanrivertrust.wa.gov.au/docs/technical-reports/development-of-biotic-indices-

1009   for-establishing-and-monitoring-ecosystem-health-of-the-swan-canning-estuary.pdf.

1010 Veale, L. J. (2013). *Inter-period comparisons of the ichthyofaunas of two nearby, modified*

1011   *estuaries and the biology of* Pelates octolineatus *(Terapontidae)*. PhD Thesis, Murdoch

1012   University, Western Australia, Australia. Available at

1013   http://researchrepository.murdoch.edu.au/18716/2/02Whole.pdf.

Table

Table I. Life-cycle guilds (LC), rankings by abundance (*R*), mean densities (*D;* numbers of fish 100 m^{-2}) and percentage contributions to the total catch (*%*) of the fish species caught between the Austral winter (July) of 2008 and the Austral autumn (April) of 2010 in all four regions of the Leschenault Estuary collectively, and of each region individually. Numbers of fish species and overall mean density (number of fish 100 m^{-2}) are also given. In this and subsequent tables, life-cycle guilds are abbreviated as follows: E, solely estuarine; E&M, estuarine & marine; E&F, estuarine and freshwater; MEO, marine estuarine-opportunist and MS, marine straggler. The ten most abundant species in the whole estuary and in each of the four regions individually are highlighted in bold.

Species Name	LC	Whole estuary			Base			Lower			Upper			Apex		
		R	*D*	*%*	*R*	*D*	*%*	*R*	*D*	*%*	*R*	*D*	*%*	*R*	*D*	*%*
Atherinosoma elongata	E	**1**	**64.0**	**34.0**	6	6.1	4.0	**1**	**67.8**	**23.4**	**1**	**74.8**	**49.9**	**1**	**107.2**	**67.0**
Craterocephalus mugiloides	E	**2**	**24.0**	**12.7**	8	3.1	2.0	3	39.9	13.8	**2**	**27.1**	**18.1**	**2**	**25.8**	**16.1**
Hyperlophus vittatus	MEO	**3**	**21.4**	**11.4**	**2**	**40.4**	**26.4**	2	45.2	15.6						
Aldrichetta forsteri	MEO	**4**	**15.7**	**8.4**	**1**	**45.6**	**29.8**	7	7.5	2.6	7	4.3	2.9	**4**	**5.5**	**3.5**
Leptatherina presbyteroides	E&M	**5**	**15.0**	**8.0**	**3**	**20.2**	**13.2**	4	33.4	11.5	6	4.7	3.1	7	1.6	1.0
Favonigobius lateralis	E&M	**6**	**12.8**	**6.8**	**4**	**14.2**	**9.3**	6	27.9	9.6	4	8.2	5.5	8	0.9	0.5
Atherinomorus vaigiensis	MEO	**7**	**9.1**	**4.8**	**5**	**6.5**	**4.3**	12	3.3	1.1	**3**	**15.0**	**10.0**	**3**	**11.5**	**7.2**
Ostorhinchus rueppellii	E&M	**8**	**7.9**	**4.2**	18	0.1	0.1	**5**	**30.9**	**10.7**	12	0.7	0.4			
Sillaginodes punctatus	MEO	**9**	**3.3**	**1.7**	**9**	**3.0**	**2.0**	8	7.2	2.5	8	2.8	1.8	11	0.1	0.1
Mugil cephalus	MEO	**10**	**3.0**	**1.6**	15	0.6	0.4	15	1.0	0.3	**5**	**7.5**	**5.0**	**6**	**2.8**	**1.8**
Torquigener pleurogramma	MEO	11	2.7	1.4	**7**	**4.2**	**2.8**	11	5.3	1.8	**10**	**1.0**	**0.6**	10	0.2	0.1
Leptatherina wallacei	E&F	12	2.3	1.2	12	1.2	0.8	**10**	**6.0**	**2.1**	9	1.6	1.1	9	0.4	0.3
Gymnapistes marmoratus	MEO	13	2.1	1.1	11	1.5	1.0	**9**	**6.2**	**2.1**	11	0.7	0.5			
Sillago schomburgkii	MEO	14	1.1	0.6	17	0.3	0.2	26	0.1	<0.1	14	0.3	0.2	**5**	**3.7**	**2.3**
Haletta semifasciata	MEO	15	0.6	0.3				13	2.6	0.9						
Arripis truttaceus	MEO	16	0.6	0.3	**10**	**2.5**	**1.7**									
Pseudogobius olorum	E&F	17	0.6	0.3	14	0.9	0.6	14	1.3	0.4	16	0.2	0.1			
Ammotretis elongatus	MS	18	0.4	0.2	13	1.1	0.7	18.5	0.4	0.1						
Stigmatopora argus	MS	19	0.3	0.1	20.5	<0.1	0.1	16	0.9	0.3	21	<0.1	<0.1			
Sillago burrus	MEO	20.5	0.2	0.1	30.5	<0.1	<0.1	20.5	0.2	0.1	13	0.5	0.3	12	<0.1	0.1
Gerres subfasciatus	MEO	20.5	0.2	0.1	25.5	<0.1	<0.1	17	0.7	0.2	18	<0.1	0.1			
Scobinichthys granulatus	MS	22	0.2	0.1	16	0.5	0.3	22	0.2	0.1						
Rhabdosargus sarba	MEO	23	0.1	0.1	25.5	<0.1	<0.1	18.5	0.4	0.1						
Contusus brevicaudus	MEO	24	0.1	0.1	25.5	<0.1	<0.1	24	0.2	0.1	17	0.2	0.1			
Pomatomus saltatrix	MEO	25	<0.1	<0.1	25.5	<0.1	<0.1	20.5	0.2	0.1						
Amniataba caudavittata	E	27	<0.1	<0.1							15	0.2	0.1			
Pelates octolineatus	MEO	27	<0.1	<0.1				24	0.2	0.1	19	<0.1	<0.1			
Urocampus carinirostris	E&M	27	<0.1	<0.1	25.5	<0.1	<0.1	24	0.2	0.1						
Pseudorhombus jenynsii	MEO	29	<0.1	<0.1	20.5	<0.1	0.1	28.5	<0.1	<0.1	21	<0.1	<0.1			
Siphamia cephalotes	E&M	30	<0.1	<0.1				27	0.1	<0.1						
Afurcagobius suppositus	E	32	<0.1	<0.1				28.5	<0.1	<0.1						
Cristiceps australis	MS	32	<0.1	<0.1	20.5	<0.1	0.1									
Lesueurina platycephala	MEO	32	<0.1	<0.1	20.5	<0.1	0.1									
Meuschenia freycineti	MEO	35	<0.1	<0.1				30	<0.1	<0.1						
Arenigobius bifrenatus	E&M	35	<0.1	<0.1	30.5	<0.1	<0.1	33	<0.1	<0.1						
Microcanthus strigatus	MS	35	<0.1	<0.1	25.5	<0.1	<0.1									
Arripis georgianus	MEO	40	<0.1	<0.1							21	<0.1	<0.1			
Callogobius depressus	E&M	40	<0.1	<0.1				33	<0.1	<0.1						
Ophisurus serpens	MEO	40	<0.1	<0.1				33	<0.1	<0.1						
Parablennius postoculomaculatus	MS	40	<0.1	<0.1				33	<0.1	<0.1						
Platycephalus laevigatus	MS	40	<0.1	<0.1				33	<0.1	<0.1						
Enoplosus armatus	MS	40	<0.1	<0.1	30.5	<0.1	<0.1									
Spratelloides robustus	MS	40	<0.1	<0.1	30.5	<0.1	<0.1									
Number of samples			124			31			31			31			31	
Number of species			43			32			35			22			12	
Number of fish			27044			5500			10407			5388			5749	
Mean number of fish 100 m^{-2}			188			153			289			150			160	

