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Condition of larvae of western rock lobster (*Panulirus cygnus*) in cyclonic and anticyclonic eddies of the Leeuwin Current off Western Australia

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Abstract. Changes in the offshore oceanographic processes are suspected to be the cause of a recent dramatic decline in the settlement of post-larvae of the Western Australian spiny lobster (*Panulirus cygnus*), which has greatly reduced the productivity from the world's second-largest spiny lobster fishery. The present study assessed whether there are differences in the nutritional condition of the larvae of *P. cygnus* sampled from two pairs of cyclonic eddies (CEs) and anticyclonic eddies (AEs). Morphometric and biochemical analyses were undertaken on the mid–late-stage larvae (VI, VII, VIII) sampled offshore from two pairs of adjacent counter-rotating mesoscale eddies in the Leeuwin Current off Western Australia. The results showed that larvae captured from CEs had greater average total dry mass ($P < 0.001$) than those from AEs. Stage VIII larvae from CEs contained more protein ($P < 0.008$) (38.5% $\pm$ 5.4 s.e.) and lipid ($P < 0.005$) (67.2% $\pm$ 12.1) than did those from AEs. The possible causes for these differences are uncertain but may be related to differences in water temperatures in CEs *v*. AEs influencing the ability of phyllosomas to accumulate nutritional reserves.

Additional keywords: eddy, nutritional condition, phyllosoma, spiny lobster.

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Introduction

The manner in which environmental factors affect the health of spiny lobster larvae (i.e. phyllosomas) is poorly understood. Experiments on cultured phyllosomas have indicated that their prey density and temperature are key determinants for their healthy development (Tong *et al.* 2000; Liddy *et al.* 2004). Understanding how environmental factors affect phyllosoma health is crucial, because declining numbers of settling post-larvae has recently been described in the three largest populations of spiny lobsters in the world, causing concern for the major fisheries that they support (Ehrhardt and Fitchett 2010; Linnane *et al.* 2010; Feng *et al.* 2011). The most dramatic decline has been observed in the world's second-largest spiny lobster fishery, that is for the Western Australian spiny lobster, *Panulirus cygnus* (Brown 2011). It has had an annual commercial catch varying between 5899 and 14 523 t over the past 30 years, before catch quotas were implemented (de Lestang and Melville-Smith 2006). The variation in the landings of adult lobsters from the fishery has largely been due to natural interannual fluctuations in the settlement of post-larvae, known as pueruli (Caputi *et al.* 2001). The positive relationship between pueruli settlement on the coast and the subsequent catch of adult lobsters in the commercial fishery has been used for many years as an effective means for managing the fishery (Phillips 1986; Morgan *et al.* 1982).

Interannual fluctuations in pueruli settlement, as measured with collectors deployed along the Western Australian coastline, have traditionally had a strong positive relationship with the strength of the Leeuwin Current, which flows southward along the coast of Western Australia, and to a large degree determines the oceanic conditions for the developing phyllosomas of the Western Australian spiny lobster (Pearce and Phillips

1988). However, in recent years, this relationship has broken down, with historically low annual settlement of pueruli being recorded regardless of the strength of the Leeuwin Current (Feng *et al.* 2011). The cause of the breakdown in this relationship is uncertain but appears to be related to oceanographic processes (Säwström *et al.* 2014). The phyllosomas of *P. cygnus* hatch from egg clutches held on adult females living on the continental shelf of Western Australia between December and March. After hatching, the phyllosomas are transported into offshore waters where they develop for ~9–11 months (Chittleborough and Thomas 1969). Most of this larval period is spent in oceanic waters beyond the continental shelf margin, and can extend to over 1500 km offshore from the coast of Western Australia (Phillips *et al.* 1979). Significantly, the phyllosoma period is used to accumulate sufficient nutritional reserves to fuel the subsequent active migration as non-feeding pueruli back into coastal waters where they settle into reefs, which occurs between August to January (Phillips *et al.* 2006). The nutritional status of pueruli is known to be critical to their successful cross-shelf migration (Wilkin and Jeffs 2011; Fitzgibbon *et al.* 2013). Therefore, oceanic processes that disrupt the accumulation of nutrients by phyllosomas will have a detrimental effect on subsequent onshore settlement.

During the pelagic phase, the waters that the phyllosomas occupy are highly influenced by a fairly narrow (~100 km wide) and shallow (<300-m depth) current, which is the Leeuwin Current. It transports warmer water of tropical origin southward along the shelf break of Western Australia, impinging on the surrounding subtropical surface water, particularly during the winter months when the current is stronger (Church *et al.* 1989). The offshore distribution of phyllosomas appears to be greatly affected by their entrapment in the series of counter-rotating mesoscale eddies that are established through the interaction of the Leeuwin Current (Griffin *et al.* 2001), the Leeuwin Undercurrent and topographical eddies (such as shelf topography variations and bottom shear). These features become a dominant oceanographic feature offshore of Western Australia at this time (Rennie *et al.* 2007). However, the manner in which the formation of eddies affects the nutritional condition and transport of phyllosomas is unknown. Eddies are frequently coupled, with anticyclonic eddies (AEs) comprising a core of warmer water derived from a mixture of water from the Leeuwin Current and shelf, and cyclonic eddies (CEs) with a core of cooler water upwelled from the Leeuwin Undercurrent (Rennie *et al.* 2007). May is a peak time for the generation of long-lived CE and AE pairs in the Leeuwin Current that can persist for 5–18 months (Morrow *et al.* 2003; Cresswell and Griffin 2004; Feng *et al.* 2007; Moore *et al.* 2007). In the region of 28–32°S off the coast of Western Australia, almost all eddies have an average radius of 100 km, with little fluctuation from their first formation and typically for up to 8 months, which is a sufficient period to encompass almost the whole larval phase of *P. cygnus* (Fang and Morrow 2003). Therefore, each eddy is sufficiently large and persistent to create a localised marine environment that is partially isolated from the surrounding subtropical surface waters. Each eddy develops its own distinctive food web, which very frequently includes entrapped phyllosomas of *P. cygnus* (Strzelecki *et al.* 2007; Waite *et al.* 2007*a*).

