1 **Morphometric relationships and seasonal variation in size, weight, and a condition index of**
2 **post-settlement stages of the Caribbean spiny lobster**
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19 **ABSTRACT**

20 Spiny lobsters have a protracted pelagic, oceanic larval phase. The final larval stage

21 metamorphoses into a non-feeding postlarva (puerulus) that actively swims towards the coast to

22 settle in shallow habitats and does not resume feeding until after the molt into the first-stage

23 juvenile. Therefore, the body dimensions and nutritional condition of both settled pueruli and first

24 juveniles are likely to vary over time, potentially playing a crucial role in the recruitment to the

25 benthic population. We compared carapace length (CL), height (CH), and width (CW); total

26 length (TL), and body weight (W) between pueruli and first juveniles of the Caribbean spiny

27 lobster, *Panulirus argus*, as well as morphometric relationships between both developmental

28 stages. Except for CL, all other dimensions were larger in first juveniles, but most markedly CH

29 and W. The slopes of the CH vs CL, CW vs CL, and W vs CL regressions differed significantly

30 between stages, and all log-transformed relationships showed isometry in both stages, except for

31 the CH vs CL relationship, which showed positive allometry. These results reflect a

32 morphological change from the flatter, more streamlined body of the puerulus, to the heavier,

33 more cylindrical body of the juvenile. We also analyzed seasonal variations in CL, W, the W/CL

34 index (a morphometric condition index), and a modified W/CL index (i.e. after controlling for a

35 significant effect of CL) of both stages using individuals monthly collected over 12 consecutive

36 seasons (Autumn 2010–Summer 2013). In both stages, all three variables exhibited significant

37 seasonal variation. For pueruli, the modified W/CL index differed from average in only two

38 seasons, winter 2011 (higher) and summer 2013 (lower), but showed great within-season

39 variation (larger coefficients of variation, CVs), potentially reflecting variability in nutritional

40 condition of larvae prior to metamorphosis and in the distances swum by individual pueruli to the

41 settlement habitats. For first juveniles, the modified W/CL index was higher than average in

42 winter and spring 2011, and lower in autumn 2011 and winter 2012, but showed less within-

43 season variation (smaller CVs), suggesting a combination of carry-over effects of puerulus

44 condition and effects of local conditions (e.g. food availability and predation risk). These findings

45 warrant further investigation into factors potentially decoupling settlement from recruitment

46 processes.

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48 **INTRODUCTION**

49 Spiny lobsters (family Palinuridae) are large decapod crustaceans that constitute valuable fishing

78   resources wherever they occur (Holthuis, 1991). Palinurids have a protracted oceanic larval phase

79   that consists of multiple flattened, leaf-like larval stages (phyllosoma) morphologically very

80   different from the adults (Phillips et al., 2006). The final phyllosoma stage metamorphoses into a

81   highly transparent swimming postlarva (the puerulus), morphologically similar to the adult. This

82   metamorphosis has been considered the most dramatic morphological transformation in a single

83   molt across crustaceans (Gurney, 1942). The puerulus is a secondary lecithotrophic (i.e., non-

84   feeding) phase, consisting of a single stage that actively swims to the coast, where it settles in

85   shallow habitats (Phillips et al., 2006). After settlement, the transparent postlarvae gradually

86   become pigmented and the molt from puerulus to first-stage juvenile (hereafter "first juvenile")

87   occurs within days or weeks. The first juveniles resume feeding shortly after molting.

88   During the post-settlement molt from puerulus to the first juvenile stage, the body of the

89   lobster undergoes substantial changes that make it more suitable for a benthic existence. Most

90   papers reporting on such changes have put emphasis on the development of the mouthparts and

91   other external and internal structures (e.g., Lemmens & Knott, 1994; Nishida, Takahashi &

92   Kittaka, 1995; Abrunhosa & Kittaka, 1997; Cox, Jeffs & Davis, 2008), but changes in

93   morphometric dimensions and allometry have been much less explored (Grobler & Ndjaula,

94   2001; Groeneveld et al., 2010; Anderson et al., 2013).