Table II. Global and pairwise *R*-statistic values and significance levels (*P*) for one-way ANOSIM tests for region, employing separate Bray-Curtis similarity matrices constructed from the fish abundances in each season. Significant pairwise comparisons are highlighted in grey.

(a) Winter *R* = 0.122, *P* = 0.033	Apex	Upper	Lower
Upper	0.143		
Lower	0.230	0.139	
Base	0.089	-0.070	0.282

(b) Spring, *R* = 0.560, *P* = 0.001	Apex	Upper	Lower
Upper	0.245		
Lower	0.941	0.609	
Base	0.778	0.504	0.278

(c) Summer, *R* = 0.455, *P* = 0.001	Apex	Upper	Middle
Upper	-0.012		
Lower	0.633	0.446	
Base	0.888	0.697	0.137

(d) Autumn, *R* = 0.369, *P* = 0.001	Apex	Upper	Lower
Upper	0.090		
Lower	0.420	0.340	
Base	0.648	0.674	0.199

Table III. Life-cycle guilds (LC), rankings by abundance (*R*), mean densities (*D*; number of fish 100 m^{-2}) and percentage contributions to the total catch (*%*) of the fish species caught in the base and lower regions of the Leschenault Estuary in each season in 1994 and over two consecutive 'years' between winter 2008 and autumn 2010. Numbers of fish species and overall mean density (number of fish 100 m^{-2}) in each period are also given. The eleven most abundant species in each period are highlighted in bold.

