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Linking environmental flows with the distribution of black bream *Acanthopagrus butcheri* eggs, larvae and prey in a drought affected estuary

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ABSTRACT: Estuaries are under threat from changes in freshwater flows resulting from anthropogenic impacts and climate change, with unknown consequences for estuarine biota. In the past decade, significant rain deficits in south-eastern Australia have coincided with a decrease in commercial catches of black bream *Acanthopagrus butcheri*, an important commercial and recreational fish species that spawn in estuaries. We investigated the temporal and spatial distribution of black bream eggs and larvae, and copepods — preferred larval prey — in relation to the hydrology of the Mitchell River, a drought-stricken tributary of Australia's largest estuarine lagoon system. We collected eggs, larvae, zooplankton and water quality data at multiple depths from 8 sampling sites over 7 fieldtrips from August to December 2008. The hydrology of the Mitchell River during this study was highly complex and influenced by freshwater flow. Spatial coupling between black bream larvae, copepods and the halocline was observed in the upper estuary. Nauplii of the copepod *Gladioferens pectinatus*, an important prey species for larval fish, dominated the zooplankton assemblage (>80%) and larval gut contents. This study demonstrates that freshwater flows and the generation of salinity stratification have a large influence on the size of suitable habitat for larval bream. Drought, water abstraction and climate change could potentially reduce flows to the point where salinity stratification in the estuary is diminished, resulting in declines in replenishment to populations of black bream and possibly other estuarine-dependent fish.

KEY WORDS: Ichthyoplankton · Larvae · Prey · Copepod · Stratification · Zooplankton · Halocline

INTRODUCTION

Productivity of coastal marine ecosystems, and the fisheries they support, depends on freshwater flows (Loneragan & Bunn 1999, Robins et al. 2005, Meynecke et al. 2006, Gillson et al. 2009). The ecological mechanisms linking freshwater flows with year class strength are not well known, but evidence suggests that episodic wind and freshwater flow events influence larval survival and recruitment of estuarine-dependent species by retaining eggs and larvae in an area of increased prey (North & Houde 2001, North et al. 2005). Freshwater flow is an important process resulting in stratification and transport of sediments and nutrients that promote retention and primary productivity, respectively, in estuaries (Cloern et al. 1983). As a consequence, freshwater flows could result in strong 'bottom-up' effects and the timing and extent of flows could influence larval growth and survival.

Estuaries are characterised by a salinity structure that is influenced by variable freshwater flows (Kurup et al. 1998). A common feature in many estuaries is a salt-wedge, where differences in water density

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create a highly stratified water column (Geyer & Farmer 1989, Kurup et al. 1998). This density gradient can prevent vertical mixing by acting as a physical and biological barrier (Kurup et al. 1998). Salinity structure has also been linked to recruitment variability (Kimmerer et al. 2001, North & Houde 2003, Jenkins et al. 2010). Jenkins et al. (2010) reported a positive relationship between freshwater flow, the level of stratification (the difference between bottom salinity and surface salinity) and recruitment of black bream *Acanthopagrus butcheri* Munro, 1949, an estuarine resident, with the greatest recruitment occurring at intermediate flows. Sakabe et al. (2011) reported a link between high freshwater flow and low recruitment of black bream on the east coast of Tasmania. Both studies suggest a link between flow and year class strength (Jenkins et al. 2010, Sakabe et al. 2011).

Natural variability in environmental conditions has long been recognised as a driver of variation in year class strength and recruitment in fishes. Variability in hydrography and water chemistry as well as prey and predator abundance can impact the success of egg production and larval survival (Hjort 1914, Cushing 1975, Iles & Sinclair 1982, Sissenwine 1984, Cushing et al. 1990, Houde 2008, Leggett & Frank 2008). Hjort (1914) was the first to recognize that the early larval period was a 'critical period' in the life history, where survival rates between hatching and first feeding could ultimately determine recruitment rates into the adult life stage. Cushing's match/mismatch hypothesis (1975) extended this concept by further hypothesising that an overlap (i.e. match) in the timing of primary and secondary productivity with fish spawning could increase larval survival from post-hatch through to the juvenile stage. The spatial alignment of larval and prey populations has also been shown to influence recruitment, with larval survival and recruitment enhanced when physical processes transport and retain larvae in areas of high prey availability (Iles & Sinclair 1982, Sirois & Dodson 2000, North & Houde 2001, 2003, 2006).

Black bream is an estuarine fish species that completes its entire life-cycle within an estuary and is endemic to temperate estuaries in southern Australia. Spawning in black bream is highly variable on both spatial and temporal scales. Spawning usually occurs in salinities >10 and is usually triggered once water temperature rises to >15°C (Haddy & Pankhurst 1998, Sarre & Potter 1999, Walker & Neira 2001, Nicholson et al. 2008). Mature fish are capable of spawning multiple times from August to December by releasing millions of pelagic eggs that take up to 48 h to hatch (Butcher 1945, Sarre & Potter 1999, Walker & Neira 2001). The larval duration and growth rates are highly dependent on temperature and prey availability, but as a guide, it takes ~22 to 28 d to reach 10 mm total length (TL) (Jenkins et al. 1999). Black bream eggs and larvae commonly occur in the upper sections of estuaries, often closely associated with the salt- and freshwater interface (Sherwood & Backhouse 1982, Newton 1996, Nicholson et al. 2008). Recent evidence suggests that the formation of the salt-wedge or stratification of the water column, due to freshwater flows, is an important process for larvae and successful recruitment (Jenkins et al. 2010, Williams et al. 2012). However, the ecological mechanisms behind this relationship remain unknown.

Newton (1996) suggested that food supply is critical to black bream spawning success and the variable year-to-year recruitment. The dietary composition of larval black bream is usually dominated by calanoid copepods, in particular nauplii and copepodites (Newton 1996, Willis et al. 1999). In the Hopkins River, south east Australia, peak concentrations of black bream larvae occurred in the upper estuary coinciding with the re-establishment of calanoid copepod populations after flooding (Newton 1996). Combined, these findings suggest that black bream may time spawning to coincide with freshwater flow that generates salinity stratification (Sakabe et al. 2011), creating optimal conditions for the production of prey as well as retention of larvae.

In this study we investigated the hypothesis that the spatio-temporal distribution of black bream eggs, larvae and their prey are influenced by the location of the salt-wedge and its halocline. On the basis of prior knowledge of black bream spawning ecology, we postulated that freshwater flow and salt-wedge structure are important variables that influence the distribution of eggs and larvae and their prey. We first determined the spatial distribution of eggs, larvae and copepods in relation to depth and distance from the river mouth, then assessed which environmental factors influenced these distributions.