Studies of mesoscale eddies formed by the Leeuwin Current have consistently found that, within AEs, there is higher primary productivity that supports a greater biomass of zooplankton, than in CEs (Paterson *et al.* 2007; Strzelecki *et al.* 2007; Waite *et al.* 2007*a*, 2007*b*). The marked differences in the physical and biological conditions within an individual eddy could reduce the opportunities of phyllosomas to feed and accumulate sufficient nutrients to facilitate their subsequent migration and recruitment as pueruli into coastal waters.

The aim of the current study is to assess whether there are differences in the nutritional condition of phyllosomas of *P. cygnus* sampled from two pairs of CEs and AEs in the Leeuwin Current, to understand the role of eddy systems in the subsequent survival and settlement of pueruli.

Materials and methods

Sample collection

Spiny lobster phyllosomas (*Panulirus cygnus*) were sampled from the RV *Southern Surveyor* in the Indian Ocean off Western Australia from 27 August to 1 September 2011 (Fig. 1). The

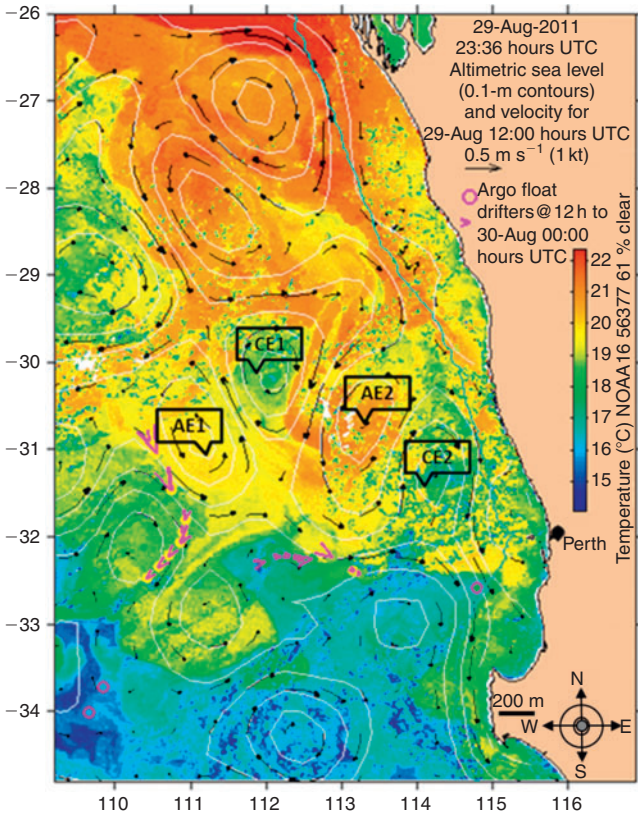

Fig. 1. Position of the four phyllosoma sampling sites off Western Australia against a background of satellite-derived sea surface temperature and sea surface geostrophic current flows for 31 August 2011. AE1, anticyclonic Eddy #1, sampled on 27 August 2011; AE2, anticyclonic Eddy #2, sampled on 29 August 2011; CE1, cyclonic Eddy #1, sampled on 1 September 2011; and CE2, cyclonic Eddy #2, sampled on 30 August 2011. Satellite data were derived from National Oceanic and Atmospheric Administration (NOAA) and analyses supplied under the auspices of the Australian Integrated Marine Observing System (IMOS).

sampling sites were two adjacent pairs of counter-rotating eddies in the Leeuwin Current (Table 1). All samples were collected in a consistent manner by deploying a surface net (1-m diameter, 1-mm mesh towed at 0–3-m depth) at night for ~10 min at less than 3.7 km h^{-1}. On recovery of the net, phyllosomas were immediately sorted from the catch, their development stage determined by examination under a dissecting microscope (onboard the vessel) according to the key of Braine *et al.* (1979), and then frozen on board the vessel at −80°C for later morphometric and biochemical analysis. From the total catch of phyllosomas, 18 individuals collected from each of the four sampled eddies were used for morphometric and biochemical analyses, i.e. six phyllosomas for each of Stages VI, VII and VIII that were randomly selected from the samples taken at each of the four eddies. In total, 72 phyllosomas were analysed.

Morphometric analyses

Digital images of each phyllosoma held against a reference grid were captured using a camera (Canon DIGITAL IXUS 95 IS, Tokyo, Japan). The body length (from the anterior margin of the cephalic shield between the eyestalks to the posterior tip of the pleon) and cephalic shield width (the widest section of the cephalic shield) of phyllosomas were measured from the images by using Image J software (Image J 1.44 p, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). All pereiopods were removed from each phyllosoma before weighing the wet mass of the remaining body to ensure consistency among individuals, because during sampling some of the fragile pereiopods were broken off or damaged on some specimens. Phyllosomas were then lyophilised for 24 h and re-weighed for dry mass, and then used for biochemical analyses.

Biochemical analyses

The lipid content of each individual lyophilised phyllosoma was determined gravimetrically with a modified (Bligh and Dyer 1959) one-phase methanol–chloroform–water extraction. After lipid extraction, individual phyllosomas were lyophilised again and then homogenised with a micropestle. Half the sample was used for analysis of protein content and the other half for carbohydrate content.