Species name	LC	1994			2008-10		
		R	*D*	*%*	*R*	*D*	*%*
Atherinosoma elongata	E	**4**	**10.8**	**4.1**	**1**	**46.2**	**19.1**
Hyperlophus vittatus	MEO	**2**	**85.1**	**32.4**	**2**	**38.9**	**16.0**
Aldrichetta forsteri	MEO	**5**	**4.4**	**1.7**	**3**	**30.8**	**12.7**
Leptatherina presbyteroides	E&M	**3**	**41.4**	**15.8**	**4**	**30.7**	**12.7**
Craterocephalus mugiloides	E				**5**	**25.4**	**10.5**
Favonigobius lateralis	E&M	**1**	**95.8**	**36.5**	**6**	**19.7**	**8.1**
Ostorhinchus rueppellii	E&M	**9**	**2.8**	**1.1**	**7**	**17.6**	**7.3**
Sillaginodes punctatus	MEO	**7**	**4.0**	**1.5**	**8**	**5.4**	**2.2**
Atherinomorus vaigiensis	MEO				**9**	**5.4**	**2.2**
Torquigener pleurogramma	MEO	12	1.4	0.5	**10**	**4.8**	**2.0**
Leptatherina wallacei	E&F	**8**	**2.9**	**1.1**	**11**	**4.6**	**1.9**
Gymnapistes marmoratus	MEO	14.5	0.8	0.3	12	4.3	1.8
Arripis truttaceus	MEO				13	1.6	0.7
Haletta semifasciata	MEO	19	0.2	0.1	14	1.5	0.6
Pseudogobius olorum	E&F	**10**	**2.6**	**1.0**	15	1.3	0.5
Mugil cephalus	MEO	**11**	**2.2**	**0.9**	16	1.0	0.4
Ammotretis elongates	MS	28	<0.1	<0.1	17	0.8	0.3
Stigmatopora argus	MS	13	0.8	0.3	18	0.4	0.2
Sillago schomburgkii	MEO	24	<0.1	<0.1	19	0.3	0.1
Sillago burrus	MEO	16	0.4	0.2	20.5	0.3	0.1
Scobinichthys granulatus	MS	26	<0.1	<0.1	20.5	0.3	0.1
Rhabdosargus sarba	MEO	21	0.1	<0.1	22	0.2	0.1
Urocampus carinirostris	E&M	17	0.4	0.1	23	0.1	<0.1
Pelates octolineatus	MEO	22	0.1	<0.1	24	<0.1	<0.1
Pseudorhombus jenynsii	MEO	24	<0.1	<0.1	25	<0.1	<0.1
Afurcagobius suppositus	E	**6**	**4.1**	**1.6**	27	<0.1	<0.1
Contusus brevicaudus	MEO	14.5	0.8	0.3	27	<0.1	<0.1
Siphamia cephalotes	E&M	18	0.3	0.1	27	<0.1	<0.1
Pomatomus saltatrix	MEO	31.5	<0.1	<0.1	30	<0.1	<0.1
Gerres subfasciatus	MEO				30	<0.1	<0.1
Meuschenia freycineti	MEO				30	<0.1	<0.1
Spratelloides robustus	MS	24	<0.1	<0.1	34	<0.1	<0.1
Arenigobius bifrenatus	E&M	31.5	<0.1	<0.1	34	<0.1	<0.1
Platycephalus laevigatus	MS				34	<0.1	<0.1
Callogobius depressus	E&M				34	<0.1	<0.1
Ophisurus serpens	MEO				34	<0.1	<0.1
Enoplosus armatus	MS	20	0.2	0.1			
Sillago bassensis	MS	28	<0.1	<0.1			
Callogobius mucosus	MEO	28	<0.1	<0.1			
Notolabrus parilus	MS	31.5	<0.1	<0.1			
Cristiceps australis	MS	31.5	<0.1	<0.1			
Number of samples			48			48	
Number of species			33			36	
Number of fish			14601			13483	
Mean number of fish 100 m^{-2}			262			242	

List of Figures

Figure 1. Map showing the base, lower, upper and apex regions of the Leschenault Estuary that were sampled using a 21.5 m seine net in each consecutive season between the Austral winter of 2008 and Austral autumn of 2010. Arrow in inset shows location of the Leschenault Estuary in south-western Australia.

Figure 2. Mean seasonal values for (a) salinity, (b) water temperature, (c) numbers of species and (d) densities of fishes in the base (◇), lower (○), upper (▲) and apex (■) regions of the Leschenault Estuary between the Austral winter of 2008 and Austral autumn of 2010. Overall mean ± 95 % confidence intervals are presented for each variable (back-transformed for the numbers and densities of fish). The horizontal line in (a) denotes full strength sea water, *i.e.* 35.

Figure 3. Numbers of species representing the marine (▲) and estuarine (△) categories and the percentage contributions of the number of individuals of the marine (■) and estuarine (□) categories (shown as stacked bar graphs) in each region of the Leschenault Estuary; (a) throughout the year and (b-e) in each season. B, base region; L, lower region; U, upper region; A, apex region.

Figure 4. nMDS ordination plots, constructed from separate Bray-Curtis similarity matrices derived from pre-treated (see text) and then meaned abundances recorded for each fish species in: (a) each season (averaged over years and sites) in the base (◆), lower (●), upper (▲) and apex (□) regions of the Leschenault Estuary; (c) each region (averaged over seasons and sites) in 2008/09 (◆) and 2009/10 (◆); and (e) each season (averaged over regions and sites) in 2008/09 (◆) and 2009/10 (◆). The nMDS stress function in (c) is mixed with a trace amount (5%) of metric MDS (mMDS) stress to avoid a degenerate solution for this small number of points. W, winter; Sp, spring; S,

summer; A, autumn. Arrows show seasonal cycling or trend up the estuary. (b) and (d) Bubble plots of mean salinities for the relevant samples are superimposed on the nMDS ordinations in (a) and (c) respectively.

Figure 5. Line plots for coherent groups of species (I-VI) identified by Type 3 SIMPROF tests, showing, for each species, the percentage contributions to its total abundance (untransformed) across the 16 combinations of four seasons (W, Sp, S, A) over four regions (base, lower, upper and apex) of the Leschenault Estuary. The species retained for the SIMPROF tests all accounted for more than 0.1% of the total abundance in at least one of the 16 (averaged) samples. This resulted in 13 coherent groups; the omitted plots are typically of singleton groups and all involve few occurrences and low total abundance (all species with >100 individuals are present in one of the displayed groups).