MATERIALS AND METHODS

Study site

This study was completed in the Mitchell River, the largest unregulated river in Victoria, Australia (Fig. 1). The Mitchell River provides a significant

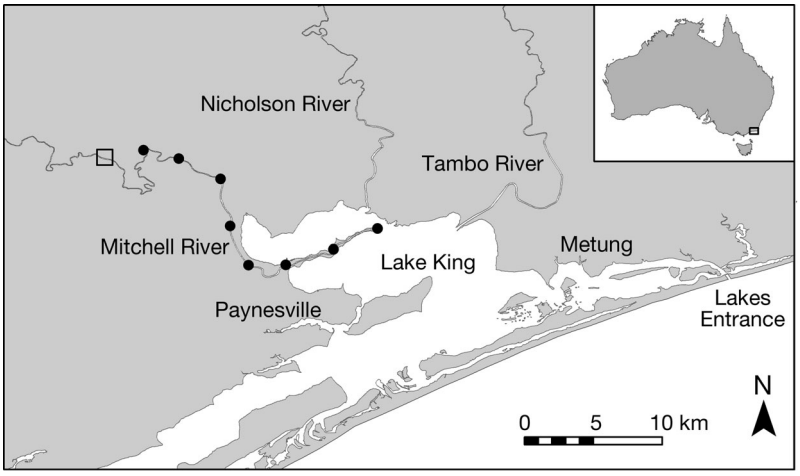

Fig. 1. Gippsland Lakes and the freshwater flow gauge (□) in south-eastern Australia. Eight sampling sites (●) were located along the Mitchell River from the river mouth to 21 km upstream

proportion of the freshwater flow into the Gippsland Lakes, the largest estuarine lagoon system in Australia covering over 340 km^2 (Fig. 1). The Mitchell River flows into Lake King, which is continuously connected to the ocean via a narrow man-made channel. The Gippsland Lakes are a micro-tidal system with tides commonly <30 cm. The Mitchell River is a popular recreational fishing site for black bream and there is no commercial fishing in the river itself, as commercial fishing is restricted to the lakes. The Mitchell River estuary has a narrow channel that varies in depth from 2 to 8 m. Freshwater flow in the Mitchell River has decreased dramatically over the last decade. The average flow from 1937–2000 was 2204 megalitres (Ml) d^{-1} compared to the period 2000–2008 when the average was 1367 Ml d^{-1}, with prolonged periods of flow <100 Ml d^{-1} (www.vicwaterdata.net). Data on the salinity structure of the Mitchell River is limited. Anecdotal evidence suggests that saline water has been gradually moving farther upstream, and over the last decade it has been common for saline water to be present as far as 25 km upstream, where a man-made rock barrier restricts further intrusion (Lovell & McAlister 2003).

Eight sampling sites were established at regular 3 km intervals from the river mouth to 21 km upstream (Fig. 1). All 8 sites were sampled on the same day during daylight hours. Black bream eggs, larvae, copepods and hydrological data were collected from the same site and at approximately the same time (within 1 h). Each site was sampled every 3 wk to ensure we captured each spawning event in 2008, based on the larval duration of 20 to 30 d.

Sampling for black bream eggs and larvae

Stratified ichthyoplankton samples were collected below the surface, approximately 30 cm from the bottom, and an extra mid-water column tow was done at sites >4 m depth. A conical net with a 750 mm diameter opening, 333 µm mesh and with a flow meter (General Oceanics 2030) was towed at approximately 1.5 to 2.0 knots. The volume of water sampled ranged from 70 to 120 m^{-3}. Samples were fixed in a 5% seawater formalin solution in the field and preserved in 70% ethanol in the laboratory post-sorting.

Eggs and larvae were counted in the laboratory using a compound microscope with 20× magnification. Large samples were split into a manageable size using a folsom plankton splitter. A subsample of approximately 250 ml (ranging from a whole sample to no more than a 1/32 split) of plankton was sufficient to count and identify eggs and larvae adequately. To test the accuracy of the plankton splitter, multiple splits were sorted to ensure that there was less than 5% discrepancy. Black bream larvae were identified, counted and staged as either yolk-sac, pre-flexion or post-flexion based on morphometrics and fin meristics (Miskiewicz & Neira 1998). Black bream eggs were identified and counted based on total size, oil globule size and shape (Miskiewicz & Neira 1998). A sub-sample of eggs was sent to the Museum of Victoria for DNA barcoding to confirm identification. DNA testing found 70% of eggs tested were identified as black bream to a confidence level of 99.8%. The other 30% were contaminated with an aquatic bacteria and the DNA could not be identified.

Sampling for larval fish prey

The zooplankton was sampled for copepods on the same day and time as the ichthyoplankton sampling from 1 October to 21 December. Zooplankton samples were collected using a submersible plankton pump to draw water from the desired depth. Samples were collected at 1 m depth intervals from directly below the surface to 30 cm above the substrate. Water was pumped for 2 min and sieved through 40 µm mesh. Approximately 150 to 200 l of water was sampled (the pump was calibrated once per field-

trip). The contents of the sieve were washed into a sample jar and preserved in 5% formalin and seawater solution in the field. In the laboratory, samples were sorted by taking 2 ml sub-samples from a fixed sample volume using a Hensen-Stempel pipette until >100 individuals had been counted. Zooplankton was identified down to the level of order, and copepods were staged as nauplii, copepodite or adult. We also randomly selected 20 samples to qualitatively examine species composition. Twenty-five larvae were haphazardly collected from across all surveys to investigate the gut contents of larvae from this study so as to confirm what would be classified as prey.

Hydrological data

Hydrological data were collected at all sites at 1 m depth intervals. A Hydrolab data sonde fitted with temperature, salinity, turbidity, dissolved oxygen and fluorometry probes was used. Data were logged manually using a field PDA and the software Hydras 3. The Hydrolab data sonde was calibrated regularly using standard solutions. Data from the Hydrolab were post-processed to remove any spurious values (i.e. outliers) that may have been recorded. Flow data were logged daily from the Rosehill gauging station and are freely available online from the Victorian Water Warehouse (www.vicwaterdata.net).

Data analysis

Egg and larval data were converted to concentration per cubic meter. This was calculated as the total numbers of eggs or larvae divided by total volume of water sampled (calculated from the flow meter). Copepod data were converted to concentration per litre by dividing total number of copepods by the volume of water pumped.

Full factorial repeated measures analysis of covariance (RM-ANCOVA) was used to test for spatial and temporal effects. Each of egg, larval and copepod concentrations were treated as the dependant variables, fieldtrip was treated as the repeated measure and the fixed factor, depth was the between-subjects effect with distance from river mouth (DRM) treated as the covariate. As mid-water tows were only taken from 3 sites, to allow for a balanced design, the mid-water tow and bottom tow at each site were averaged. The test of sphericity for the repeated measures ANOVAs was always assumed to be violated as it is very sensitive to deviations from multivariate normality (Quinn & Keough 2002). Therefore, *F*-ratios were always adjusted using the conservative Greenhouse-Geisser correction factor. When significant within-subjects interactions occurred, each fieldtrip was analysed separately with ANCOVA.

All ANCOVAs and correlation analyses were done using SPSS 16. All data were natural log transformed $\ln(x + 1)$ to meet the assumptions of normality and homogeneity of variances. Fieldtrips that contained all zeros or <2 samples with eggs or larvae were removed for ANCOVA analysis to assist with meeting the assumptions of normality and homogeneity of variance.

The hydrological data including fieldtrip date, depth and DRM were examined for correlations and colinearity using Pearson's correlation analysis and sequential Bonferroni adjusted p values. To visually display the salinity structure of the Mitchell River, longitudinal profiles for each fieldtrip were created using Surfer 8 (Golden Software). Salinity data were gridded using triangulation and linear interpolation. To visualise the location and abundance of eggs and larvae in relation to the salinity structure of the Mitchell River, expanding spheres were overlaid. Also, to visualise the location and abundance of prey in relation to salinity structure and location of eggs and larvae, prey data were interpolated using triangulation and linear interpolation, and the results were overlaid as contour lines.

