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Preliminary insight into the relationship between environmental factors and the nutritional condition and growth of *Gilchristella aestuaria* larvae in the upper reaches of South African estuaries

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Abstract Estuarine systems play a critical role as nursery areas for some fish species. Nursery function, nevertheless, is likely to vary among estuaries, mostly due to differences in habitat quality. High quality nursery habitats are those in which growth and survival of early stages of fish are enhanced. The nutritional condition of larval fishes has vital implications for their mortality and growth, and thereby their recruitment. This study aimed to compare the nutritional condition of *Gichristella aestuaria* larvae, using individual RNA-DNA ratios and growth rates in the upper reaches of six estuaries in South Africa to find the environmental factors that better determine the nutritional condition of fish larvae. Physico-chemical factors as well as calorific value of zooplankton were used to correlate to fish body condition. Results showed that the larvae of *G. aestuaria* from the freshwater rich Gamtoos and the Sundays estuaries were in better nutritional condition than the larvae from other estuaries, while larvae from the Swartkops Estuary, a highly eutrophic system, presented the worst nutritional condition of all studied larvae. Salinity and the abundance of zooplankton were the major factors determining the nutritional condition of *G. aestuaria* larvae in these warm temperate estuaries. In addition, our results suggest that the match-mismatch hypothesis might also be important in estuarine systems. This study represents one of the few studies worldwide that applied a multispecies growth model for fish larvae in warm temperate estuaries.

Keywords Nursery habitats · Estuarine round-herring · Fish larvae · Food patches · RNA-DNA ratio · Habitat quality

Introduction

Estuarine systems play a critical role in the dynamics of coastal environments and are particularly important for some fish species that are widely tolerant of the high environmental variability that characterizes estuaries (Whitfield 1998; Beck et al. 2001; Able et al. 2006; Vasconcelos et al. 2010). The ecological dynamics of these fish species in southern Africa has been a longstanding focus of research (Whitfield 2010). As a result, southern African estuaries act as essential nursery habitats for several coastal fish species (Whitfield 1999; Strydom et al. 2003; Harrison 2005; Harrison and Whitfield 2012; Strydom 2014). Nursery function, nevertheless, is likely to vary among estuaries, mostly due to differences in habitat quality (Gibson 1994; Vasconcelos et al. 2011).

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High quality nursery habitats are those in which growth and survival of early stages of fish are enhanced (Gibson 1994; Heck et al 2003; Vasconcelos et al. 2010). However, the effects of the major agents determining habitat quality are not well studied in estuarine systems. For example, the availability of food is a critical link to the growth and survival of larval fishes (O'Connell and Raymond 1970; Buckley and Durbin 2006; Baumann et al. 2007). The quality or nutritional value of the available prey also affects the condition of fish larvae (Cruz-Rivera and Hay 2000; Vos et al. 2000) and is often expressed in terms of energetic value or caloric content of the prey (Davis 1993). Other important factors that might influence the quality of a nursery are abiotic traits, particularly temperature, salinity, oxygen and turbidity (Beck et al. 2001; Summers et al. 1995).

The most common approaches used to evaluate nursery quality compare the habitat-specific growth and/or mortality rates of selected fish species (Able et al. 1999; Beck et al. 2001). The study of the condition of early stages of fish has important implications for the prediction of their mortality and growth, and therefore their recruitment success (Houde 2008). Condition indices based on biochemical parameters are generally a very effective method of evaluating the condition status of aquatic animals (Ferron and Leggett 1993; Suthers 1992; Catalán et al. 2007). Amidst the biochemical methods available, the RNA-DNA ratio has been extensively used for the assessment of the nutritional condition of fish larvae in the ocean especially because of its high sensitivity, and because it allows for individual quantifications and responds to changes in feeding levels in a short period (Buckley 1984; Ferron and Leggett 1993; Clemmesen 1994; Catalán et al. 2007; Costalago et al. 2014).

Whilst the concentration of DNA is considered constant in somatic tissues, and is directly related to the numbers of cells of an individual, the amount of RNA is linked to the protein synthesis, and thereby to growth (Catalán et al. 2007). Thus, RNA/DNA serves as a proxy of the cell's protein synthetic capacity and of individual growth (Buckley et al. 1999, 2008). Consequently, nutritional condition of fish larvae, assessed through the analysis of nucleic acids, can be used as an indicator of habitat quality (Amara et al. 2009). In addition, some of the most recent studies of larval fish condition (Chícharo et al. 2012; Grote et al. 2012; Paulsen et al. 2014) relate the RNA-DNA ratio with individual growth rates and environmental temperature through a multispecies larval fish growth model (Buckley et al. 2008). This approach allows for comparisons of larval fish RNA-DNA ratio among habitat types at different temperatures.