Figure 6. (a) nMDS ordination plot constructed from the Bray-Curtis similarity matrix derived from the (pre-treated) mean abundances of each fish species in each season (averaged for regions) in the Leschenault Estuary in 1994 (□), 2008/09 (●) and 2009/10 (◆). Arrows show direction of seasonal cycling. (b) Segmented bubble plot, superimposed on the ordination in (a), with segment sizes, on a common scale, proportional to the means of the (pre-treated) abundances for each of 6 species, identified by a two-way SIMPER analysis (removing seasonal effects) as primarily contributing to the difference between the 1994 and 2008-2010 periods. *Favonigobius lateralis* (🟨), *Craterocephalus mugiloides* (🟦), *Aldrichetta forsteri* (🟪), *Atherinomorus vaigiensis* (🟥), *Atherinosoma elongata* (🟦) and *Torquigener pleurogramma* (🟩). (c) Bubble plot as in (b), with segment sizes on the same common scale, but for 6 species identified by two-way SIMPER (removing period effects) as primarily contributing to the observed seasonal changes. *Hyperlophus vittatus* (🟨),*Gymnapistes marmoratus* (🟦),*Sillaginodes punctatus* (🟪), *Pseudogobius olorum* (🟥),*Ostorhinchus rueppellii* (🟦) and *Stigmatopora argus* (🟩).
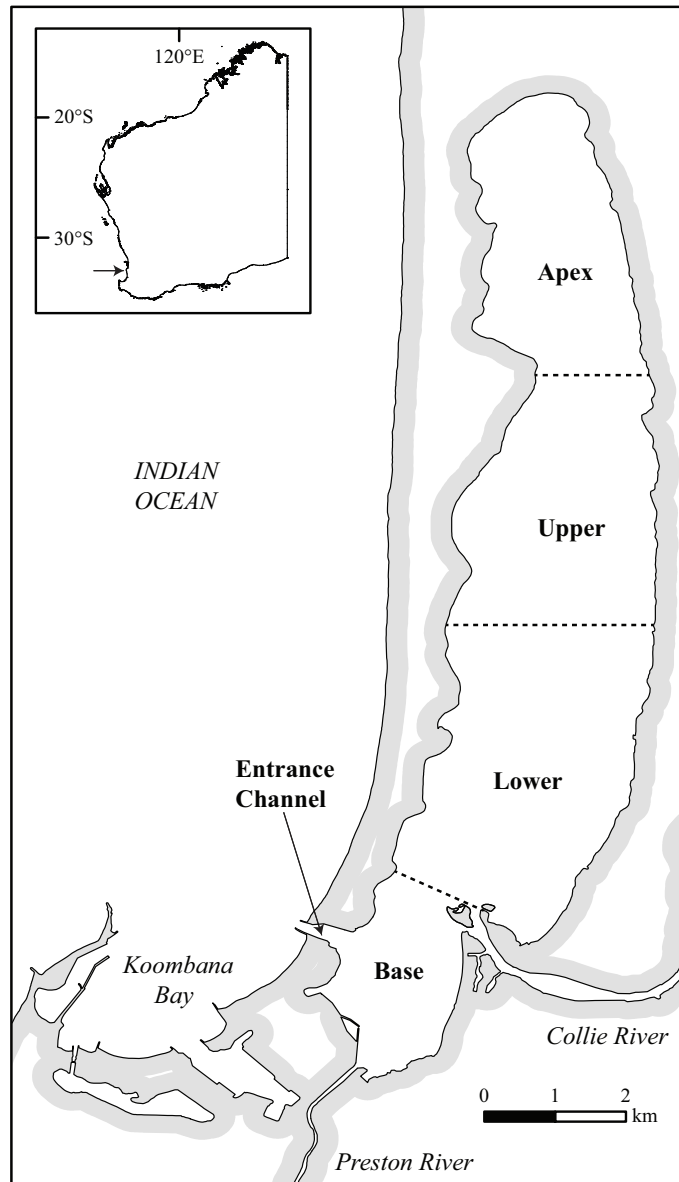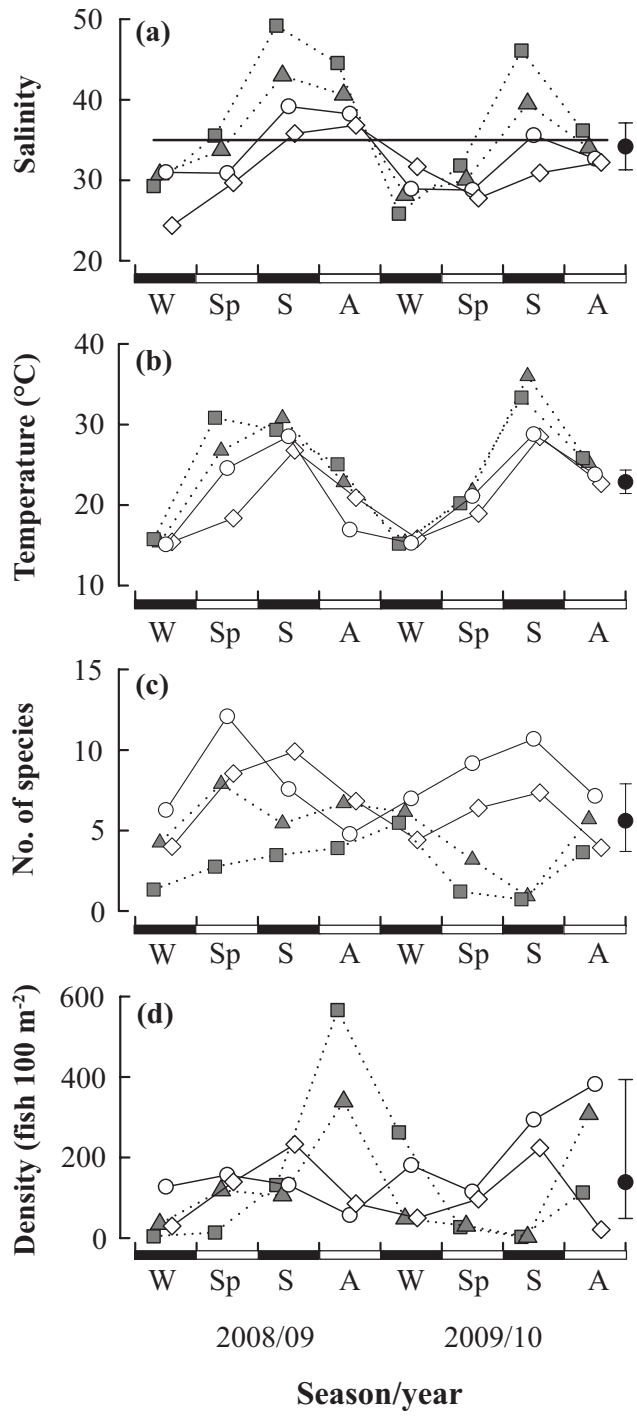
Figure