Scatterplots revealed that concentrations of eggs, larvae and copepods across environmental variables were highly skewed and variable, and therefore traditional regression would not be appropriate. As we were interested in determining if there were limiting or maximum concentrations for each environmental variable, we developed quantile regression models. Quantile regression is an extension of the classical regression of a centrally fitted line (Cade et al. 2005). Quantile regression spline models were fitted to concentrations of eggs, larvae and prey for each environmental variable. The 95th percentile was used based on Anderson (2008) as it predicts the maximum concentrations along each environmental gradient without being overly influenced by outliers. B-splines were used to fit a piecewise polynomial of a specified degree. The appropriate degree for the polynomial was decided using the corrected Akaike's information criterion AIC_c (Lancaster & Belyea 2006, Anderson 2008). The model with the lowest AIC_c of the set of models having a polynomial of degree = 2, 3, 4, or 5 was chosen. If 2 models had an AIC_c within 2 units of each other, the lowest order model was chosen. For each model, the value at which the predicted concen-

trations reached an absolute maximum was identified and used as the estimated maximum occurrence for each hydrological variable. Bootstrap confidence intervals (95%) were obtained for the predicted absolute maximum concentrations using bias-corrected percentiles from re-application of the model to each of 10000 bootstrapped sample pairs, using the polynomial degree that was used in the original model (Anderson 2008). The models were fitted using the 'quantreg' package (Koenker 2011) and the 'splines' package (Hastie 1993) in R Project software (www.r-project.org). Concentrations of eggs, larvae and copepods were natural log transformed $\ln(x+1)$ prior to analysis. Multiple regressions of each subset of variables (including copepod concentration for egg and larvae data) were analysed to determine the relationship between environmental variables and concentrations of eggs, larvae and copepods. Model selection was based on the model with the lowest AIC_c; if any other models had an AIC_c within 2 units, the model with the lowest number of variables was selected. Multiple regressions were done using the 'best subset' function in Systat 13.

RESULTS

Hydrology of the Mitchell River

Throughout this study, freshwater flows into the Mitchell River were highly variable (Fig. 2). In the months preceding this study, from February to July, freshwater flow was well below the seasonal average of ~1000 Ml d^{-1} (Fig. 2). Freshwater flow increased throughout the winter month of July and fluctuated between 1500 and 2500 Ml d^{-1} before decreasing to <1000 Ml d^{-1} in October, midway through the spawning season (Fig. 2). Sampling was also characterised by a significant flow event in early December of >10000 Ml d^{-1}. The longitudinal salinity profiles demonstrate the highly stratified salinity structure of the Mitchell River during this study (Fig. 3). Throughout the study, a freshwater layer and halocline were always detected. There was a significant relationship between freshwater flow and the location of the salt-wedge toe (where marine and freshwaters meet at the substrate; $R^2 = 0.959$).

Hydrological data revealed intra-estuary spatial and temporal variability. Significant correlations were observed between salinity, depth and DRM (Table 1). Water temperature was positively correlated with fieldtrip date (Table 1) increasing from ~9°C to ~22°C throughout this study, with vertical stratification varying between ~1 and 3°C. Levels of dissolved oxygen decreased with depth (Table 1). The surface layer was often >9 mg l^{-1} or >100% saturation, while deeper sites within the estuary often had levels of dissolved oxygen <4 mg l^{-1} or <50% saturation. A positive relationship with freshwater flow and dissolved oxygen was detected (Table 1).

Turbidity and fluorescence displayed significant temporal variability influenced by freshwater flow (Table 1). During periods of high flows turbidity levels increased and fluorescence decreased and vice versa. Turbidity was highest after significant flow events on 8 September and 3 December 2008 (58 and 60.4 nephelometric turbidity units (NTU), respectively). Fluorescence levels were the highest during the period of lowest freshwater flow during the 30 October and 15 November fieldtrips.

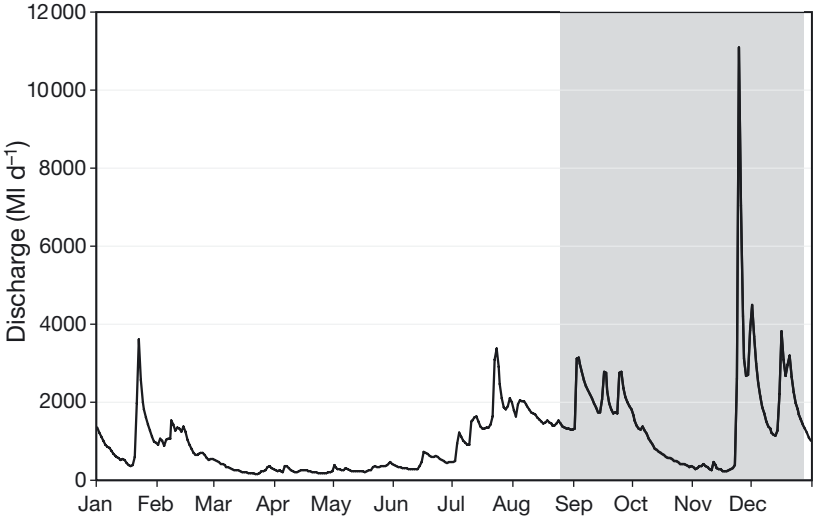

Fig. 2. Daily freshwater flow for the Mitchell River in 2008 (www.vicwaterdata.net). The shaded area represents the sampling period for this study

Spatio-temporal distributions of eggs, larvae and their prey

Black bream eggs where collected on 6 of the 7 fieldtrips, and concentrations peaked on 30 October 2008 (Fig. 4a). Eggs were collected from all sites and depths during this study, although fieldtrips on 18 August and 8 September contained very few or no eggs and hence were not used in analyses. The bottom and mid-water tows