95   The Caribbean spiny lobster, *Panulirus argus* (Latreille, 1804), occurs throughout the

96   wider Caribbean region and is by far the most heavily fished of the 24 extant species of spiny

97   lobsters (Phillips et al., 2013). The larval phase of *P. argus* comprises 10 phyllosoma stages

98   (Goldstein et al., 2008) and metamorphosis occurs in oceanic waters. The puerulus swims to the

99   coast and settles in shallow vegetated substrates (macroalgal beds, seagrass meadows, coastal

100   mangroves), where the early benthic juveniles remain for a few months before shifting to coral

101   reef habitats (Butler & Herrnkind, 2000). Lewis, Moore & Babis (1952) described the puerulus

102   and first few juvenile stages of *P. argus*, and Anderson et al. (2013) examined allometric growth

103   of defensive and reproductive structures through ontogeny in *P. argus*, but to our knowledge the

104   relationships between weight and size dimensions and allometry in postlarvae and first juveniles

105   of this species have not been explored.

106   Artificial collectors mimicking the natural settlement habitats have been devised to

107   monitor long-term levels of puerulus settlement (Phillips & Booth, 1994). In a few spiny lobster

108   species from temperate or cold water regions, in which reproduction and postlarval settlement are

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strongly seasonal, the settlement data have shown relationships with the commercial catch after a lag of several years, opening the possibility of developing catch predictive models (reviewed in Phillips et al., 2013). However, this has not been the case for *P. argus*, suggesting that for tropical species characterized by year-long reproduction and postlarval settlement, a number of factors potentially mask or decouple the relationship between levels of settlement and abundance of subsequent benthic phases (Herrnkind & Butler, 1994; Briones-Fourzán, Candela & Lozano-Álvarez, 2008). These factors may include quality of settlement habitat, food availability, and predation mortality, as well as variability in nutritional condition of settling pueruli.

In crustaceans, the nutritional condition is the extent to which an individual has accumulated reserves of nutrients to allow normal physiological function and growth (Moore, Smith & Loneragan, 2000). Nutritional condition can be an indicator of past foraging success and ability to cope with environmental pressures, and can greatly affect population dynamics (Jakob, Marshall & Uetz, 1996). Because pueruli do not feed, their post-settlement nutritional condition will depend on the nutrient reserves accumulated prior to metamorphosis and the energetic cost of swimming to the coastal settlement habitats (McWilliam & Phillips, 1997; García-Echauri & Jeffs, in press). If pueruli settle with a very low nutritional condition, they may die before, during, or soon after molting into first juveniles, precluding their recruitment to the benthic populations (Lemmens, 1994; Jeffs, Nichols & Bruce, 2001; Fitzgibbon, Jeffs & Battaglene, 2014). After molting, the first juveniles need to resume feeding to replenish their energy sources, but this may depend on local availability of food and predation risk (Smith & Herrnkind, 1992; Weiss, Lozano-Álvarez & Briones-Fourzán, 2008), which may further reduce the nutritional condition of first juveniles, potentially precluding their molt into second-stage juveniles (Espinosa-Magaña, Lozano-Álvarez & Briones-Fourzán, 2017). Therefore, monitoring the levels of nutritional condition of benthic pueruli and first juveniles may provide insight into the potential factors decoupling the relationships between the levels of settlement and the abundance of subsequent benthic phases of *P. argus*.

Several studies have examined the use of nutrients (lipids, proteins, and carbohydrates) during the transition from phyllosoma to puerulus (e.g., Jeffs, Wilmott & Wells, 1999; Limbourn & Nichols, 2009; Espinosa-Magaña et al., 2018), or the contents of these nutrients as biochemical indicators of nutritional condition over time in settled pueruli and first juveniles (Limbourn et al., 2009). However, this type of biochemical analyses destroys the animals and can be expensive and

time-consuming, making their use impractical to monitor condition over the long term. Other biochemical nutritional indices, such as blood refractive index, can be accurate but are strongly affected by the molt cycle (Oliver & MacDiarmid, 2001; Lorenzon, Martins & Ferrero, 2011). Alternative morphometric techniques avoid injuring the animals and can be more practical to use over the long term (Jakob, Marshall & Uetz, 1996). Some studies have found significant variation in the size of benthic (settled) pueruli over time (e.g., Booth & Tarring, 1986; Yeung et al., 2001, Groeneveld et al., 2010), but size alone is not a good reflection of nutritional condition.