Indian Ocean map figure showing estuary with labeled regions: Apex, Upper, Lower, Base, Entrance Channel, Koombana Bay, Collie River, Preston River. Inset map shows coordinates 120°E, 20°S, 30°S.

Figure 1.

Figure

Figure 2.

Figure

Figure 3.
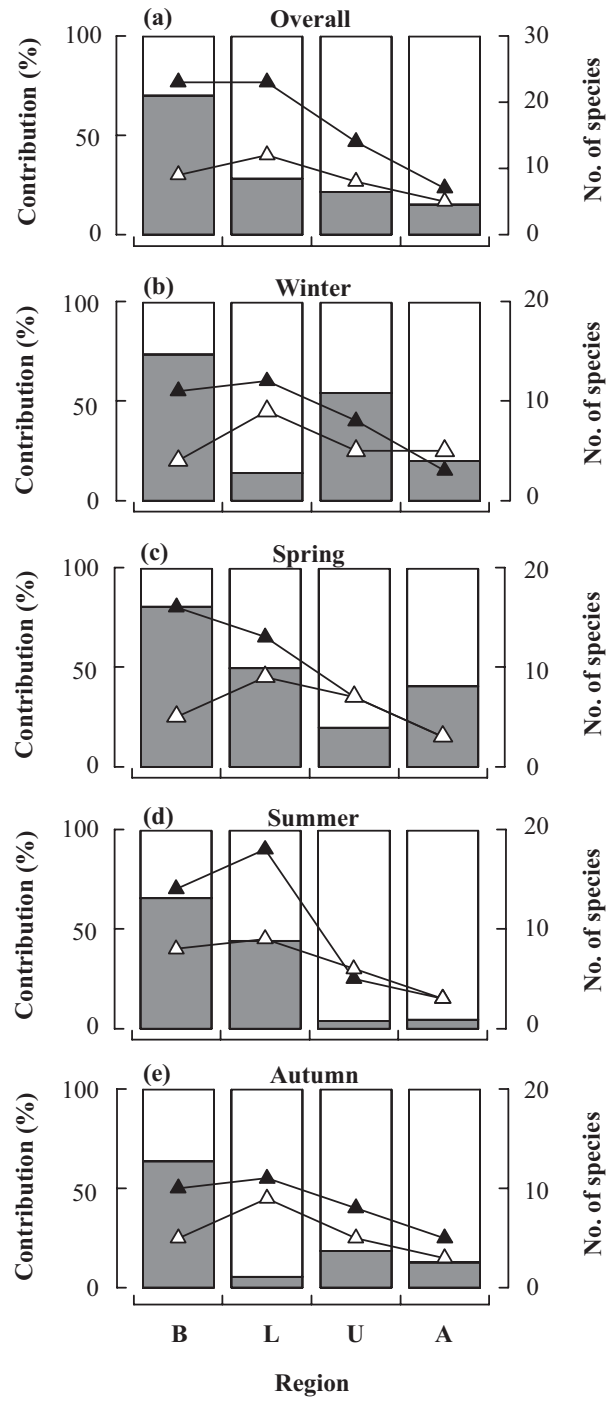
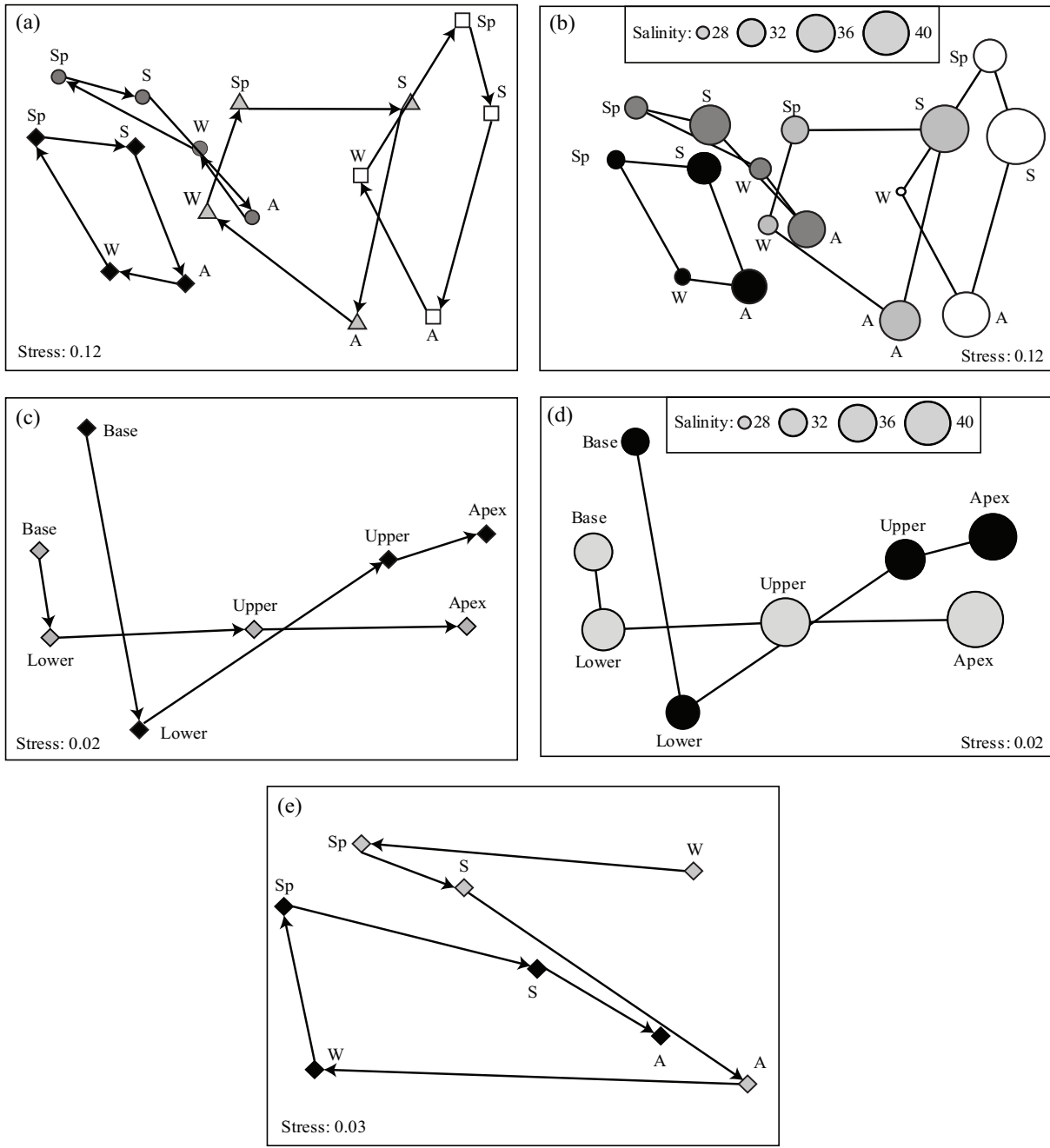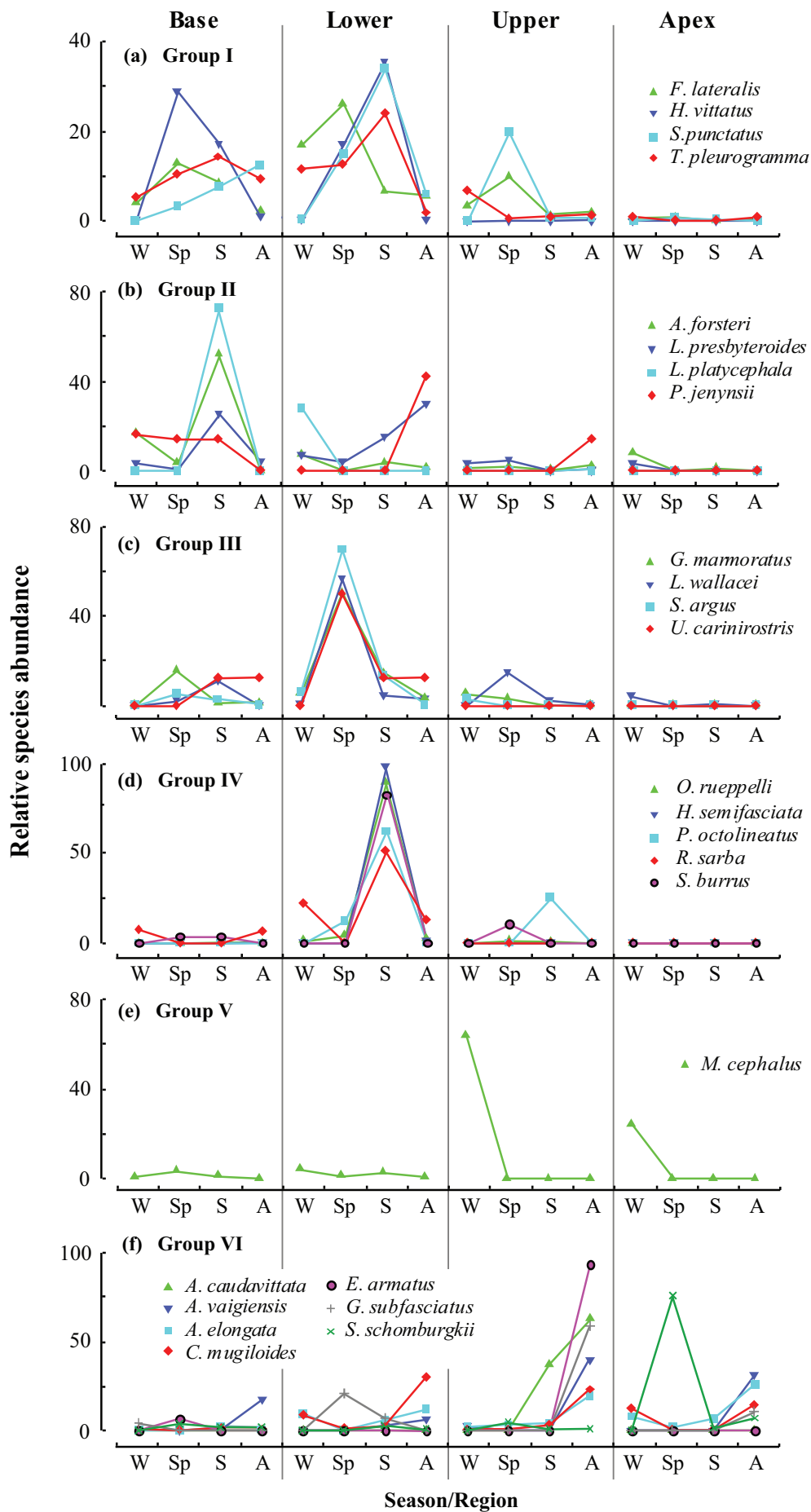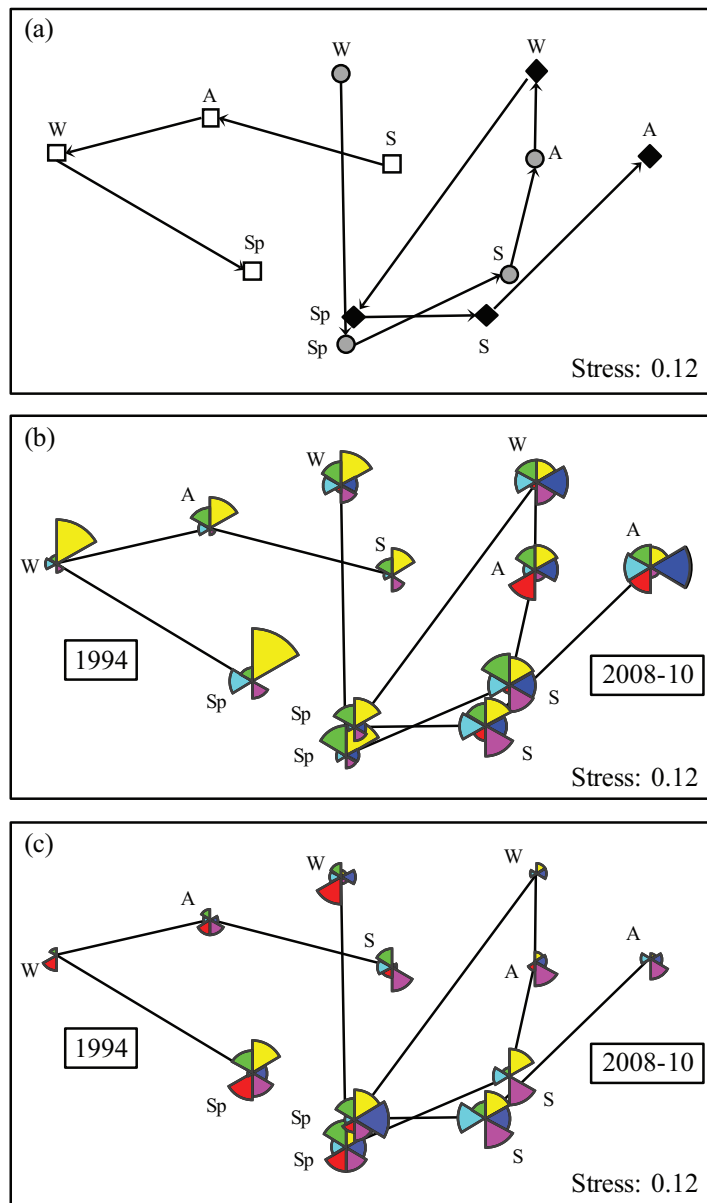Figure