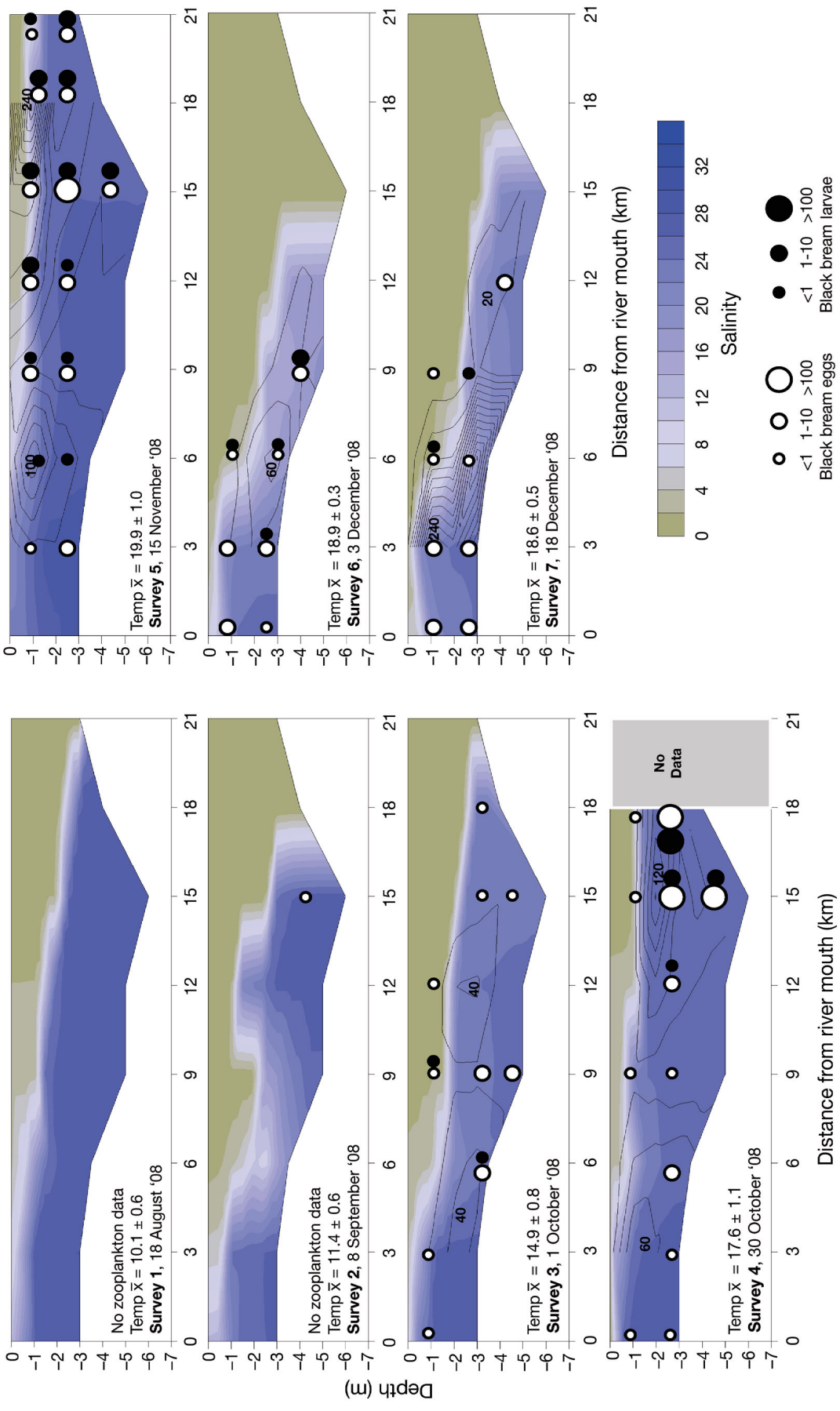

Fig. 3. Two-dimensional cross sections of the Mitchell River salinity structure through time. Each plot represents a single snapshot in time of the salinity, egg, larvae, and zooplankton distribution. The black contour lines represent zooplankton concentrations, with the gap between lines being concentrations of 20 copepods l^{-1}. $\bar{x}$: sample mean

Table 1. Pearson correlation coefficients for all hydrographical data and spatial and temporal variables such as fieldtrip, depth and distance from river mouth (DRM). *Correlation was significant at $p < 0.05$ (2-tailed); **correlation was significant at $p < 0.01$ (2-tailed). DO: dissolved oxygen

	Fieldtrip	Depth	DRM	Flow	Temperature	Salinity	DO	Turbidity
Depth	0.03							
DRM	−0.04	0.11						
Flow	0.24**	−0.03	−0.01					
Temperature	0.88**	−0.01	−0.07	−0.10				
Salinity	−0.10	0.55**	−0.39**	−0.43**	0.04			
DO	−0.09	−0.67**	−0.18**	0.33**	−0.26**	−0.62**		
Turbidity	0.29**	0.04	0.33**	0.47**	0.03	−0.41**	0.16**	
Fluorescence	−0.21**	0.22**	−0.18**	−0.36**	−0.08	0.64**	−0.25**	−0.23**

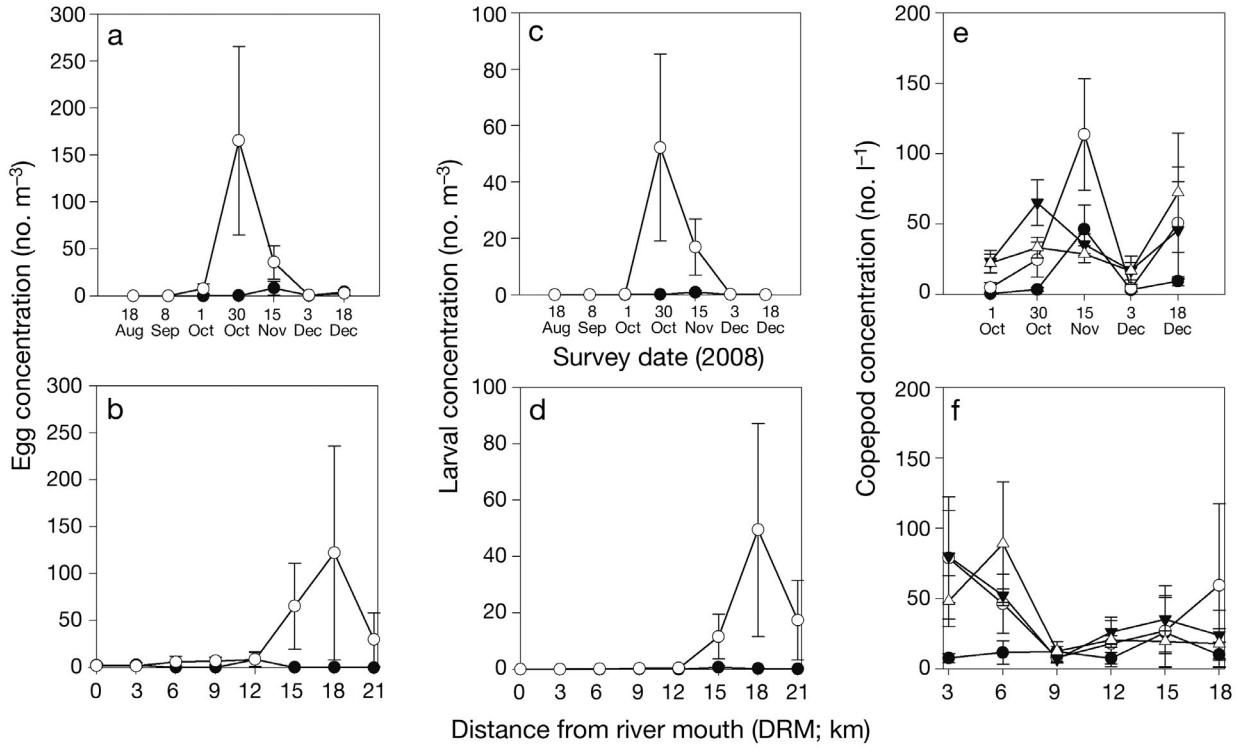

Fig. 4. Mean (±SE) concentration of black bream *Acanthopagrus butcheri* (a,b) eggs and (c,d) larvae from 18 August to 18 December 2008 for ichthyoplankton tows below the surface (●) and bottom and mid-water (○). (e,f) Mean (±SE) concentration of copepods from 1 October to 18 December 2008 from below the surface (●), 1 m (○), 2 m (▼), and 3 m (△). Error bars are standard error (n = 8)

contained significantly more eggs than surface tows (Table 2, Fig. 4a) with no effect of the covariate DRM (Table 2, Fig. 4b). Significant temporal differences in egg concentration occurred among fieldtrips (Table 2, Fig. 4a) as well as interactions among fieldtrips and DRM (Table 2) and among fieldtrips and depth (Table 2). Analysing each fieldtrip separately, due to the significant interaction between fieldtrips and depth, revealed significantly more eggs in bottom and mid-water tows on 30 October, as well as a significant effect of the covariate (DRM), with more eggs being collected at sites >12 km upstream (Fig. 5b, Table 3). Coinciding with a flow event, field trips on 3 and 18 December had significantly more eggs collected from sites at 0 to 9 km compared to sites further upstream (Fig. 5d,e, Table 3).