One of the simplest morphometric indices of condition is the "ratio index", which has long been used to assess nutritional condition in fishes (Froese, 2006) and is calculated as body mass divided by a linear dimension of body size. The ratio index has also been used in crustaceans such as prawns and lobsters, in which this index is usually estimated as the total weight of the animal (W) divided by its carapace length (CL). CL is typically preferred over total length because, unlike the highly flexible abdomen, the rigid carapace can be measured with less error. Therefore, we herein refer to this index as the "W/CL index". The W/CL index has been shown to reflect nutritional condition in shrimps (Araneda, Pérez & Gasca-Leyva, 2008), prawns (da Rocha et al., 2015), and spiny lobsters (Robertson, Butler & Dobbs, 2000; Oliver & MacDiarmid, 2001; Briones-Fourzán, Baeza-Martínez & Lozano-Álvarez, 2009; Lopeztegui-Castillo, Capetillo-Pinar & Betanzos-Vega, 2012). This is because unlike the blood refraction index, the W/CL index is independent of the molt cycle and may be a more direct reflection of nutritional health (Oliver & MacDiarmid, 2001).

The W/CL index is also useful for comparing the nutritional status between groups or populations (e.g. Robertson, Butler & Dobbs, 2001), although Briones-Fourzán, Baeza-Martínez & Lozano-Álvarez (2009) showed that its use for these purposes was only warranted when the size range of the groups was similar because the W/CL index increases with size. Given that pueruli and first juveniles have a small and similar size range, the W/CL index could be useful for long-term monitoring of their condition. Therefore, the aims of the present study were twofold: to compare several morphometric relationships between pueruli and first juveniles of *P. argus* and examine allometric changes during this ontogenetic transition, and to examine seasonal variations in size, weight, and the W/CL index of recently settled pueruli and first juveniles of *P. argus*. We predicted that both pueruli and first juveniles would exhibit significant variation in all three variables, the former because of the varying environmental conditions during their long

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217 larval phase and the potential variation in the distance swum by different individuals, and the

218 latter because of carry-over effects (Marshall & Morgan, 2011) in conjunction with local effects

219 after the post-settlement molt (e.g., predation risk, changes in food availability).

MATERIALS AND METHODS

Sampling of organisms

223 Settled pueruli (transparent and pigmented) and first juveniles of *P. argus* were obtained from

224 GuSi-type postlarval collectors (Gutiérrez-Carbonell, Simonín-Díaz & Briones-Fourzán, 1992)

225 that are used to monitor monthly postlarval settlement indices on the Caribbean coast of Mexico

226 (eastern coast of the Yucatan peninsula) (Briones-Fourzán, Candela & Lozano-Álvarez, 2008).

227 These collectors, in sets of six, were deployed leeward of the coral reef tract at two locations:

228 Puerto Morelos (at 20° 50.9' N, 86° 52.1' W) and Bahía de la Ascensión (at 19° 49.8' N, 87°

229 27.1'W). The GuSi collectors simulate intricate marine vegetation and are colonized by very

230 large numbers of small invertebrates (mostly less than 5 mm long), such as isopods, amphipods,

231 shrimps, crabs, ostracods, harpacticoid copepods, small gastropods, nudibranchs, ophiuroids, and

232 many types of worms (Mendoza-Barrera & Cabrera, 1999), thus providing abundant natural prey

233 for the first juveniles after the post-settlement molt (Marx & Herrnkind, 1985; Lalana & Ortiz,

234 1991). A permit for pueruli collection was issued by Comisión Nacional de Acuacultura y Pesca

235 (DGOPA.06695.190612.1737). We had access to all samples taken from collectors at Puerto

236 Morelos between November 2010 and September 2013, but only to samples taken from collectors

237 at Bahía de la Ascensión between August 2012 and September 2013. Individuals were