Figure 4.

Figure

Figure 5.

Figure

Figure 6.

Appendix 1. Mean squares (MS), *F* values (F*)* and significance levels (*P*) for three-way PERMANOVA tests employing the Euclidean distance matrices constructed from (a) number of species and (b) mean densities of fishes (numbers 100 m^{-2}) in the base, lower, upper and apex regions of the Leschenault Estuary in each season over the two consecutive years between the Austral winter of 2008 and Austral autumn of 2010. *df* = degrees of freedom. Significant differences (<0.05) are highlighted in bold.

Main Effects	df	(a) Number of species MS	F	P	(b) Density MS	F	P
Year	1	**1.58**	**9.1**	**0.013**	1.56	0.9	0.357
Season	3	0.23	1.1	0.358	**6.72**	**6.4**	**0.002**
Region	3	**8.22**	**20.1**	**<0.001**	**8.70**	**6.2**	**0.008**
Site (Region)	12	**0.41**	**2.8**	**0.009**	1.41	1.9	0.071
Interactions							
Year × Season	3	**1.33**	**9.0**	**<0.001**	**10.28**	**13.9**	**<0.001**
Year × Region	3	0.55	3.2	0.065	3.96	2.3	0.121
Season × Region	9	**1.17**	**5.5**	**<0.001**	**8.03**	**7.7**	**<0.001**
Year × Site (Region)	12	0.17	1.2	0.337	**1.71**	**2.3**	**0.026**
Season × Site (Region)	36	0.21	1.5	0.148	1.06	1.4	0.155
Year × Season × Region	9	**0.60**	**4.0**	**0.001**	**5.64**	**7.6**	**<0.001**
Residuals	32	0.15			0.74		

Appendix 2. Mean squares (MS), Pseudo-F (pF) values, and significance levels (*P*) for four-way PERMANOVA tests on the Bray-Curtis similarity matrices constructed from the (pre-treated) fish abundances recorded in (a) the base, lower, upper and apex regions of the Leschenault Estuary in each season over two consecutive years between the Austral winter of 2008 and Austral autumn of 2010 and (b) those in the base and lower regions in each season in 1994, 2008/09 and 2009/10. *df* = degrees of freedom. Significant results (<0.05) are highlighted in bold. Asterisks indicate some missing cells in the unbalanced design.