Black bream larvae were collected from 5 of the 7 fieldtrips, with concentrations peaking on the 30 Oc-

Table 2. Results from the repeated measures (RM)-ANCOVA comparing concentrations of eggs and larvae of black bream *Acanthopagrus butcheri* and copepods across distance from river mouth (DRM), depth and fieldtrip. The degrees of freedom are in subscript

Factors	Eggs		Larvae		Copepods	
	F	p	*F*	p	*F*	p
DRM	$0.86_{1,13}$	0.37	$11.39_{1,13}$	<0.01	$26.71_{1,19}$	<0.01
Depth (D)	$13.90_{1,13}$	<0.01	$6.74_{1,13}$	0.02	$6.27_{3,19}$	<0.01
Fieldtrip (F)	$4.21_{4,10}$	0.03	$0.57_{2,12}$	0.58	$5.23_{4,76}$	<0.01
F × DRM	$5.73_{4,10}$	0.01	$7.32_{2,12}$	<0.01	$15.85_{4,76}$	<0.01
F × D	$3.33_{4,10}$	0.06	$2.45_{2,12}$	0.13	$4.69_{12,76}$	<0.01

Table 3. ANCOVA comparing black bream *Acanthopagrus butcheri* egg and larvae as well as copepod concentrations across 2 factors: depth and distance from river mouth (DRM). Fieldtrips without eggs or larvae were excluded from the analyses. All data were ln(x + 1) transformed (*p < 0.05, **p < 0.01)

	1 Oct		30 Oct		15 Nov		3 Dec		18 Dec	
	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Eggs										
Depth	1	2.64	1	9.45**	1	2.82	1	0.01	1	<0.01
DRM	1	0.32	1	7.46*	1	4.42	1	8.27*	1	18.51**
Larvae										
Depth			1	6.19*	1	5.49*	1	0.48		
DRM			1	8.15*	1	14.96**	1	0.32		
Copepods										
Depth	3	14.20**	3	10.23**	3	2.65	3	2.44	3	1.52
DRM	1	26.32**	1	2.50	1	3.36	1	24.72**	1	33.63**

tober field trip (Fig. 4c). During the course of this study, larvae were collected at all sites and depths, with the exception of the site at the river mouth. Fieldtrips on 18 August, 8 September and 18 December were not used in the analysis as they contained no or very few larvae. Significant differences in concentrations of larvae occurred between depths with more larvae in mid- and bottom tows compared to surface tows (Table 2, Fig. 4c) and DRM (Table 2, Fig. 4d). No significant differences occurred among fieldtrips (Table 2, Fig. 4c) or with fieldtrip and depth (Table 2). However, a significant interaction occurred with fieldtrip and DRM (Table 2). Analysis of individual fieldtrips revealed significantly more larvae in mid- and bottom tows compared to surface tows (Fig. 6a,b, Table 3), with significantly more larvae collected from sites 15 to 21 km upstream (Fig. 6a,b).

Copepods were highly abundant in the zooplankton, with copepod populations dominated by nauplii, on average >80% of the total sample. Calanoid copepodites accounted for

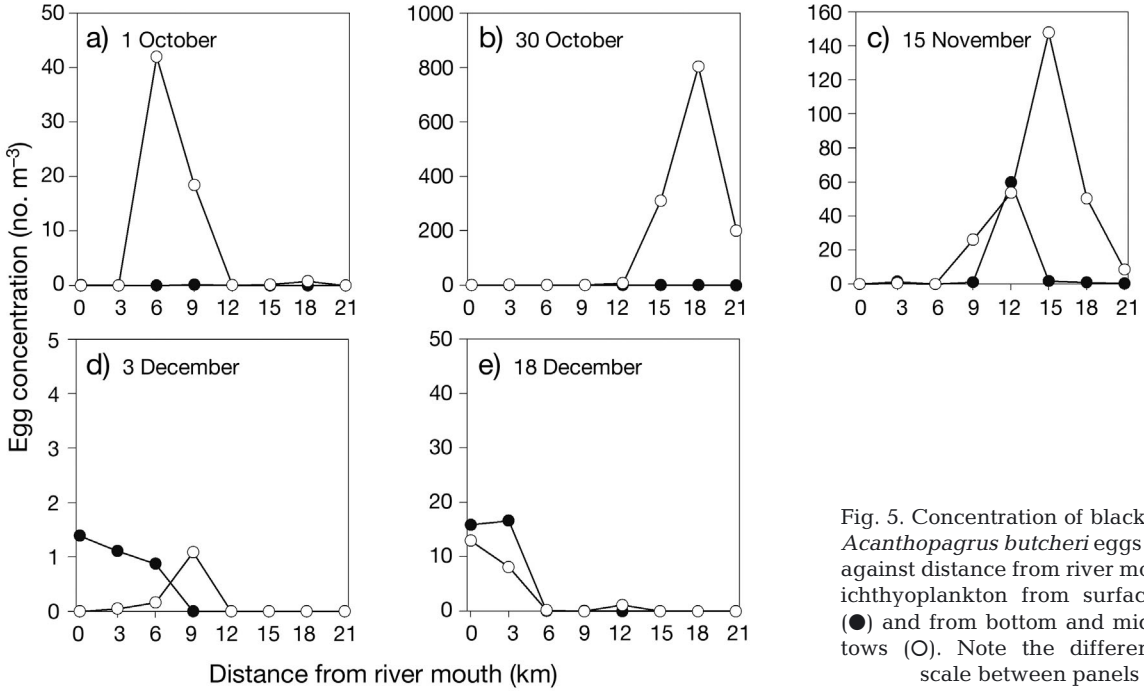

Fig. 5. Concentration of black bream *Acanthopagrus butcheri* eggs plotted against distance from river mouth for ichthyoplankton from surface tows (●) and from bottom and mid-water tows (○). Note the differences in scale between panels