Many South African estuarine systems are being increasingly altered by global and regional environmental disturbances, such as declining freshwater inflows, fishing, agricultural and industrial practices, pollution or climate change (Whitfield 1992, 2010). Thus, it is imperative to understand the consequences of these changes for feeding young fishes, particularly changes that affect food productivity (Strydom and Whitfield 2002). Mesohaline zones (salinity ranging 5–18) occurring in the middle and upper reaches of temperate South African estuaries have been suggested as important feeding areas for larval fishes based on zooplankton and larval fish density maxima in these areas (Strydom 2014). Freshwater rich estuaries have prominent mesohaline zones but the implications of richer zooplankton stocks on body condition of fish larvae has not been explored in South Africa. It was therefore necessary to explore the relationship between the value of zooplankton (density and calorific value) and its effect on instantaneous body condition of the larvae in the estuary at that time. Given that estuarine productivity and freshwater supply are positively correlated (Strydom and Whitfield 2002), the abovementioned relationship could only be explored by selecting estuaries ranging in freshwater supply and therefore zooplankton productivity.

The estuarine round-herring, *Gilchristella aestuaria* (Family Clupeidae) was selected as a candidate species to examine the quality of these ecosystems as nursery habitats through the assessment of the nutritional condition of the larvae. This species is an estuary resident species and the most abundant fish in southern African estuaries. It is an important forage fish and plays a key ecological role as mid-trophic species (Harrison 2005). The species occurs all along the coast of southern Africa (Smith 1965) and completes its entire life cycle in estuaries (Haigh and Whitfield 1993), feeding primarily on zooplankton (Coetzee 1982; Strydom et al. 2014). *Gilchristella aestuaria* spawn in the middle reaches of estuaries, with peak larval abundance typically in mesohaline zones of estuaries (Strydom 2014).

The present study thus aimed to compare the nutritional condition and growth rates of *G. aestuaria* larvae in six well studied estuarine systems in South Africa (Harrison 2005; Whitfield 2010; Strydom 2014) using

the RNA/DNA method in order to ascertain which environmental factors best correlate with the nutritional condition of larvae. In addition, this study provides RNA-DNA ratio-based growth rates that allow for comparison of fish larvae from environments with different temperature regimes. This is one of the few studies worldwide that has applied the multispecies growth model to fish larvae at temperatures over 20 °C (Buckley et al. 2008).

Materials and methods

Study sites and field sampling

Samples were collected from known *G. aestuaria* breeding areas in the upper reaches of six permanently open estuaries with varying freshwater supply along the warm temperate coast of South Africa (Fig. 1). The Kromme and Kariega estuaries are classified as freshwater poor estuaries; the Swartkops receives a moderate supply of freshwater and the Gamtoos, Sundays and Great Fish estuaries are classified as freshwater rich (Strydom and Whitfield 2002). Further in-depth information about the physico-chemical and geomorphological characteristics of these systems can be found in Strydom (2014). Samples were collected after dark on six consecutive nights in November 2013. Physico-chemical parameters (temperature, salinity, turbidity and dissolved oxygen) were measured at each station in the upper reaches where larvae were collected. Measurements were recorded from surface to bottom every 0.5 m of depth with a YSI 6600 multi-parameter probe (Strydom and

Whitfield 2002). Averaged values from the stations where fish larvae were found were used for analyses. To provide an estimation of the average depth of the estuaries at the sampling stations where larvae were collected, the last (closest to the bottom) measurements of the probe were taken at 2, 1, 2.5, 2, 2 and 1.5 m in the Kromme, Gamtoos, Swartkops, Sundays, Kariega and Great Fish Estuaries, respectively.

Fish larvae and zooplankton were collected at a similar time in each estuary, to avoid diel variations in RNA contents (Bergeron 1997), from the subsurface waters (<4 m) by means of two modified WP2 plankton nets (570 mm mouth diameter and 0.2 mm mesh aperture) fitted with Kahlsico 005 WA 130 flowmeters. Both nets were lowered simultaneously with two short booms (1.5 m length) fixed on either side of the bow of a single-hulled boat and towed horizontally. Sampling speed was approximately 2 knots and each tow lasted 5–10 min (Strydom et al. 2003). Samples (zooplankton and fish larvae) from one net were preserved in 5 % buffered formalin for quantitative analyses. Zooplankton from the other net were kept on ice until arrival at the laboratory, where it was frozen at −20 °C until calorimetric analysis was performed. *Gilchristella aestuaria* larvae were preserved individually in vials containing RNAlater (Sigma-Aldrich) for nucleic acids analysis. Once in the laboratory, all fish larvae were stored at −80 °C.