(a) Fish compositions in 2008/09 and 2009/10				
Main Effects	***df***	**MS**	**pF**	***P***
Year	1	**4904**	**3.8**	**0.003**
Season	3	**8738**	**8.1**	**<0.001**
Region	3	**12721**	**6.2**	**<0.001**
Site (Region)	12	**2091**	**2.4**	**<0.001**
Interactions				
Year × Season	3	**4000**	**4.7**	**<0.001**
Year × Region	3	**2211**	**1.7**	**0.034**
Season × Region	9	**3186**	**3.0**	**<0.001**
Year × Site (Region)	12	**1283**	**1.5**	**0.003**
Season × Site (Region)	36	**1082**	**1.3**	**0.012**
Year × Season × Region	9	**1833**	**2.1**	**<0.001**
Residuals	32	858		
(b) Fish compositions in 1994, 2008/09 and 2009/10				
Main effects	***df***	**MS**	**pF**	***P***
Year	2	**7843**	**3.5**	**0.001**
Season	3	**6165**	**4.0**	**<0.001**
Region	1	**6892**	**2.5**	**0.036**
Site (Region)	6	2784	1.5	0.049
Interactions				
Year × Season	6	**3026**	**2.4**	**<0.001**
Year × Region	2	2411	1.1	0.362
Season × Region	3	**3051**	**2.0**	**0.006**
Year × Site (Region)*	10	2201	1.2	0.198
Season × Site (Region)	18	1531	0.8	0.881
Year × Season × Region	6	**1752**	**1.4**	**0.034**
Year × Season × Site (Region)*	28	1864	0.7	0.997
Residuals	24	2662		

Appendix 3. Species which, on the basis of one-way SIMPER, typified (shaded) the fish assemblages in each region of the Leschenault Estuary in (a) winter, (b) spring, (c) summer and (d) autumn of 2008-10 and distinguished between each pair of assemblages (un-shaded). The region in which each species was most consistently found and abundant is given in superscript for each pairwise comparison. Asterisks denote the relative consistency of each species in either typifying or distinguishing the faunal compositions in each region, as measured by the similarity to standard deviation ratio and dissimilarity to standard deviation ratio, respectively; 1.0-1.5*, 1.5-2.0**, 2.0-2.5***, >2.5****.

(a) W

(a) W	Apex	Upper	Lower	Base
Apex	A. forsteri L. presbyteroides* A. elongata C. mugiloides			
Upper		F. lateralis*** L. presbyteroides* T. pleurogramma A. elongata		
Lower	F. lateralis^{L*} A. forsteri^{A*} T. pleurogramma^{L}		F. lateralis** C. mugiloides O. rueppellii	
Base			F. lateralis^{B*} C. mugiloides^{B} A. elongata^{B}	F. lateralis* T. pleurogramma

(b) Sp

(b) Sp	Apex	Upper	Lower	Base
Apex	A. elongata			
Upper	F. lateralis^{U*} S. punctatus^{U*} A. elongata^{U} A. forsteri^{U*}	F. lateralis** A. elongata* S. punctatus		
Lower	F. lateralis^{L**} G. marmoratus^{L*} S. argus^{L*} T. pleurogramma^{L*}	F. lateralis^{M} C. mugiloides^{M} A. elongata^{M*}	F. lateralis*** G. marmoratus**** S. argus H. vittatus*	
Base	S. argus^{B*} F. lateralis^{B*} G. marmoratus^{B*} T. pleurogramma^{B*} H. vittatus^{B*}	H. vittatus^{L*} F. lateralis^{L*} S. punctatus^{U} A. elongata^{U} S. granulatus^{B}	S. argus^{L*} F. lateralis^{L*} G. marmoratus^{L*} L. wallacei^{L*}	F. lateralis**** H. vittatus T. pleurogramma

(c) S

(c) S	Apex	Upper	Lower	Base
Apex	A. elongata*			
Upper		A. elongata* C. mugiloides		
Lower	F. lateralis^{L} S. punctatus^{L} O. rueppellii^{L} H. vittatus^{L}	F. lateralis^{L} S. punctatus^{L*} O. rueppellii^{L*} T. pleurogramma^{L} H. vittatus^{L}	F. lateralis* O. rueppellii A. elongata	
Base	F. lateralis^{B**} A. forsteri^{B} H. vittatus^{B} L. presbyteroides^{B*}	A. forsteri^{B} F. lateralis^{B*} H. vittatus^{B*} L. presbyteroides^{B*}		F. lateralis** H. vittatus* A. forsteri S. punctatus

(d) A

(d) A	Apex	Upper	Lower	Base
Apex	A. elongata*** C. mugiloides* A. vaigiensis			
Upper		A. elongata*** C. mugiloides**** A. vaigiensis**** F. lateralis*		
Lower	F. lateralis^{L**} A. elongata^{A**} A. vaigiensis^{A*} C. mugiloides^{L*}	A. elongata^{U****} F. lateralis^{M*} C. mugiloides^{M*}	F. lateralis* C. mugiloides T. pleurogramma	
Base	A. elongata^{A**} C. mugiloides^{A*} T. pleurogramma^{B*} S. punctatus^{B}	A. elongata^{U***} C. mugiloides^{U***} A. vaigiensis^{U**} T. pleurogramma^{B*}	C. mugiloides^{L} A. vaigiensis^{B*} L. presbyteroides^{L} F. lateralis^{L*}	F. lateralis T. pleurogramma A. vaigiensis