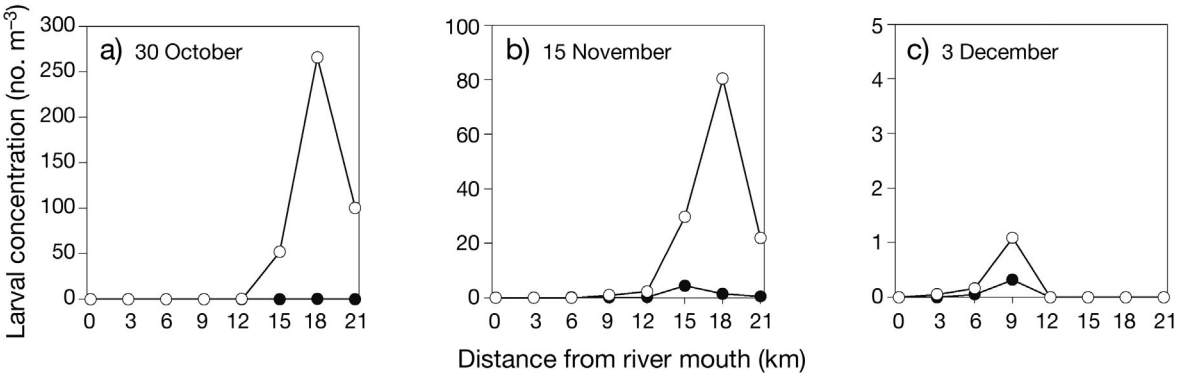

Fig. 6. Concentration of black bream *Acanthopagrus butcheri* larvae plotted against distance from river mouth for ichthyoplankton from surface tows (●) and from bottom and mid-water tows (○)

8% and adult copepods 6% of the copepod population. A visual qualitative assessment of 20 randomly selected samples revealed that the copepod assemblage was dominated by 1 species, *Gladioferens pectinatus* (>90%). Inspection of 25 larval guts confirmed diet composition was dominated by copepod nauplii (72%). Other prey items included cladocerans (7%), polychaetes (7%), copepodites (5%), parts of adult copepods including egg sacs (5%), and phytoplankton (3%). On average, larvae had 3 prey items in their guts, with a maximum of 8 and a minimum of 1.

Analysis of the copepod data also revealed spatiotemporal trends. The first fieldtrip sampling copepods on 1 October found relatively low concentrations of ~20 to 40 l^{-1} (Fig. 4e). Concentrations gradually increased and peaked as high as 290 l^{-1} on 15 November, 18 km upstream from the river mouth (Fig. 4e). A freshwater flow event prior to the fieldtrip on 3 December displaced saline water downstream, and the mean concentration of copepods decreased to <40 l^{-1} (Fig. 4e). During the final fieldtrip on 18 December copepod concentrations had increased to 270 l^{-1}. Differences between depth of sample (Table 2, Fig. 4e) and distances from river mouth (Table 2, Fig. 4f) were significant. Significant differences also occurred among fieldtrips (Table 2, Fig. 4e) as well as a strong interaction between fieldtrip and DRM (Table 2) and between fieldtrip and depth (Table 2). Due to the significant interactions each fieldtrip was analysed separately with an ANCOVA (Table 3). ANCOVA revealed a significant difference among depths for fieldtrips on 1 and 30 October, with more copepods being located between 2 and 3 m depth (Table 3, Fig. 7a,b). A significant effect of DRM occurred with significantly more copepods located in the lower half of the estuary on 1 October, 3 December and 18 December (Table 3, Fig. 7a,b,e).

Distribution of eggs, larvae and prey in relation to hydrology

Overlaying expanding circles to represent concentrations of eggs and larvae on the salinity profiles demonstrates that eggs and larvae were distributed throughout the saline layer (Fig. 3). Copepods were not sampled until 1 October and concentration data were interpolated and overlaid on the salinity profiles using contour plots. Overlaying the interpolated data revealed hotspots of copepods as well-defined areas of much higher concentrations than other regions within the estuary (Fig. 3). There was clear spatial coupling among high concentrations of larvae, prey hotspots and the halocline (Fig. 3). The salinity profiles visualise how freshwater flows determine the location and extent of the halocline and therefore the distribution of larval and prey hotspots (Fig. 3). The highest concentrations of eggs, larvae and copepods occurred during low flow conditions when there was a very shallow layer of freshwater and the saline layer occurred throughout the estuary (Fig. 3). A large flow event displaced the salt-wedge and consequently, eggs, larvae and copepods downstream to the mid to lower estuary (Fig. 3).

The quantile regression spline models provided a good 'envelope', for our scattered data, of the maximum concentrations of eggs, larvae and copepods along each of the hydrological variables (Fig. 8). Water temperature was an effective indicator of temporal patterns as it increased throughout the study. Black bream eggs and larvae were collected in water temperature ranging from 13 to 22°C and displayed strong temporal patterns with concentrations increasing and reaching absolute maximum at 17°C (Fig. 8, Table 4) before decreasing in concentrations. The concentration of copepods gradually increased and peaked and leveled at an absolute maximum of 20.2°C (Fig. 8, Table 4).

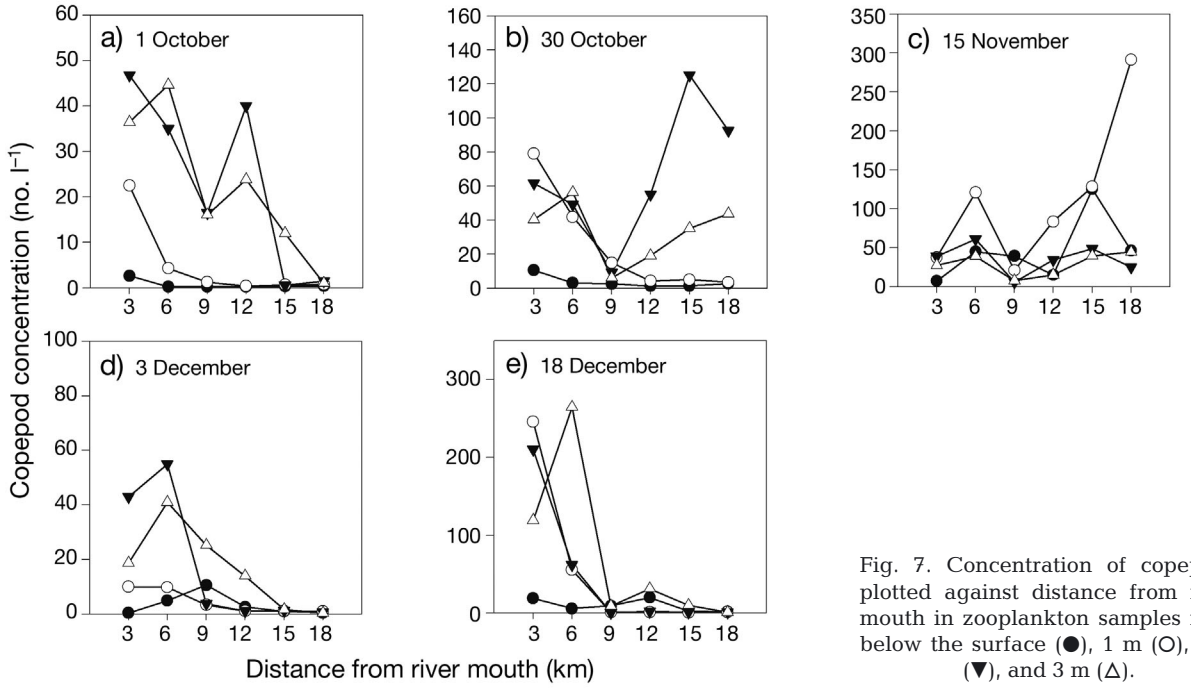

Fig. 7. Concentration of copepods plotted against distance from river mouth in zooplankton samples from below the surface (●), 1 m (○), 2 m (▼), and 3 m (△).