Zooplankton analyses

Quantification and identification of major groups of zooplankton were carried out under a Leica M80 stereo
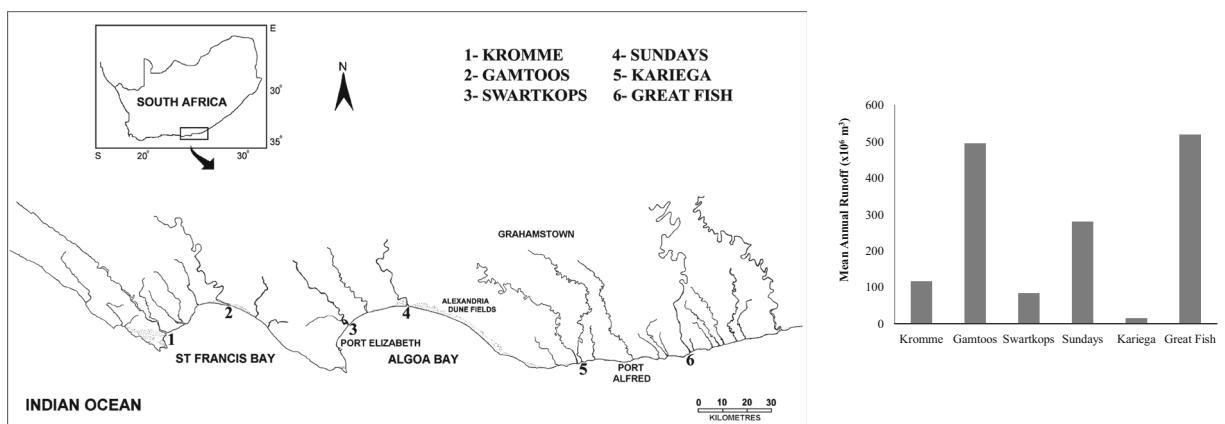

Fig. 1 Locations of the six sampled estuaries in the South East coast of South Africa and bar graph showing the mean annual runoff ($\times 10^6$ m^3) in each estuary to illustrate the differences in freshwater richness

microscope at ×100 magnification. Zooplankton samples were analyzed in three aliquots representing each about 10 % of the total volume and individuals identified to the lowest possible taxonomical level until counting at least 400 copepods and mysids were counted. For the calorimetric analysis, frozen zooplankton samples were thawed and sieved to separate organisms larger than 355 μm, which are not feasible prey for *G. aestuaria* larvae because of their size (Strydom et al. 2014). Copepods were also separated from the samples. Three replicates of zooplankton and copepod samples from each estuary were dried in an oven at 60 °C over 24 h and homogenized using mortar and pestle. Dry weight of replicates ranged from 55 to 131 mg. Calorimetry analyses of dried replicates were performed with a Parr 1425 semi-micro bomb calorimeter. Zooplankton and copepod energy contents are reported as cal/g dry weight.

Nucleic acids extraction

Once thawed, the standard length of 80 larvae from each estuary was measured to the nearest 0.01 mm with a Mitutoyo digital calliper. Individual samples were freeze-dried and then homogenized using the protocol of Caldarone et al. (2001) adapted by Costalago et al. (2014). 1.50 ml of sarcosil Tris-EDTA buffer pH 8.0 (containing 0.1 % of N-lauroylsarcosine sodium salt in 0.05 M Tris, 0.01 M EDTA, and 0.1 M NaCl) and three glass beads (3 mm diameter) were added to each RNase-DNase free vial containing the larvae. After 5–6 min for rehydration of the larvae, vials were vortexed in a balanced box for 10 min, sonicated for 1 min at 4 °C and then vortexed for an additional 20–30 min. After total homogenization, samples were centrifuged in a cooled centrifuge at 4 °C for 20 min at 4000 rpm (12.3 g) in an Eppendorf 5804R centrifuge. Supernatant was collected and stored at −80 °C for nucleic acids quantification.

Nucleic acids quantification

RNA and DNA quantification was completed using the procedure described by Berdalet et al. (2005) and adapted by Costalago et al. (Costalago et al. 2014). SYBR Green II fluorochrome (Lonza) and standards DNA from herring sperm and RNA from baker's yeast (Sigma-Aldrich) were used. Standards were diluted at six different concentrations, ranging from 0.38 to 6.06 μg/ml, to obtain the standard curve. Enzymes

RNase and RNase-free DNase were acquired from Qiagen. Duplicate (50 μL) samples, standards and blank (containing 0.01 % sarcosil Tris-EDTA) were pipetted into black 96-well plates (Greiner Bio-One). Fifty microliter of SYBR Green II were added to each well and the fluorescence (Ex_{492nm}:Em_{520nm}) were measured using a BioTek PowerWave XS microplate reader linked to the Gen5 2.0 Data Analysis software. In a second plate, 7.5 μL RNase per well was added; and 5 μL DNase per well in the third plate. These plates were incubated for 30 min at 37 °C until enzymes degraded nucleic acids completely, then 50 μL of SYBR Green II per well added and fluorescence measured. The amounts of DNA and RNA were calculated, respectively, as the difference between the total nucleic acids in the first plate (without enzymes) and the amount of nucleic acids in the second plate (which contained RNase) and in the third plate (which contained DNase). The RNA-DNA ratios were then standardized (sRD) using this information and the reference slope ratio of 3.9, according to Caldarone et al. (2006).

Growth rate calculation

To minimize possible bias due to the differences in temperature and mean larval size among estuaries, the larval instantaneous growth rates were calculated according to Buckley et al. (2008). The best-fit multispecies growth model that was chosen for further calculation was:

$$G_i = 0.0145 \times sRD + 0.0044 \times (sRD \times T) - 0.078,$$

where G_i is the instantaneous growth rate, sRD the standardized RNA-DNA ratio and T the temperature at the given site. Results have to be interpreted in the way that a value of 0 would mean no growth at all and a value of 1 would be a doubling of the weight of the larva per day.