238 transported in aerated seawater to the laboratory within 1 to 3 h of collection, and categorized

239 into transparent pueruli (which are completely transparent except for the eyes), pigmented pueruli

240 (with dark stripes of coloration in the new cuticle, visible below the old, transparent cuticle), first

241 juveniles (Fig. 1), and older juveniles. First juveniles were distinguished from older juveniles

242 based on morphological differences described by Lewis, Moore & Babis (1952), mainly the

243 length of the medial flagella (inner branch) of the antennules relative to the lateral flagella (outer

244 branch), the appearance of the grooves in the abdominal tergites, and the degree of development

245 of the pleopods. Older juveniles were not further considered in this study.

Morphometric relationships and allometry

247 Morphometric relationships allow testing the relationship between two variables and predicting

the value of one (Y) from the other (X), whereas allometry is useful to examine how one variable scales with another (Warton et al., 2006). To examine morphometric relationships and allometry, we used individuals collected from both locations between August 2012 and September 2013. For these individuals, we measured the following length dimensions: carapace length (CL, from between the rostral horns to the posterior edge of the cephalothorax), carapace height (CH, across the mid-point between the bases of the first pair of pereopods and the highest point in the cephalothorax), carapace width (CW, across the widest part of the cephalothorax), and total length (TL, from between the rostral horns to the posterior edge of the telson) with digital Vernier calipers (± 0.01 mm). All length dimensions were measured under a stereoscopic microscope to reduce measurement error. We also measured total wet weight (W) of individuals with a digital scale (± 0.001 g) after blotting excess moist.

Exploratory analyses revealed that transparent and pigmented pueruli did not differ significantly in any of the body dimensions that we measured (Student's t-tests, p > 0.05 in all cases); therefore, we pooled their data into one category ("pueruli") for further analyses. Prior to regression analyses, we used separate general linear models (GLM) to test for potential effects of location (Puerto Morelos and Bahía de la Ascensión) and developmental stage (pueruli and first juveniles) on each body dimension. As the effect of location was not significant (see Results), we pooled the data from both locations and used ordinary least-squares regression (OLR) to examine morphometric relationships (Warton et al., 2006). For each stage, CH, CW, TL, and W were regressed against CL. For each relationship, the slopes of regressions were compared between stages with Student's t-tests; if the slopes did not differ significantly, we compared the elevations (Zar, 1999). We then used the log-transformed data of all dimensions to test for allometry, which involves testing if the slope equals a specific value (i.e., isometry: $b = 1$ for length-length relationships and $b = 3$ for length-weight relationships) (Hartnoll, 1982). The appropriate method to estimate slopes for this purpose is the reduced major axis regression (RMA, also known as standardized major axis regression) (Warton et al., 2006). Slopes were then tested for departures from isometry with Student's t-tests.

Seasonal variation in CL, W, and W/CL index

To examine seasonal variation in size, weight, and the W/CL index, we used samples from Puerto Morelos only, as this location was sampled uninterruptedly for three years. Given that the monthly catch of the different stages tends to be low or even zero (Briones-Fourzán, Candela &

Lozano-Álvarez, 2008), we pooled the data by astronomical season (spring: March 20 to June 20; summer: June 21 to September 21; autumn: September 22 to December 20; winter: December 21 to March 19) based on the day of the month when the collectors were sampled. This procedure resulted in data from 12 consecutive seasons.

We used separate GLMs to examine the potential effects of season (12 levels) and stage (2 levels, pueruli and first juveniles) on each variable (CL, W, and W/CL). However, because the W/CL index is affected by size (Briones-Fourzán, Baeza-Martínez & Lozano-Álvarez, 2009), the seasonal variation in the W/CL index was further evaluated with an analysis of covariance (ANCOVA), using CL as a covariate to control for its significant effect. Least squares means and confidence intervals for seasonal W/CL were then computed for covariates at their means, yielding a modified W/CL index. For each stage and season, the coefficient of variation ($CV = \left(\frac{SD}{mean}\right) \times 100$) was estimated as a measure of within-seasonal variability.