The protein content of each phyllosoma was measured by first digesting the samples for 12 h in 0.1 mol L^{-1} NaOH at 50°C and then assaying with the bicinchoninic acid (BCA) method with a micro-BCA protein assay kit (Thermo Scientific, Pierce, Rockford, IL, USA) and by using bovine serum albumin as the reference protein.

The dissolved carbohydrate fraction of each phyllosoma was measured with a phenol sulphuric acid reagent method according to DuBois *et al.* (1956) and was referenced against a D-glucose standard.

Lipid : protein ratio was calculated as the measured mass of lipid *v.* protein for each phyllosoma.

The measured masses of protein, lipid and carbohydrate were converted to energy equivalents using the estimated combustion enthalpy coefficients of 23.9 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.5 kJ g^{-1} for carbohydrate (Gnaiger 1983), such that total energy (kJ) = (protein mass × 23.9) + (lipid mass × 39.5) + (carbohydrate mass × 17.5).

Statistical analyses

To test whether the stage of phyllosoma development and the location from where it was sampled influenced the mean body length, cephalic shield width, wet and dry body mass, and biochemical composition (i.e. protein, lipid, carbohydrate), a two-way ANOVA were used. Separate two-way ANOVAs were used to compare among phyllosomas from within the two CEs, and from within the two AEs. These analyses indicated a lack of differences in the means of the variables within respective eddy types, so the data were pooled by eddy type to allow for a more rigorous comparison of these data for CEs *v.* AEs. The data analyses made use of the software SigmaPlot for Windows (v. 11.0, Systat Software Inc., Erkrath, Germany). Prior to analyses, the normality of the data was verified with a Shapiro–Wilk's test, and homogeneity of variances with Levene's test. When data departed from the requirements for ANOVA, a $\log_{10}$ transformation was performed on the data and the transformed data were tested again to confirm compliance with assumptions required for ANOVA. A statistical significance level of $\alpha = 0.05$ was employed for all analyses, except where the same data were used in multiple ANOVA analyses, where a Bonferroni correction was applied to adjust the significance level to control for inflated Type II errors (Holm 1979). Where ANOVA results were significant, *post hoc* comparisons of pairs of means were undertaken using a Holm–Sidak test. All data have been presented as mean ± standard error.

Results

In total, 449 phyllosomas from Stages V to IX were caught from the two CEs ($n = 94$) and two AEs ($n = 355$) (Table 1).

Comparison of phyllosomas between similar eddies

Comparisons for each of the morphometric and biochemical variables (i.e. cephalic shield width, body length, dry mass, wet

Table 1. Total catch of phyllosomas of each larval developmental stage, location of capture with estimated distance from the shore of the four phyllosoma sampling sites, and average water temperature at 40- and 100-m depth recorded from CTD (conductivity, temperature and depth) casts

Eddy	Latitude	Longitude	Distance from the shore (km)	Average temperature at 40 m (°C)	Average temperature at 100 m (°C)	Number of phyllosomas				
						Stage V	Stage VI	Stage VII	Stage VIII	Stage IX
AE #1	−31.14	111.38	350	20.05	20.03	5	69	71	28	1
AE #2	−30.32	113.11	150	21.18	20.69	0	25	73	83	0
CE #1	−29.48	112.10	250	19.56	19.19	0	6	10	17	0
CE #2	−31.17	114.18	100	18.36	18.13	1	16	23	20	1

Table 2. Results of two-way ANOVAs applied to compare the effects of stage (VI, VII, VIII) and location (cyclonic eddy (CE) #1 and #2) on the morphometric and biochemical parameters of western rock lobster phyllosoma (*Panulirus cygnus*) sampled from two CEs in the Leeuwin Current off Western Australia

Significant effects are shown in bold

Source of variation	Stage			Location			Stage × Location		
	d.f. = 2			d.f. = 1			d.f. = 2		
	SS	*F*-value	*P*-value	SS	*F*-value	*P*-value	SS	*F*-value	*P*-value
Wet mass (mg)	**0.253**	**5.761**	**0.008**	0.011	0.507	0.482	0.031	0.702	0.503
Dry mass (mg)	0.132	3.658	0.038	0.000	0.025	0.874	0.013	0.359	0.701
Width (mm)	**24.034**	**23.301**	**<0.001**	0.698	1.354	0.254	0.202	0.196	0.823
Length (mm)	**0.080**	**23.900**	**<0.001**	0.003	1.598	0.216	0.001	0.222	0.802
Protein (mg)	**0.155**	**4.525**	**0.019**	0.002	0.100	0.754	0.008	0.240	0.788
Lipid (mg)	**0.558**	**8.228**	**0.001**	0.005	0.161	0.691	0.036	0.534	0.592
Carbohydrate (mg)	**0.070**	**6.075**	**0.006**	0.003	0.466	0.500	0.008	0.695	0.507
Lipid : protein	**0.063**	**6.196**	**0.006**	0.001	0.192	0.664	0.010	0.980	0.387
Energy (kJ)	**0.401**	**8.324**	**0.001**	0.004	0.167	0.686	0.020	0.408	0.668

Table 3. Results of 2-way ANOVAs applied to compare the effects of stage (VI, VII, VIII) and location (anticyclonic eddy (AE) #1 and #2) on the morphometric and biochemical parameters of western rock lobster phyllosoma (*Panulirus cygnus*) sampled from two AEs in the Leeuwin Current off Western Australia