Statistical analysis

After testing whether data fit to normal distribution with a Shapiro-Wilks test, one-way analysis of variance was performed in order to identify significant differences among estuaries in the zooplankton caloric content and in the standard length of fish larvae, which were the only variables following a normal distribution. Differences in abiotic variables (temperature, salinity, turbidity and oxygen) among estuaries were analysed using a

Kruskal-Wallis test and, when significant (** $p<0.05$), Mann–Whitney U post-hoc was applied on pairs of estuaries, with a new value of significance of $\alpha=$ 0.003. A non-parametric test (Kruskal-Wallis) was applied in order to determine if there were statistically significant differences in the sRD among estuaries. Spearman's rank-order correlation analysis was performed to identify and select relevant correlations between the mean sRD in each estuary and the studied variables (i.e., temperature, salinity, turbidity, oxygen concentration, density of zooplankton, density of copepods, caloric content of zooplankton and density of *G. aestuaria* larvae). All the statistical analyses were performed using the OpenStat free software.

Results

Physical-chemical variables

Mean temperature in the areas of highest density of larval *G. aestuaria* was significantly lower ($p<0.003$) in the Swartkops Estuary (range 20.77–21.56 °C) than in the Sundays Estuary (21.88–24.36 °C) and showed high variability within the Gamtoos Estuary (Fig. 2a). Mean salinity was significantly higher in the Kromme Estuary (30.07–34.83) than in the other estuaries, and also was significantly higher in the Sundays Estuary (11.12–24.24) than in the Kariega (7.92–10.86) and Great Fish (1.47–7.03) estuaries. Variability of salinity in the water column in the Gamtoos Estuary was very high, as with temperature, but not within the other estuaries (Fig. 2b). Mean dissolved oxygen concentration (Fig. 2c) was significantly higher in the Kromme Estuary (7.47–8.25 mg/L) than in the Swartkops Estuary (3.49–8.83 mg/L). Turbidity (Fig. 2d) in the Kromme Estuary (0) was significantly lower than in all the other estuaries, it was also significantly lower in the Swartkops Estuary (1.6–3.9), and the highest significant turbidity was measured in the Great Fish Estuary (42.3–72.9). Other significant differences in the abiotic variables among estuaries were not found (Table 1).

Plankton analyses

Sundays (77878.90 ind/m³) and Kromme estuaries (36764.00 ind/m³) presented the highest densities of zooplankton (Table 1), and also of copepods (38367.99 ind/m³ and 5789.50 ind/m³, respectively)

(Table 1). *Gilchristella aestuaria* larvae were more abundant in the Sundays Estuary (371.11 ind/m³), followed by the Gamtoos Estuary (33.59 ind/m³) and the Kromme Estuary (15.72 ind/m³); lower densities of *G. aestuaria* larvae were found in the Kariega Estuary (10.82 ind/m³), the Swartkops Estuary (4.50 ind/m³) and the Great Fish Estuary (1.56 ind/m³).

Significant differences ($\alpha=0.05$) were identified in the zooplankton caloric content among estuaries (Table 1), with the mean value in the Great Fish Estuary (5628.23 cal/g) being significantly higher than in the Gamtoos (4772.64 cal/g), Swartkops (3549.26 cal/g) and Kariega (1722.14 cal/g) estuaries, while in the Kariega Estuary zooplankton caloric content was significantly lower than in the other estuaries (Table 1).

Nucleic acids quantification and growth rates

Mean standard length of *G. aestuaria* larvae was significantly different ($p<0.05$) among all estuaries except between the Kromme and the Gamtoos estuaries and between the Swartkops and the Kariega estuaries (Table 1).

The sRD was higher in the larvae of the Gamtoos (1.41) and the Sundays (1.44) estuaries than in the other estuaries, with intermediate values in the Kromme (1.29) and Kariega (1.11). The lowest values were recorded in the Swartkops Estuary (0.79) and the Great Fish Estuary (0.97) (Fig. 3, Table 1). There were significant differences in the sRD between the Gamtoos Estuary and the Swartkops and Kariega estuaries (Fig. 3).

The Spearman-ranks correlations did not show significant correlation between the sRD and any of the environmental variables (Table 2) except with larval fish density, for which a positive correlation was found ($r_s=0.943$, $p<0.05$). Among the non-significant correlations, the highest one was the positive correlation between sRD and salinity ($r_s=0.66$) and between oxygen, and copepod and zooplankton densities ($r_s=0.37$) (Table 2).