Statistical analyses were done with the software Statistica v.10 (StatSoft Inc., 2011) except for the OLS and RMA regression analyses, which were done with the software PAST v.3.20 (Hammer, Harper & Ryan, 2001). In all cases, results were considered significant if p < 0.05.

RESULTS

Size distribution, morphometric relationships, and allometry

Between August 2012 and September 2013, we obtained 606 individuals, 380 pueruli (278 from Puerto Morelos and 102 from Bahía de la Ascensión) and 226 first juveniles (154 from Puerto Morelos and 72 from Bahía de la Ascensión). Size ranges of pueruli and first juveniles were 5.01–6.90 mm CL and 5.31–6.98 mm CL, respectively (Fig. 2). Results from the GLMs examining the effects of location and developmental stage on body dimensions (Table 1) indicated that, except for CL, which did not differ significantly between pueruli and first juveniles, all other body dimensions were significantly affected by stage, but not by location, and the interaction term was also not significant. Therefore, the data from both locations were pooled prior to regression analyses.

In general, morphometric relationships of pueruli exhibited a greater dispersion of data around the OLS regression line (Fig. 3A-D), resulting in lower coefficients of determination than for first-stage juveniles (Table 2). In the TL vs CL relationship, the slopes of the OLS regressions

did not differ significantly between pueruli and first juveniles, but the elevations did (Fig. 3A, Table 2). The slopes of all other regressions differed significantly between both stages (Table 2). In particular, W (Fig. 3B) and CH (Fig. 3C) increased more steeply with increasing CL in juveniles compared to pueruli. Results of RMA regressions revealed that for both stages, all relationships with log-transformed data exhibited isometry, except for the Ln CH vs Ln CL relationship in both stages and the Ln CW vs Ln CL relationship in pueruli, which showed positive allometry (Table 3).

Seasonal variation in CL, W, W/CL, and corrected W/L index

Between 20 and 45 pueruli per season, and between 12 and 35 first juveniles per season were obtained from samples at Puerto Morelos between autumn 2010 and summer 2013. Results of GLMs on these data showed a significant effect of both main factors (stage and season) but not of their interaction, on CL, W and W/CL (Table 4). First juveniles had higher mean values of all three variables than pueruli, with a similar trend for both developmental stages over the sampling period (Fig. 4). For both stages, all three variables were low in summer 2011 and tended to decrease between spring 2012 and summer 2013 (Fig. 4). Results of ANCOVA showed that, for each developmental stage, the effect of season on W/CL remained significant after controlling for the significant effect of CL (the covariate) (Table 5). However, the modified W/CL index of pueruli only differed significantly from the average value in two seasons: winter 2011 (higher), and summer 2013 (lower) (Fig. 5A), whereas within-season variability (seasonal CVs) ranged from 10.1% to 11.6%. For first juveniles, the modified W/CL index was more variable over time, with higher than average values in winter and spring 2011, and lower than average values in autumn 2011 and winter 2012 (Fig. 5B), whereas within-season variability ranged from 6.7% to 7.7%.

DISCUSSION

We compared several body dimensions and morphometric relationships between pueruli and first juveniles of *Panulirus argus*, and examined seasonal variation in a morphometric condition index in both stages. When comparing data from Puerto Morelos and Bahía de la Ascensión, location had no effect on any of the measured body dimensions. Stage significantly affected all dimensions with the exception of CL, which remained virtually unchanged after the post-settlement molt, as previously reported by Lewis, Moore & Babis (1952). In contrast, Abrunhosa

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In all, we obtained 823 individuals, 641 from Puerto Morelos (98 transparent pueruli, 311 pigmented pueruli, and 252 first juveniles) and 182 from Bahía de la Ascensión (9 transparent pueruli, 95 pigmented pueruli, and 78 first juveniles). Considering the entire sample, the size range of transparent pueruli, benthic pueruli, and first juveniles was 5.46–6.90 mm CL, 5.14–6.89 mm CL, and 5.0–7.15 mm CL, respectively. Figure 2 shows the great overlap in size range between stages. Exploratory analyses revealed that transparent and pigmented pueruli did not differ significantly in any of the body dimensions that we measured (Student's *t*-tests, p > 0.05 in all cases), supporting their pooling into one category (benthic pueruli).¶