Significant effects are shown in bold

Source of variation	Stage			Location			Stage × Location		
	d.f. = 2			d.f. = 1			d.f. = 2		
	SS	*F*-value	*P*-value	SS	*F*-value	*P*-value	SS	*F*-value	*P*-value
Wet mass (mg)	**1703.163**	**7.520**	**0.002**	35.522	0.314	0.580	80.258	0.354	0.705
Dry mass (mg)	33.309	3.748	0.035	21.252	4.783	0.037	0.699	0.079	0.925
Width (mm)	**15.187**	**22.967**	**<0.001**	0.303	0.918	0.346	0.657	0.994	0.382
Length (mm)	**108.298**	**26.484**	**<0.001**	2.421	1.184	0.285	3.194	0.781	0.467
Protein (mg)	**2.604**	**4.251**	**0.024**	1.042	3.403	0.075	0.011	0.018	0.982
Lipid (mg)	**4.952**	**8.364**	**0.001**	1.138	3.843	0.059	0.019	0.032	0.969
Carbohydrate (mg)	0.008	0.489	0.618	0.006	0.787	0.382	0.011	0.712	0.499
Lipid : protein	**0.140**	**10.542**	**<0.001**	0.027	4.119	0.051	0.003	0.202	0.818
Energy (kJ)	**0.019**	**7.637**	**0.002**	0.004	3.500	0.071	0.000	0.009	0.991

mass, protein, lipid, and carbohydrate content) for the three stages of phyllosomas (i.e. Stages VI, VII and VIII) found no significant differences among any of these means between the two CEs (2-way ANOVA, Table 2). This was also the case for the two AEs (2-way ANOVA, Table 3). Consequently, the data were pooled for each type of eddy for subsequent comparison of CEs with AEs.

Phyllosomas from CEs v. *AEs: phyllosoma morphometrics*

Comparison of mean phyllosoma cephalic shield width and body length showed overall significant differences by stage, but not for CEs *v*. AEs, or for the interaction term (2-way ANOVA, Table 4).

There was a significant increase in mean cephalic shield width from phyllosoma Stages VI to VII (0.7 mm ± 0.2, 9.6% ± 2.9 wider, $P < 0.001$), from Stages VII to VIII (1.0 mm ± 0.3, 13.0% ± 3.8 wider, $P < 0.001$), and from Stages VI to VIII ($P < 0.001$), regardless of location of capture. The body length of phyllosomas increased significantly from Stages VI to VII (1.5 mm ± 0.4, 9.5% ± 2.9 longer, $P = 0.001$), from Stages VII to VIII (3.0 mm ± 0.7, 17.0% ± 4.1, $P < 0.001$), and from Stages VI to VIII ($P < 0.001$), regardless of location of capture. The body length of the analysed phyllosomas ranged from 14.0 to 18.8 mm, from 14.7 to 20.9 mm, and from 17.8 to 26.4 mm for Stages VI, VII and VIII respectively.

Phyllosomas from CEs v. *AEs: phyllosoma mass*

Comparisons of both mean wet and mean dry mass showed significant differences for CEs *v*. AEs, significant increases by stage, but not for the interaction term (2-way ANOVA, Table 4).

The mean wet mass of phyllosomas for CEs was significantly higher than that for AEs ($P = 0.002$, $P = 0.024$, $P = 0.002$ for Stages VI, VII, and VIII respectively; Fig. 2). The mean wet mass of phyllosomas from CEs increased significantly from Stages VI to VIII ($P = 0.002$), and from Stages VII to VIII ($P = 0.013$), and in AEs from Stages VI to VIII ($P = 0.001$). Over all four eddies, the wet mass of sampled Stage VII phyllosomas was 18.6% ± 10.2 greater than that of Stage VI, and wet masses of Stage VIII phyllosomas were 37.4% ± 7.7 greater than those of Stage VII.

Table 4. Results of 2-way ANOVAs applied to compare the effects of stage (VI, VII, VIII) and eddy (cyclonic (CE) and anticyclonic (AE) eddy) on the morphometric and biochemical parameters of western rock lobster phyllosoma (*Panulirus cygnus*) sampled from CEs and AEs in the Leeuwin Current off Western Australia

Significant effects are shown in bold

Source of variation	Stage			Eddy			Stage × Eddy		
	d.f. = 2			d.f. = 1			d.f. = 2		
	SS	*F*-value	*P*-value	SS	*F*-value	*P*-value	SS	*F*-value	*P*-value
Wet mass (mg)	**0.487**	**13.873**	**<0.001**	**0.455**	**25.903**	**<0.001**	0.011	0.301	0.741
Dry mass (mg)	**0.180**	**6.748**	**0.002**	**0.289**	**21.612**	**<0.001**	0.017	0.638	0.532
Width (mm)	**0.366**	**44.372**	**<0.001**	0.009	2.265	0.137	0.026	3.120	0.051
Length (mm)	**0.136**	**49.347**	**<0.001**	0.002	1.277	0.262	0.007	2.451	0.094
Protein (mg)	**0.350**	**14.073**	**<0.001**	**0.107**	**8.567**	**0.005**	0.026	1.036	0.361
Lipid (mg)	**0.863**	**16.554**	**<0.001**	**0.300**	**11.509**	**0.001**	0.055	1.058	0.353
Carbohydrate (mg)	**0.060**	**4.544**	**0.014**	0.009	1.423	0.237	0.018	1.353	0.266
Lipid : protein	**0.116**	**14.938**	**<0.001**	**0.0488**	**12.569**	**<0.001**	0.00615	0.791	0.457
Energy (kJ)	**0.6**	**15.823**	**<0.001**	**0.201**	**10.592**	**0.002**	0.0415	1.094	0.341