Larvae from the Gamtoos and the Sundays estuaries had the highest sRD and instantaneous growth rates (ranging from −0.05 to 0.23 d⁻¹ and −0.02 to 0.22 d⁻¹, respectively) and larvae from the Swartkops Estuary presented the lowest sRD instantaneous growth rate ($-0.07 - 0.46$ d⁻¹) (Table 1). Ranges of instantaneous growth rates of larvae from the Kariega, Kromme and Great Fish Estuaries were $-0.04 - 0.26$ d⁻¹, $-0.07 - 0.25$ d⁻¹ and $-0.08 - 0.50$ d⁻¹, respectively.
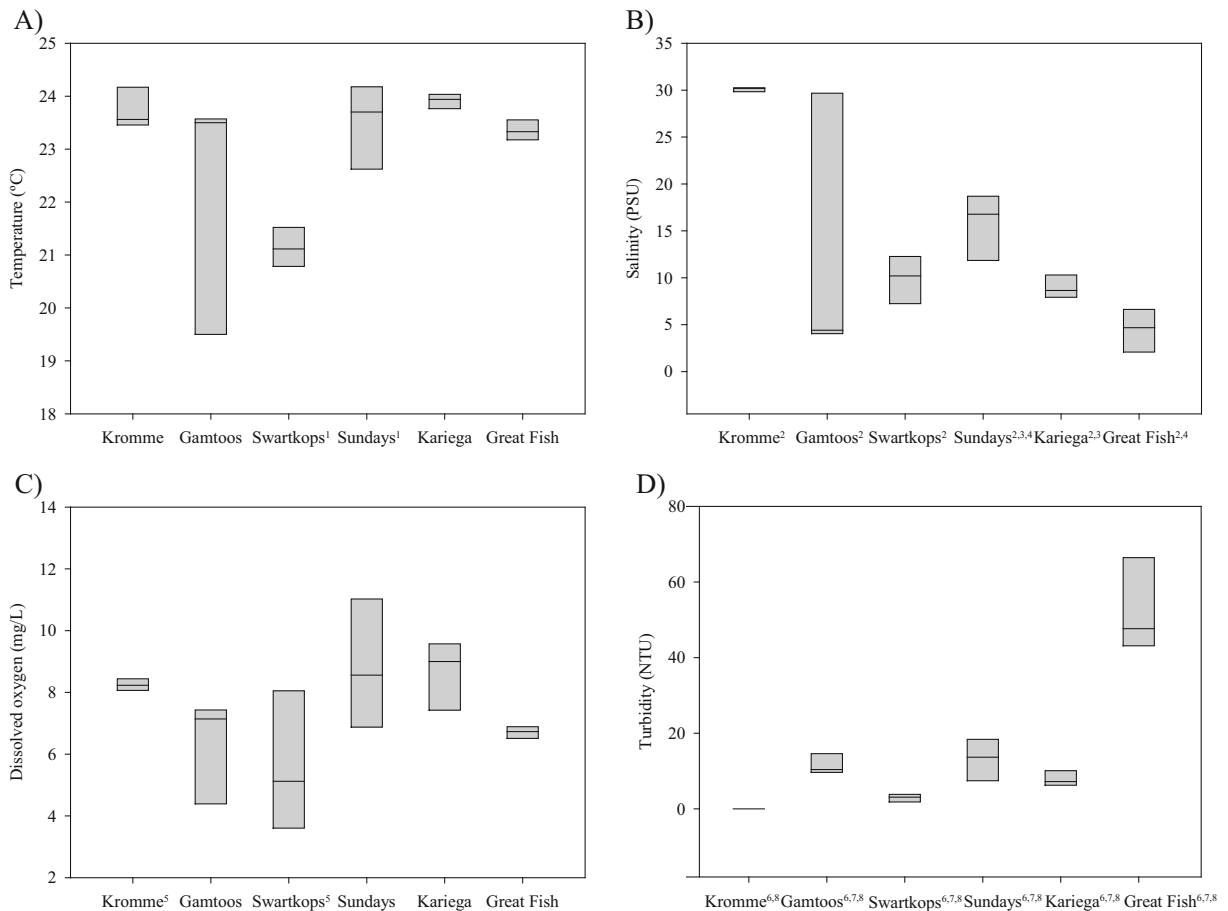
Fig. 2 Box-plots showing the ranges and median of the abiotic parameters: (a) Temperature, (b) Salinity, (c) Dissolved oxygen and (d) Turbidity, in the stations where *Gilchristella aestuaria* larvae were collected in each of the six estuaries. Estuaries sharing a common superscript (1–8) differ significantly (see Table 1)

Discussion

The environmental factors affecting nutritional condition, estimated through the RNA-DNA ratio of *G. aestuaria* larvae, were determined in nursery areas of *G. aestuaria* in the upper reaches of six warm temperate South African estuaries during the peak summer breeding season (Wooldridge and Bailey 1982; Cyrus et al. 1993). The values of the environmental variables measured in the six estuaries during this study were all within the normal ranges for the summer season in these estuaries (Strydom 2014). Concurrently, the values of sRD obtained in this study are also within an acceptable range for the development and survival of *G. aestuaria*, according to the work of Meyer et al. (2012), which shows that, with water temperatures above 20 °C, the survival threshold of sRD (the lowest possible

biochemical condition sustaining life) for the fish larvae is around 0.5, a value that is lower than what we have found for *G. aestuaria* larvae at temperatures of 21–24 °C in our study.

Temperature influences the growth of fish larvae (Buckley 1984; Buckley et al. 2008; Costalago et al. 2011; Strydom et al. 2014), but only extreme temperatures have a direct impact on the mortality of fish. However, some authors (Thiel et al. 1995; Esteves et al. 2000) found significant correlations between water temperature and larval fish abundance in estuaries. Since the temperature in the Kariega Estuary was the highest of all estuaries, but the larval condition in this system was significantly lower than in the Gamtoos Estuary, the relationship between temperature and larval fish condition appears especially complex and constrained by other factors such as food availability.