Deleted: The analyses of seasonal variation in CL, W, and the W/CL index encompassed 12 consecutive seasons, from November (autumn) 2010 to September (summer) 2013. During this period, we obtained 329 benthic pueruli and 252 first-stage juveniles at Puerto Morelos. Results of ANOVAs showed a significant effect of both main factors (Developmental stage and Season), but not of their interaction, on each of the three variables (Table 1). First juveniles had higher mean values of all three variables than benthic pueruli, with a similar trend for both developmental stages over the sampling period (Fig. 3). For both stages, all three variables were low in summer 2011 and also tended to decrease between spring 2012 and summer 2013 (Fig. 3). However, results of ANCOVA showed that, after controlling for the significant effect of CL (the covariant), the corrected W/CL index of benthic pueruli varied significantly with season, with higher than average values in autumn 2010 and winter of 2011, and lower than average values in summer 2011 and spring-summer 2013 (Fig. 4A). The corrected W/CL index of first juveniles also varied significantly with season after controlling for the significant effect of CL, with higher than average values in autumn 2010, and winter-spring 2011, and lower than average values in autumn 2011 and winter 2012 (Fig. 4B).¶
Morphometric relationships¶
Results from the factorial ANOVAs examining the effects of location and developmental stage on body dimensions (Table 2) indicated that, except for CL, which did not differ significantly be ... [2]

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& Kittaka (1997) reported an increase in mean CL of 7.2% between the puerulus and first juvenile of *P. cygnus*, and Groeneveld et al. (2010) reported an increase in mean CL of 4–16% in *Jasus lalandii*. Although CW and TL were larger in first juveniles than in pueruli, the main differences between the two stages were an increase in CH and in W after the post-settlement molt. The increase in CH reflects the change from the relatively flattened body of the puerulus, which provides a more streamlined form for this forward-swimming stage, to the more cylindrical body of the benthic juveniles, which lose the ability to swim forward. This ontogenetic change was previously observed by Grobler & Ndjaula (2001) and Groeneveld et al. (2010) in *J. lalandii*. The increase in weight is greatly due to the uptake and incorporation of minerals into the cuticle of the first juveniles, which becomes thicker and heavier (Lemmens, 1995), and also potentially to the development of internal structures (Lemmens & Knott, 1994).

In the morphometric analyses, all dimensions increased linearly with CL and the slopes of all OLS regressions differed between stages with the exception of the TL vs CL regression, in which the intercepts differed significantly. Although the relationship W vs CL is typically described with a power function in spiny lobsters (e.g., Zetina-Moguel, Ríos-Lara & Cervera-Cervera, 1996; Cruz et al., 2007), we used a linear function for this relationship because of the small range in size and weight of pueruli and first juveniles. The small measured range gave rise to wide variability in most relationships (values of $r^2 < 0.7$), as was also the case for W vs CL relationships in pueruli of *J. lalandii* (Grobler & Ndjaula, 2001; Groeneveld et al., 2010). Because allometry was examined for each stage, we expected relationships between log-transformed variables to be isometric. This was the case for all relationships except for Ln CH vs Ln CL, which exhibited positive allometry in both stages, and Ln CW vs Ln CL, which exhibited positive allometry in pueruli. These results suggest that larger pueruli emerge from metamorphosis with a proportionally broader carapace than smaller pueruli, potentially reflecting individual heterogeneity among phyllosomata.

Morphometric changes between the puerulus and the first juvenile could be considered as subtle compared with the dramatic morphological change from phyllosoma to puerulus, but the post-settlement molt does involve considerable structural and morphological changes (Lewis, Moore & Babis, 1952; Lemmens & Knott, 1994; Nishida, Takahashi & Kittaka, 1995; Abrunhosa & Kittaka, 1997; Jeffs, Nichols & Bruce, 2001) not necessarily evident in the body dimensions that we measured. Interestingly, Ventura et al. (2015) found that the genes expressed during the

716 molt between puerulus and first juvenile in *Sagmariasus verreauxi* are the same genes expressed

717 during metamorphosis in other crustaceans, whereas the more dramatic phyllosoma-puerulus

718 morphological shift relies on a different, yet to be identified metamorphic mechanism.