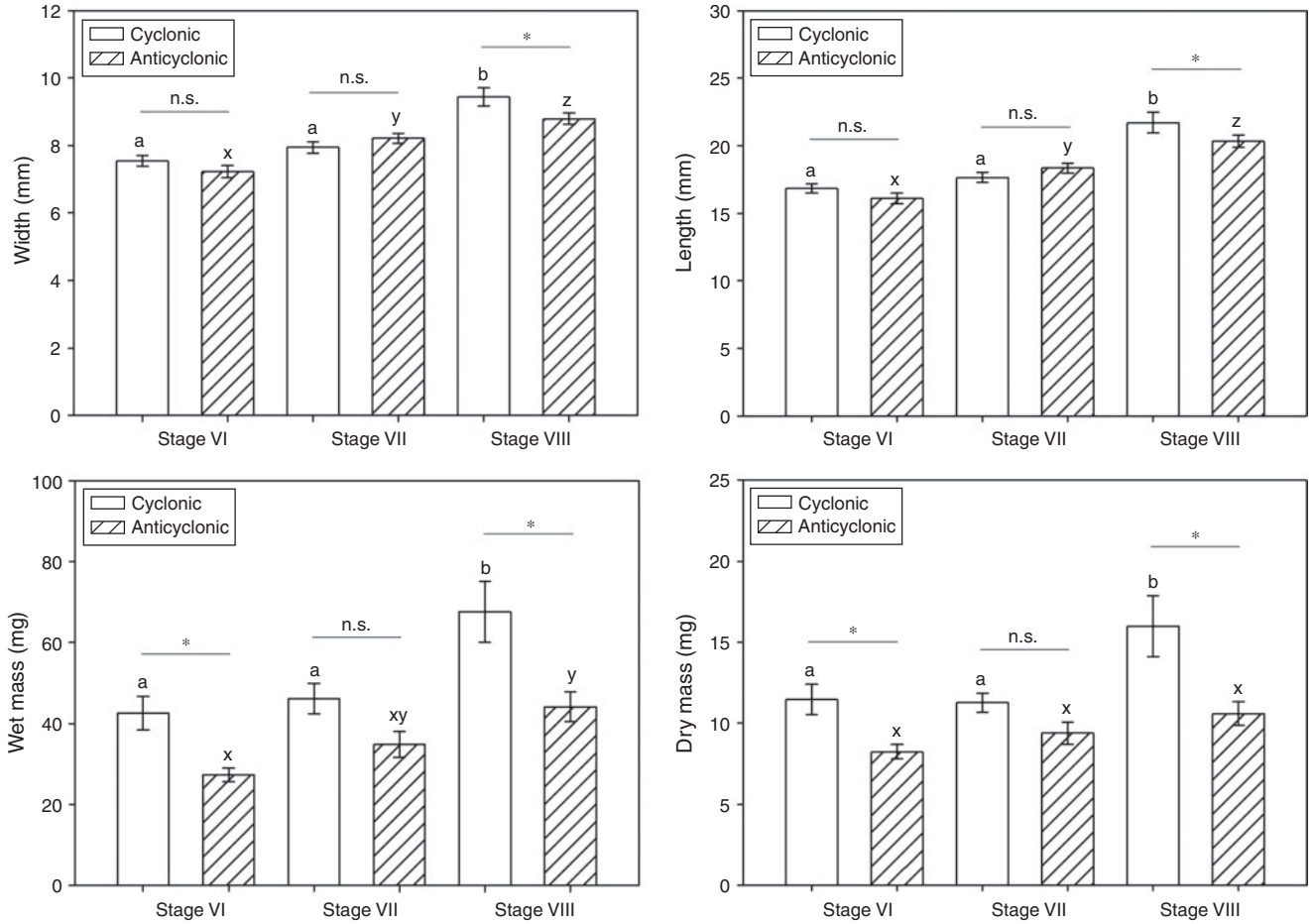

Fig. 2. Mean (±s.e.) cephalic shield width, body length, body wet mass and dry mass for phyllosomas of *Panulirus cygnus* of three larval stages (VI, VII, VIII) sampled from cyclonic (CEs) and anticyclonic (AEs) eddies off Western Australia. Significant ($P < 0.05$) results of pairwise comparisons of means between CEs and AEs for each of the three larval stages are represented by as asterisk, with Bonferroni correction applied; n.s., non-significant. Significant ($P < 0.05$, with Bonferroni correction applied) results of pairwise comparisons of means between each of the three larval stages within each of the two types of eddies are represented by those mean values not possessing an alphabetical letter in common.