Table 1 Mean values of standard length, sRD (standardized RNA-DNA ratio) and G_i (instantaneous growth rate) of individual larvae, zooplankton caloric content and of abiotic variables, and total densities of copepods and *Gilchristella aestuaria* larvae measured at each estuary

Estuary	Standard length (mm) **	sRD	G_i (d^{-1})	Zooplankton caloric content (cal/g) **	Zooplankton density (ind/m^3)	Copepods density (ind/m^3)	*G. aestuaria* larvae (ind/m^3)	Temperature (°C) **	Salinity (PSU) **	Dissolved oxygen (mg/L) **	Turbidity (NTU)
Kromme	10.50 (1.39)	1.28 (0.93)	0.075 (0.111)	4799.74 (632.95)[a]	36764.00	35789.75	15.72	23.76 (0.41)	30.07 (0.23)[2]	8.25 (0.20)[5]	0 (0)[6]
Gamtoos	10.90 (1.07)	1.41 (0.62)	0.079 (0.069)	4772.64 (413.01)[b]	1677.61	1087.59	33.59	22.04 (2.09)	14.44 (14.18)	6.23 (1.53)	11.82 (3.09)
Swartkops	13.24 (1.01)	0.79 (0.90)	0.007 (0.097)	3549.26 (350.79)[c]	11633.91	7915.71	4.50	21.14 (0.37)[1]	9.87 (2.51)	5.66 (2.30)	2.90 (0.96)[7]
Sundays	12.09 (1.82)	1.44 (1.34)	0.090 (0.157)	4285.83 (203.10)[d]	77878.90	38370.93	371.11	23.45 (0.92)	16.32 (4.36)	8.65 (2.53)	13.40 (6.17)
Kariega	13.79 (1.12)	1.11 (1.14)	0.055 (0.137)	1722.14 (230.38)	369.05	173.75	10.82	23.91 (0.15)	9.02 (1.27)[3]	8.69 (1.19)	8.14 (2.50)
Great Fish	8.75 (1.12)	0.97 (0.53)	0.035 (0.062)	5628.23 (142.57)[e]	13607.58	13223.85	1.56	23.35 (0.20)	4.46 (2.37)[4]	6.71 (0.20)	52.62 (13.85)[8]

Statistical differences in caloric content were analysed with ANOVA (** $p<0.05$; superscripts a-e). Values in parentheses correspond to standard deviations, and superscripts 1–5 show post-hoc significant relations

** Significant differences in standard length were found between all estuaries except between Kromme-Gamtoos and between Swartkops-Kariega

[1] Sundays; [2] Gamtoos, Swartkops, Sundays, Kariega, Great Fish; [3] Gamtoos, Swartkops, Kariega, Great Fish; [4] Swartkops, Sundays, Kariega, Great Fish; [5] Gamtoos, Swartkops, Sundays, Kariega, Great Fish; [6] Gamtoos, Swartkops, Sundays, Kariega, Great Fish; [7] Gamtoos, Sundays, Kariega, Great Fish; [8] Kromme, Gamtoos, Swartkops, Sundays, Kariega

Therefore, in our study, although a particular range of water temperature might be important for larval fish occurrence in estuaries linked with summer spawning adults (Strydom 2014), when it comes to explaining the nutritional condition and growth of fish larvae, it is likely that coupled factors play a more determinant role.

Salinity in the areas where *G. aestuaria* larvae were collected was higher in the Kromme (29.74–30.2), Gamtoos (3.91–32.37) and Sundays (11.12–24.24) estuaries, where *G. aestuaria* presented the highest sRD and mean growth rates, although the average salinities for the Gamtoos and the Sundays estuaries were within the normal ranges of the highly productive mesohaline zone (5–18), which is where the highest densities of *G. aestuaria* larvae are known to occur (Strydom 2014). Similarly, in the Swartkops and the Great Fish estuaries, with the lowest sRD and growth rates, salinity was comparatively low (ranging 6.61–12.45 and 1.47–7.03, respectively). The correlation analysis has shown a positive correlation ($r_s=0.66$) between salinity and sRD. The correlation is non-significant likely because salinity has limited direct effects on fish growth (Gibson 1994). However, it is one of the main physico-chemical variables structuring fish communities in open estuaries, affecting the distribution of several species (Strydom et al. 2003; Harrison 2005) and influencing productivity in estuaries (Wooldridge and Bailey 1982; Strydom et al. 2003). Salinity is known to be positively correlated with the abundance and biomass of *G. aestuaria* in several South African estuaries (Strydom and Whitfield 2000; Harrison and Whitfield 2006). In this study, the strongest correlations were between sRD and salinity and between sRD and larval abundance. Therefore our results support the hypothesis of salinity as an important regulator of the estuarine ecological structure. Interestingly, the only significant correlation reported in this study was between sRD and larval abundance, suggesting that higher salinity is associated with a higher presence of *G. aestuaria* larvae and that this positively affects the condition of the individual larvae.