Table 4. Estimated absolute maximum (Abs. max.) for measured variables that would result in theoretical highest concentrations of eggs, larvae and copepods taken from the quantile regression spline models. Only the model with the best fitting polynomial degree ('Degree') is stated for each variable. DO: dissolved oxygen

Variable	Degree	Abs. max.	95% CI
Eggs			
Temperature	5	17.0°C	15.0, 20.2
Salinity	5	25.0 ‰	24.5, 26.4
DO	4	2.8 mg l^{-1}	1.5, 9.9
Turbidity	3	8.1 NTU	0, 29.6
Fluorescence	4	34.0 relative units	20.2, 36.5
Larvae			
Temperature	5	16.9°C	15.1, 20.3
Salinity	4	26.4 ‰	23.6, 28.6
DO	4	2.3 mg l^{-1}	1.0, 3.2
Turbidity	3	10.1 NTU	1.0, 23.6
Fluorescence	4	34.0 relative units	5.8, 39.9
Copepods			
Temperature	2	20.2°C	12.4, 21.2
Salinity	5	20.0 ‰	8.1, 27.6
DO	4	8.0 mg l^{-1}	5.4, 14.9
Turbidity	3	4.4 NTU	0, 18.5
Fluorescence	4	9.3 relative units	0, 32.3

A large peak at higher salinities and a lower peak at lower salinities defined each of the salinity models. Highest concentrations of eggs and larvae were restricted to salinities between 15 and 30 (Fig. 8, Table 4). Copepods were found over a large range of salinities (0 to 26), with the absolute maximum concentration occurring at 20 (Fig. 8, Table 4). The spline models revealed a second minor peak between 0 and 10 that is most likely a result of samples that were collected from near the halocline where the net is sampling a wide range of salinities (Fig. 8). No eggs and larvae were found in salinities <1 and these samples over-influenced the models and were therefore removed from further analysis of dissolved oxygen, turbidity and fluorescence.

Models for dissolved oxygen and turbidity were indicators for the position of eggs, larvae and copepods within the salt-wedge. Absolute maximum concentrations of eggs and larvae occurred in water with 2 to 3 mg l^{-1} of dissolved oxygen (Fig. 8, Table 4). Absolute maximum copepod concentration occurred at levels around 8 mg l^{-1} (Fig. 8, Table 4). Absolute maximum concentrations of eggs and larvae occurred in water characterised by turbidity between 5 and 10 NTU and fluorescence between 10 and 34 relative units (Fig. 8, Table 4), while maximum copepod concentrations occurred in water characterised by turbidity of 0 NTU and fluorescence of 9 relative units (Fig. 8, Table 4). Multiple regressions revealed that the 'best subset' of environmental variables that explain the variability in egg and larval concentration with environmental conditions was a model containing dissolved oxygen and copepod concentration (Table 5). The 'best subset' of variables that describe the variability of copepod concentration was a model containing salinity, temperature, dissolved oxygen, turbidity and fluorescence (Table 5).

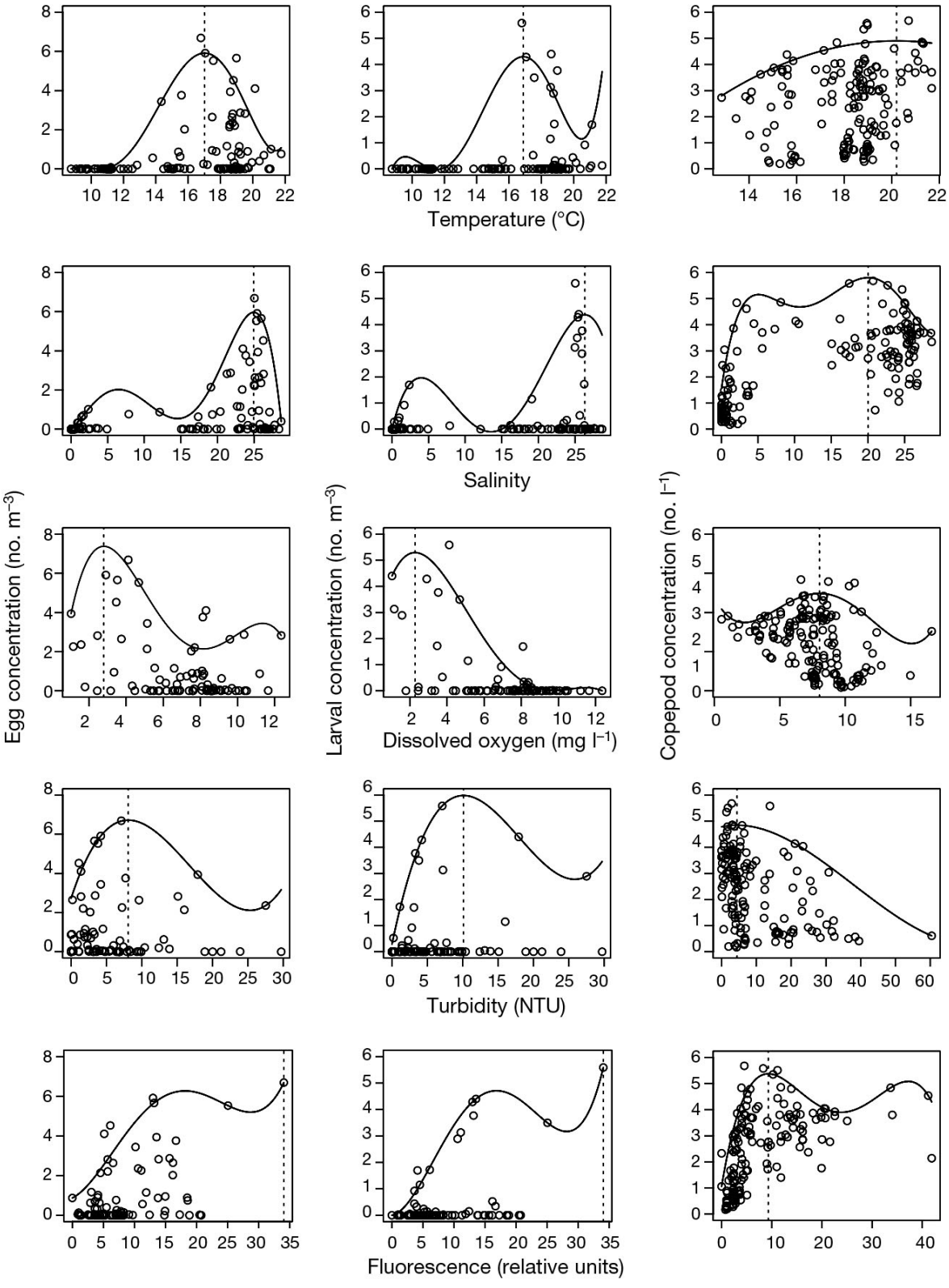

Fig. 8. Concentrations of black bream *Acanthopagrus butcheri* eggs and larvae and copepods across hydrological variables: temperature, salinity, dissolved oxygen, turbidity and fluorescence. Quantile regression spline models have been fitted to each plot to represent the 95th percentile. The vertical dashed line represents the estimated absolute maximum concentration

Table 5. The best subset of variables from all multiple regression subsets chosen using corrected Akaike Information Criterion (AIC_c). DO: dissolved oxygen; Cop: copepod concentration; Sal: salinity; Temp: temperature; Turb: turbidity; Fluo: fluorescence