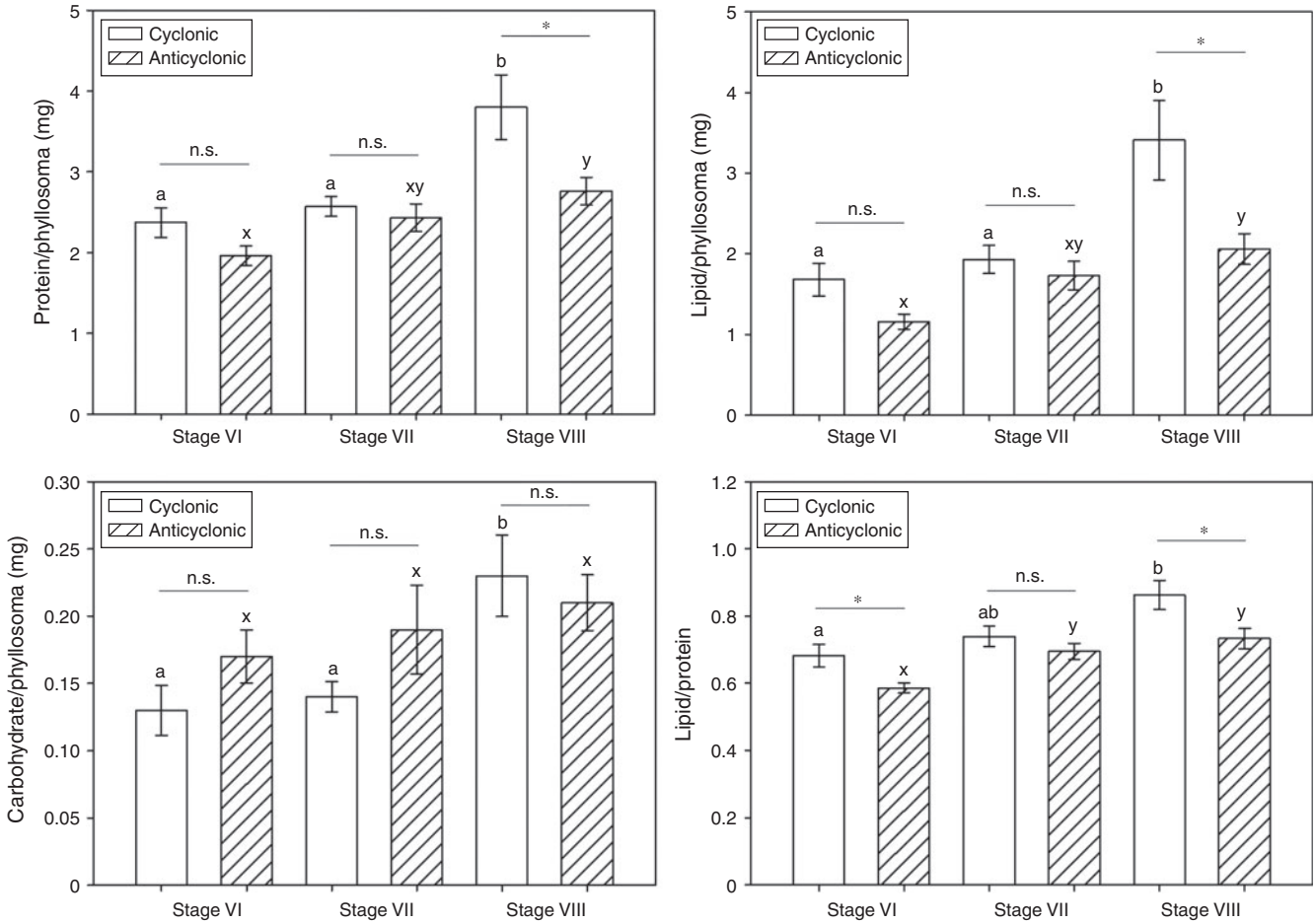

Fig. 3. Mean (±s.e.) protein, lipid and carbohydrate amount (larvae^{-1}), and lipid : protein ratio of phyllosomas of *Panulirus cygnus* of three larval stages (VI, VII, VIII) sampled from cyclonic (CEs) and anticyclonic (AEs) eddies off Western Australia. Significant ($P < 0.05$) results of pairwise comparisons of means between CEs and AEs for each of the three larval stages are represented by an asterisk, with Bonferroni correction applied; n.s., non-significant. Significant ($P < 0.05$) results of pairwise comparisons of means between each of the three larval stages within each of the two types of eddies are represented by those mean values not possessing an alphabetical letter in common.

The mean dry mass of phyllosomas from CEs was significantly higher than that from AEs for Stages VI and VIII ($P = 0.006$, $P = 0.001$; Fig. 2). There was a significant increase in the mean dry mass of phyllosomas from CEs from Stage VI to Stage VIII ($P = 0.024$), and from Stage VII to Stage VIII ($P = 0.017$).

Phyllosomas from CEs v. *AEs: proximate composition*

Comparisons of both mean lipid and protein contents per phyllosoma showed significant differences between CEs and AEs, and increases by larval stage, but not for the interaction term (2-way ANOVA, Table 4). The mean carbohydrate content per phyllosoma showed significant increases by stage, but there was no difference between CEs and AEs, or for the interaction term (2-way ANOVA, Table 4).

The mean lipid content per phyllosoma for CEs was significantly higher than that for AEs for Stage VIII ($P = 0.005$; Fig. 3). There was a significant increase of lipid content of phyllosoma from CEs from Stages VII to VIII ($P = 0.004$) and from Stages VI to VIII ($P < 0.001$), and in AEs from Stages VI to VIII ($P = 0.002$; Fig. 3).

The mean protein content per phyllosoma for CEs was significantly higher than that for AEs for Stage VIII ($P = 0.008$; Fig. 3). There was a significant increase of protein content in phyllosoma from CEs from Stages VI to VIII ($P < 0.001$), and from Stages VII to VIII ($P = 0.003$), and in AEs from Stages VI to VIII ($P = 0.006$; Fig. 3).

For all of the analysed phyllosomas, regardless of location of capture, mean carbohydrate content (larva^{-1}) of Stages VI, VII and VIII was respectively 0.15 mg ± 0.06, 0.16 mg ± 0.08 and 0.22 mg ± 0.08 (Fig. 3). There was a significant increase of carbohydrate content for phyllosomas from CEs from Stages VI to VIII ($P = 0.015$), and from Stages VII to VIII ($P = 0.015$; Fig. 3).