Strydom et al. (2002) concluded that larvae of *G. aestuaria* are more likely than juveniles to be flushed down to the lower, more saline reaches of the Great Fish Estuary. Therefore, *G. aestuaria* larvae have difficulty maintaining their position in this highly turbid freshwater rich estuary, and feeding for early larvae may be difficult under strong flow conditions as a result of the inter basin water transfer scheme. Some studies have shown that the patchiness and habitat selection of larval

Fig. 3 Box-plots showing the median, the interquartile, the minimum and maximum values and the outliers (*black dots*) of the sRD of each estuary. **Gamtoos Estuary significantly higher than Swartkops and Kariega Estuaries, *p*<0.05
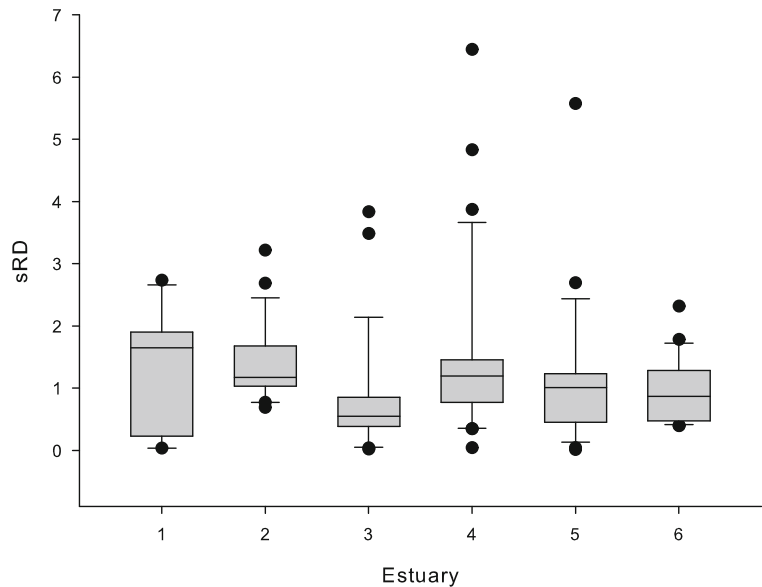
fish are adaptive to assure optimum larval survival (Vlymen 1977; Hewitt 1981; McGurk 1986). If conditions are unfavourable for movement in a highly flushed system like the Great Fish, this may affect feeding opportunities and account for lower body condition.

The values of dissolved oxygen in the Sundays Estuary was among the highest of the six estuaries; however, mean dissolved oxygen did not seem to play a key role in explaining the nutritional condition of larvae, as demonstrated with the Spearman correlation analysis. In line with this, Able et al. (1999) also claimed that low dissolved oxygen was probably not a critical factor for larval fish growth in estuaries, while Gibson (1994) stated that only in highly vegetated and shallow estuaries, or in systems affected by pollution, as is the case in the eutrophic Swartkops Estuary (Lord and Thompson 1998), some fish may suffer oxygen depletion. Although none of the sampled stations presented low levels of dissolved oxygen, it has been reported that fish can easily avoid patches of anoxic areas (Rogers and Lockwood 1990); consequently, even when low oxygen patches are detected, this is not a reason to expect poor nutritional condition in fish.

It is known that larvae of planktivorous fish species rely on their vision for particulate feeding more than the larger individuals due to the lack of well-developed gill rakers –the filtrating apparatus- in the early life stages (Costalago and Palomera 2014; Costalago et al. 2015) Thus, the nutritional condition of planktivorous fish larvae like *G. aestuaria* would likely be also affected by turbidity, partly explaining why the larvae in the Great Fish Estuary, where the turbidity was significantly higher, were in relatively low condition, in spite of being a very rich fresh water estuary. This hypothesis is also in agreement with Peck et al. (2012), who reviewed the factors that affect the fish larval dynamics and claimed that turbidity affects foraging success of fish larvae, reducing food consumption rates at higher turbidity levels.

In terms of food availability, the freshwater rich Sundays Estuary had very high zooplankton densities. This was supported by good condition larval stage *Gilchristella* in this study. Sundays River catchment is extensively used for agriculture, a relatively high input of inorganic nutrients, particularly of nitrate, is received by the estuary (Scharler and Baird 2003). Although the

Table 2 Spearman correlations of the studied variables with sRD (r_s) and level of significance of the correlations (p)

	Copepod density	Zooplankton caloric content	*G.aestuaria* density	Temperature	Salinity	Oxygen	Turbidity
r_s	0.37	0.08	0.94	0.08	0.66	0.37	0.20
p	0.49	0.92	0.02	0.92	0.17	0.49	0.71

extent of the effect of this supply of nutrients on the condition of fish larvae in the Sundays Estuary needs to be further investigated, our results suggest that this input of nitrates might be enhancing the energy flow, through a trophic cascade from plankton to fish.