	Variables	AIC_c	Adj. R^2	p
Eggs	DO, Cop.	215.218	0.236	<0.001
Larvae	DO, Cop.	164.909	0.412	<0.001
Copepods	Sal, Temp, DO, Turb, Fluo	427.584	0.550	<0.001

DISCUSSION

This study found a strong coupling in the spatial and temporal distribution of black bream eggs and larvae and their preferred prey, copepods, with the halocline in the Mitchell River estuary in 2008. In 2008, freshwater flows were well below average and during this study saline water reached the maximum distance upstream where it is restricted by a man-made barrier. Eggs were spawned throughout the saline layer with the highest concentrations occurring at the halocline in the upper estuary, suggesting increased spawning activity or convergence of eggs towards the physical barrier of the halocline. The area below the halocline also supported high productivity of copepods, predominately *Gladioferens pectinatus,* an important prey item for feeding black bream larvae. Having a well defined spatial overlap between eggs and prey may be an important life history strategy that maximises the reproductive success of black bream.

Within a spawning season, freshwater flows are highly variable and this influences the location of the salt-wedge and associated eggs, larvae and copepods within the estuary. Previous research found that highly stratified sites had the greatest concentrations of larvae and therefore freshwater flow had a large influence on the extent of optimal larval habitat (Williams et al. 2012). The results from the current study suggest that sites that are highly stratified also have optimal salinities and high concentrations of larval prey. Based on this finding, it is likely that moderate freshwater flows (~2000 Ml d^{-1}) relative to lower (<1000 Ml d^{-1}) or higher (>3000 Ml d^{-1}) flows will extend the area of the halocline and therefore maximise the extent of optimal habitat for larvae and copepods. This hypothesis is further supported by evidence that years of intermediate flow (~1000 to 2000 Ml d^{-1}) also have the highest level of recruitment of black bream in the Gippsland Lakes (Jenkins et al. 2010).

The reduction in freshwater flow at the start of the spawning season could have facilitated upstream transport of eggs in saline waters towards the halocline, particularly as bream eggs are likely to be positively buoyant in salinities >20 (Jenkins et al. 2010). The salinity profiles provide an excellent visual representation of what is occurring: eggs are likely being transported to the halocline where larvae hatch into a region of high prey availability, a beneficial strategy that maximises feeding success and growth rates (Kimmerer 2002, North & Houde 2003). A similar reproductive strategy has been widely reported for the anadromous striped bass *Morone saxatilis* in the USA (Secor & Houde 1995, North & Houde 2003).

The main factors influencing the spatial and temporal distribution of zooplankton (larval prey) in estuaries are salinity and temperature (Marques et al. 2006, Primo et al. 2009). Therefore, freshwater flows have a direct influence on zooplankton distribution. Historically, *Gladioferens pectinatus* has been the numerically dominant species of copepod in the Gippsland Lakes and has been found to be an important food source for black bream larvae (McKinnon & Arnott 1985, Newton 1996, Willis et al. 1999). In the present study, hotspots of copepods were confined to the vicinity of the halocline, and while our study was not designed to reveal the mechanism behind this, it is likely that increased nutrients and phytoplankton accumulating in the halocline assist in supporting copepod production (Cloern et al. 1983, Jassby et al. 1995, Kimmerer 2002). Therefore, the timing and location of spawning activity is crucial to maximise the spatial and temporal overlap of larvae and prey. Our findings suggest that it is a combination of complex physical, chemical and biological processes that determine spawning success and larval survival for estuarine-dependent fish. In the terms of fisheries paradigms, a combination of match/mismatch (Cushing et al. 1990) and convergence theories (Iles & Sinclair 1982) may explain a large proportion of the high natural variability in the year class strength of black bream by (1) freshwater flow determining the location and area of halocline or optimal spawning and larval habitat, and (2) upstream movement of the intermediate to high saline layer.

We observed other factors that may have limited the temporal and spatial distribution of eggs, larvae and copepods. During this study it was not uncommon for sites with high egg and larval concentration to have low dissolved oxygen saturation. Many spe-

cies of marine and estuarine fish have eggs and larvae that are sensitive to dissolved oxygen and require levels to be greater than 40% saturation (Miller et al. 2002). The Mitchell River had particularly low levels of dissolved oxygen, especially the deeper pools in the upper section of the estuary. This is of concern as more than 50% of eggs and larvae collected in our study came from water with less that 50% dissolved oxygen saturation. Laboratory experiments testing hatch rates and larval development of black bream found no eggs hatched in water with less than 30% saturation, and larvae did not survive past Day 2 in water less than 55% saturation (Hassell et al. 2008a,b). Hypoxia and anoxia are becoming increasingly problematic in highly stratified estuaries where there is high nutrient loading through agricultural activities, including the Gippsland Lakes (Longmore et al. 1990). A reduction in freshwater flow due to drought and climate change is a likely occurrence and will only result in further decreases in dissolved oxygen, creating longer periods of hypoxia and anoxia in estuaries (Kurup & Hamilton 2002, Bates et al. 2008). Newton (1996) studied the Hopkins River in non-drought conditions and found that winter rainfall was beneficial in maintaining high levels of dissolved oxygen throughout the black bream spawning season in spring and early summer. Other studies have confirmed that high levels of dissolved oxygen maximise egg hatch rates and larval survival (Hassell et al. 2008a,b).

The findings of this study are concerning, as current climate change predictions suggest parts of the world will get wetter and experience high river flows, while others are expected to experience more frequent dry periods with decreases in river flow (Bates et al. 2008). South-eastern Australia has experienced severe drought since 1997, and current climate change forecasts suggest a possible reduction in river flow of between 5 and 45% by 2030 (Jones & Durak 2005). The impacts of climate change are further exacerbated by anthropogenic impacts such as water extraction, damming and diverting flow as human need for freshwater increases (Boon 1992, Zann 1996, Nilsson et al. 2005). Our study suggests that sufficient freshwater flow needs to be delivered to the estuary to generate the stratified conditions that correspond to increased concentration of black bream eggs, larvae and their potential prey. The increasing knowledge about black bream's dependence on, and complex interactions with, their hydrological environment enable us to continually adjust water management strategies and help maintain a viable population of this important fish species.

CONCLUSION

This study found that the halocline, also referred to as salt front, is an important habitat for black bream larvae and their prey. The spatial overlap of eggs within an area of intermediate salinities and high copepod concentrations maximises the chance of successful hatching and larval survival. The timing of spawning and hatching of larvae to coincide with increasing prey availability is also important to maximise growth and survival of larvae. For black bream, optimal levels of freshwater flow for the generation of salinity stratification are likely to be a good predictor of favourable conditions for offspring survival. Because freshwater has a large influence on the location and area of the halocline, it likely explains some of the natural variability in year class strength of this and possibly other estuarine fish species. This study further suggests that low to moderate flows provide the maximum area for the halocline and therefore may increase black bream productivity. These findings imply that a marked reduction in flows through drought, abstraction of water, and future climate change may reduce the stratified area of the estuary and restrict the area of suitable environmental conditions and larval prey for black bream, and potentially also for other species that utilise estuaries to spawn or as larval nursery habitats.

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