Phyllosomas from CEs v. *AEs: lipid : protein ratio*

Comparison of mean lipid : protein ratio showed significant differences for CEs *v.* AEs, and significant increases by stage,

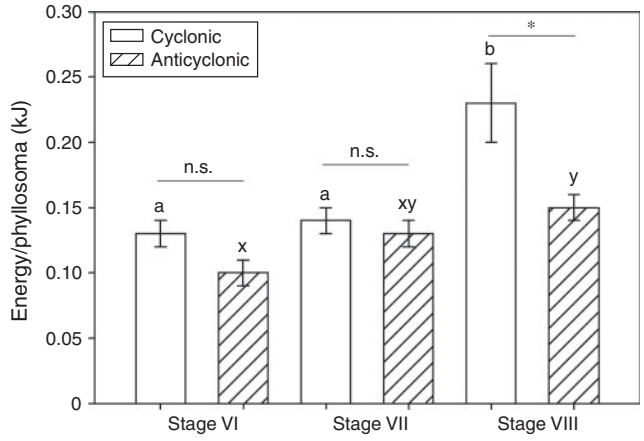

Fig. 4. Mean (±s.e.) total estimated energy content (larvae^{-1}) of phyllosomas of *Panulirus cygnus* of three developmental stages (VI, VII, VIII) sampled from cyclonic (CEs) and anticyclonic (AEs) eddies off Western Australia. Significant ($P < 0.05$) results of pairwise comparisons of means between CEs and AEs for each of the three larval stages are represented by an asterisk, with Bonferroni correction applied; n.s., non-significant. Significant ($P < 0.05$, with Bonferroni correction applied) results of pairwise comparisons of means between each of the three larval stages within each of the two types of eddies are represented by those mean values not possessing an alphabetical letter in common.

but not for the interaction term (2-way ANOVA, Table 4). The lipid : protein ratio of phyllosomas from CEs was significantly higher than that of phyllosomas from AEs for Stage VI ($P = 0.018$) and Stage VIII ($P = 0.009$). There was a significant increase of lipid : protein ratio for phyllosomas from CEs from from Stages VI to VIII ($P < 0.001$), and from AEs from Stages VI to VIII ($P = 0.001$), and from Stages VI to VII ($P = 0.012$).

Phyllosomas from CEs v. *AEs: energy content*

Comparison of mean energy content per phyllosoma showed significant differences for CEs *v*. AEs, and significant increases by stage, but not for the interaction term (2-way ANOVA, Table 4). The estimated mean energy content of phyllosomas for CEs was significantly higher than that for AEs for Stage VIII ($P = 0.005$) (Fig. 4). There was a significant increase of energy content for phyllosomas from CEs from Stages VI to VIII ($P < 0.001$), and from Stages VII to VIII ($P = 0.003$), and for phyllosomas from AEs from Stages VI to VIII ($P = 0.003$).

Discussion

Comparison between CEs or AEs replicates

Within each of the three stages of phyllosoma analysed (i.e. Stages VI, VII and VIII), there was little or no difference in the morphometric measures or biochemical condition of larvae sampled from two CEs, or from two AEs. This strongly suggests that metabolism and feeding conditions for phyllosomas are similar between the two CEs, and between the two AEs, despite the considerable differences in their distance offshore and geographic separation from one another, that is, the centres of eddies being more than 150 km apart. Furthermore, the distances between these eddies indicate that they would have been formed at different times because counter-rotating mesoscale eddies meander westward after being formed along the margins of the shelf (Fang and Morrow 2003).

Phyllosomas from CEs v. *AEs*

Phyllosomas from CEs were consistently larger in wet and dry mass, and contained more protein, lipid and total energy on average than did those from AEs for all three larval stages. These results indicated that phyllosomas in CEs are in better nutritional condition than those in AEs, especially at Stage VIII when these differences had become substantial. In addition, the higher lipid : protein ratio of phyllosomas in CEs than in AEs could suggest that there is better food availability for phyllosomas in CEs than in AEs, because lipid is known to be catabolised at a greater rate than protein and carbohydrate during food deprivation of phyllosoma (Ritar *et al.* 2003). Furthermore, experimental feeding of *P. cygnus* phyllosomas of the same developmental stages as examined in this current study showed that lipid is rapidly accumulated when prey are readily available (Saunders *et al.* 2012). To the contrary, previous research has shown that the CEs of the Leeuwin Current generally exhibit oligotrophic oceanic conditions by global standards, whereas AEs have been classed as mesotrophic in nature (Waite *et al.* 2007*a*, Waite *et al.* 2007*b*). As a result, the AEs have consistently been found to be more biologically productive than are CEs, supporting significantly higher zooplankton biomass and more abundant mesozooplankton, which could be expected to provide much better feeding conditions for a generalist predator of zooplankton, such as phyllosomas. The study of the fatty acid profiles of phyllosomas from CEs and AEs suggested that the microbial food web operating in cyclonic eddies may provide better feeding conditions for lobster larvae, which could explain this apparent contradiction (Wang *et al.* 2014).

On average, there is substantially more lipid per larva for the phyllosomas from CEs than for those from AEs for stage VIII. These results suggest that CEs are likely to produce pueruli in a better nutritional condition in readiness to actively migrate across the continental shelf to settle and recruit on the coast (Jeffs *et al.* 1999). For example, the average of 1.4 mg of additional lipid measured in Stage VIII phyllosomas from CEs *v*. AEs has the potential to support an estimated additional 35 km of cross-shelf migration for pueruli according to previous measures of energy consumption rate of 1.6 J km^{-1} (Phillips *et al.* 2006). Although this figure may vary if assistance is provided to pueruli by known shoreward-directed physical processes (Wilkin and Jeffs 2011). Regardless, the extent of the nutritional reserves available to the non-feeding pueruli of spiny lobsters appears to play a critical role in their successful completion of cross-shelf migration (Jeffs *et al.* 2001*a*, 2001*b*; Phillips *et al.* 2006; Fitzgibbon *et al.* 2013).