In South African estuaries, the peak of zooplankton abundance normally occurs between November and February (Wooldridge and Bailey 1982), coinciding with the peak of *G. aestuaria* larvae. This can be interpreted as an adaptation of *G. aestuaria* to spawn at the beginning of the period of highest zooplankton abundance [i.e., in October-November (Blaber 1979; Wooldridge and Bailey 1982; Cyrus et al. 1993)] in order for the larvae to have access to a greater food supply, resulting in a better nutritional condition, thus supporting the match-mismatch hypothesis (Cushing 1990) might also be important in estuarine systems, contrarily to what has been previously thought (Newton 1996). While the density of *G. aestuaria* larvae and the abundance of prey are not correlated, the highest larval densities, in the Gamtoos and, especially, in the Sundays Estuaries, were coinciding with the highest sRD of the larvae. In particular, the larval density in the Sunday Estuary was very high in comparison with what has been found in other studies (Whitfield 1998, 1999; Strydom 2014), and seems to imply that the survival of *G. aestuaria* larvae is not density dependent, contrarily to what happens to marine species in the ocean, which is a less productive system (Cowan et al. 2000).

Food consumption in *G. aestuaria* adults has been reported to be highest in October-December in the Sundays Estuary (Whitfield and Harrison 1996) and the Swartkops Estuary (Talbot and Baird 1985). Therefore, parental contributions to the condition of larvae might be playing an important role in these estuaries. However, has been poorly studied in estuarine systems worldwide (Gao and Munch 2013). Some studies in coral reefs (Donelson et al. 2008; Green 2008) and others with reared fish (Clemmesen et al. 2003; Probst et al. 2006) concluded that the size of preflexion larvae was significantly positively related with parental body condition. From these studies, it can be suggested that good condition in adult estuarine planktivorous fish might translate into faster growth and higher survival rates of larvae. Although this seems to be the case in the studied estuaries, further research on the adult condition should be performed.

The caloric content of the zooplankton in the studied estuaries did not contribute to the condition of the fish larvae. The Kromme Estuary, where *G. aestuaria* larvae had the highest sRD and growth rates, was characterised by an elevated value of zooplankton caloric content. On the other hand, the Great Fish Estuary had a significantly higher zooplankton caloric content than any other estuary, but the larvae in this estuary were in relatively poor condition. Copepods are known to be an important source of calories for their predators in comparison with other types of potential prey (Salonen et al. 1976; Davis 1993).

The Sundays Estuary also had *G. aestuaria* larvae with high sRD and, although the zooplankton caloric content was not as high as in the Kromme or the Great Fish estuaries, likely due to the smaller proportion of copepods in the Sundays Estuary, the total density of the zooplankton was more than twice that of the Kromme Estuary, supporting the hypothesis that food availability plays a very important role in determining the condition of fish larvae in estuaries.

In the Swartkops Estuary, where an important input of nutrients derived from sewage and agricultural activities also takes place, this additional supply of nitrates is accompanied by a variety of pollutants and inorganic dissolved nutrients from the highly industrialized and urbanized catchment area, thus, compromising the water quality in the system (Lord and Thompson 1998) and negatively affecting the condition of the fish larvae. Moreover, this estuary, as explained above, had a very low density of *G. aestuaria* larvae. As stated by Whitfield and Elliot (2002), new knowledge on larval fish dynamics in estuaries will be extremely useful for future ecological and environmental health indices, which are, as particularly seen with the Swartkops Estuaries, highly impacted systems that might show clear effects on important fish species like *G. aestuaria*.

In conclusion, larvae of *G. aestuaria* were in better nutritional condition in the freshwater rich Gamtoos and Sundays estuaries than the larvae from other estuaries. Larvae from the Swartkops Estuary presented the worst nutritional condition of all studied larvae. This estuary receives a moderate supply of freshwater and has historically been a good fish nursery area (Strydom et al. 2003), but on-going eutrophication from sewerage spills may be altering water quality. The Great Fish Estuary is characterized by particularly large volumes of fresh water derived via an inter-basin transfer. Strydom et al. (2002) demonstrated that *G. aestuaria* larvae spawned and residing in the upper reaches of a high-flow estuary, like the Great Fish Estuary, are at a much greater risk of

being flushed out to sea as a result of the excessive amount of river. Although freshwater inputs into estuarine systems are vital for the nursery function of South African estuaries (Whitfield 1994), excessive supply, and the high turbulence associated with it, can potentially alter the larval composition and the larval feeding success in the upper reaches.

Food quantity was an important was positively correlated with the nutritional condition of *G. aestuaria* larvae in these warm temperate estuaries. This supports findings from marine studies where the RNA-DNA ratios of fish larvae have also been related to food density (Buckley 1984; Clemmesen 1994; Rooker and Holt 1996; Clemmesen et al. 2003; Catalán et al. 2007). This study thus suggests that *G. aestuaria* larvae can survive and thrive in particularly variable environments, and especially within very wide ranges of salinity (4.46–30.07) and turbidity (0–52.62), inferring that estuarine fish species have remarkable adaptive capabilities (Whitfield 2015). This work is the first of its kind in South Africa and much is still needed to fully understand the drivers of a successful nursery. Food patch dynamics are poorly studied in South Africa and further research is required. Cost of biochemical analyses are however an impediment for larger sample sizes and longer term studies but the use of RNA-DNA ratio derived body condition assessment appears to be a step in the right direction to fully understand food patch dynamics in estuaries and larval fish survival.

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