Besides nutrition, water temperature is the most important extrinsic factor in the regulation of larval growth of decapod crustaceans (Anger 1998). In some crustacean species, larvae tend to grow larger in colder *v*. warmer water, corresponding with Bergman's Rule (Shirley *et al.* 1987; Furota 1996). This is thought to be due to decreasing temperature resulting in enlarged cell size, slowed development and prolonged life span, all of which lead to an increase in maximum body size (Timofeev 2001). The higher mass of phyllosomas from CEs

than from AEs in the current research may have been caused by the lower temperatures experienced in these cold-core CEs (Table 1). A previous study identified an inverse relationship between mean size of Stage VI phyllosomas of *P. cygnus* and the sea surface temperature at the location from which they were sampled (Ritz 1972). In addition, it has been found that at higher temperatures, overall feed consumption of cultured mid-stage phyllosomas decreased owing to reduced intermoult duration, resulting in reduced growth (Tong *et al.* 2000), and, consequently, the total larval duration has been found to decrease with increasing culture temperature (Matsuda and Yamakawa 1997). Furthermore, a distinct downward shift in the temperature optimum for the growth of late-stage phyllosomas has been reported in both *P. japonicas* and the packhorse spiny lobster, *Sagmariasus verreauxi* (Matsuda and Yamakawa 1997; Fitzgibbon and Battaglene 2012). At lower temperatures, phyllosomas had a lower metabolic rate but maintained their feed intake, providing for greater energy accumulation (Fitzgibbon and Battaglene 2012). The late-stage phyllosomas of *S. verreauxi* were shown to have a narrow temperature optima. Operating beyond the optima over the period of many weeks may lead to marked differences in their cumulative nutritional condition. Therefore, the cooler water temperatures in the CEs than in the AEs could be responsible for increasing the overall size and nutritional condition of phyllosomas at the same relative developmental stage.

Sea surface temperatures in the ocean region off the Western Australian coast have been warming at ~0.1°C per decade since the 1950s (Pearce and Feng 2007). It is possible that the elevated water temperatures associated with climate change in this ocean region may be exceeding the temperature optima for the development of late-stage *P. cygnus* phyllosomas, and, in turn, contributing to a decline in larval nutritional condition and subsequent successful shoreward migration and settlement of pueruli (Pearce and Feng 2007; Poloczanska *et al.* 2007; Caputi *et al.* 2010). If so, constrained nutritional condition caused by the physiological effects of elevated water temperatures could be expected to be more pronounced among those larvae living in AEs, which have generally warmer water temperatures than those in CEs. This explanation is consistent with our observations of the consistent differences in nutritional condition of larvae between the two types of eddies, but remains to be empirically tested.

Developmental stages

The analyses of biochemical content in wild phyllosomas from Stage VI to VIII in the present study highlighted some key developmental changes in the nutritional content of larvae, especially in lipid, which was accumulated rapidly in larvae from Stages VI to VIII (Fig. 3). Lipid is used as the major energy store during larval development, probably because of its high energy density and buoyancy compared with protein and carbohydrate, which both yield approximately half the energy per unit of mass compared with lipid (Crisp 1976). High lipid concentrations of late-stage *P. cygnus* phyllosomas (Stage IX) (19.8–29.3% DW; Limbourn and Nichols 2009), (7.0–22.4% DW; Phillips *et al.* 2006), and of final-stage *J. edwardsii* phyllosomas (18.6–34.4%; Jeffs *et al.* 2004) have been previously reported. Phyllosomas accumulate lipid as an energy store during their final developmental stages to power the non-feeding post-larval or puerulus stage, which undertakes an energy-intensive migration across the continental shelf (Jeffs *et al.* 1999, 2001*b*, 2004). Significant increases of lipid content of phyllosomas of *P. cygnus* were found during development from Stage VI to Stage VIII for both CEs and AEs in this current study. This amounted to an average increase of 0.41 mg (±0.20) of lipid per larva (33.9% ± 14.6) from Stage VI to VII (Fig. 3), and an even larger accumulation of lipid from Stage VII to Stage VIII of 0.9 mg (±0.33) per larva (48.4% ± 17.5), on average, over all of the phyllosomas analysed.

In other species of spiny lobster, such as *J. edwardsii*, phyllosomas from mid-developmental stages (Stages VI and VII) are thought to be capable of tackling and consuming more active prey such as krill to provide for more rapid accumulation of lipid (Jeffs *et al.* 2004). There are also large increases in the activity of digestive enzymes from this stage, which could be related to an increase in food availability or prey-handling proficiency that precedes a large increase in phyllosoma size (Johnston *et al.* 2004). The results of the current study confirmed that there is pronounced lipid accumulation from Stage VI to VIII in phyllosomas of *P. cygnus*. This result suggests a possible change in diet in late-stage phyllosomas to prey with a high lipid yield, such as krill, as there is a narrow range of potential zooplankton prey in the wild with high lipid content (Saunders *et al.* 2012; Wang *et al.* 2013).

Overall, late-stage phyllosomas of *P. cygnus* in CEs of the Leeuwin Current are larger and in a substantially better nutritional condition than are those in AEs. These marked differences could be related to the ability of phyllosomas to accumulate nutritional reserves in higher water temperatures associated with AEs, and this needs further investigation. Reduced nutritional condition, especially lipid, of the subsequent post-larval (puerulus) stage has the potential to reduce the chances of successful migration, settlement and eventual recruitment into the coastal fishery for this lobster species. A similar process may also be in operation in other important species of spiny lobsters world-wide which have recently been shown to have declining post-larval settlements, and for which the nutritional condition of the post-larvae is also thought to play a key role in their successful settlement.

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