719   Temporal variations in carapace length (CL) of pueruli have been previously documented

720 in spiny lobsters (e.g., *J. edwardsii*: Booth & Tarring, 1986; *J. lalandii*: Grobler & Ndjaula, 2001;

721 Groeneveld et al., 2010; *P. interruptus*: Guzmán del Próo et al., 1996; *P. argus*: Yeung et al.,

722 2001). In New Zealand, pueruli of *J. edwardsii* were larger in winter than during the rest of the

723 seasons (Booth & Tarring, 1986), whereas in Namibia (Grobler & Ndjaula, 2001) and South

724 Africa (Groenveld et al., 2010), pueruli of *J. lalandii* were larger in late summer. In all these

725 cases, the authors offered as possible explanations that phyllosomata metamorphosing in that

726 particular season either found more favorable feeding conditions or had more time to feed and

727 grow, or that pueruli arriving to the settlement habitats in different seasons may have originated

728 from different adult populations.

729   In the present study, the mean CL and W of pueruli of *P. argus* varied throughout the

730 study period, with the lowest values in summer 2011 and in spring-summer 2013. In pueruli of *P.*

731 *argus* collected between June 1997 and June 1999 in Florida, the smaller mean CL values also

732 occurred during the summer (Yeung et al., 2001), a result that was ascribed to variable seawater

733 temperatures and nutritional conditions during larval growth. Espinosa-Magaña et al. (2018)

734 collected nektonic pueruli of *P. argus* offshore of the Mexican Caribbean coast in autumn 2012

735 and spring 2013, and found that the pueruli were larger and contained more lipid reserves in

736 spring than in autumn. These differences were ascribed to a greater availability of basal food

737 resources (phytoplankton) across the western Caribbean between October and March than during

738 April to September, as reported by Melo-González et al. (2000), and/or to the higher water

739 temperatures during the summer, which may reduce the metabolic efficiency and the increase in

740 size at molt of late-stage phyllosomata (e.g., Matsuda & Yamakawa, 1997), potentially resulting

741 in smaller pueruli during the summer months. Indeed, in laboratory experiments, Fitzgibbon &

742 Battaglene (2012) found a downward shift in the optimum temperature for late-stage

743 phyllosomata compared to early- and mid-stage phyllosomata of *S. verrauxi*. This shift involves

744 changes in feeding and energy metabolism, resulting in larger pueruli at lower temperatures.

745   Limbourn et al. (2009) found a significant spatial and temporal variation in total lipid

746 content and fatty acid composition of pueruli and first juveniles of *P. cygnus* in western

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Deleted: TL, W, CH, and CW increased with CL in both stages. The slopes of all regressions differed significantly between developmental stages with the exception of the TL vs CL regression, in which the intercepts differed significantly. Although the relationship W vs CL is typically described with a power function in spiny lobsters (e.g., Zetina-Moguel, Ríos-Lara & Cervera-Cervera, 1996; Grobler & Ndjaula, 2001; Cruz et al., 2007), we used a linear function for this relationship because of the small range in size and weight of benthic pueruli and first juveniles. The small range in these and the rest of the variables that we measured was reflected in a relatively wide variability in some relationships (values of r² < 0.5), as was also the case for W vs CL relationships in pueruli of *J. lalandii* (Grobler & Ndjaula, 2001; Groeneveld et al., 2010). ¶

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Australia, but to the best of our knowledge, the present study is the first to examine temporal variability in a morphometric condition factor for pueruli and first juveniles of any spiny lobster species. Robertson, Butler & Dobbs (2000) experimentally demonstrated that the W/CL index reflected the nutritional condition of early benthic juveniles of *P. argus*, which is why we chose this index. However, as the W/CL index increases with size (Briones-Fourzán, Baeza-Martínez & Lozano-Álvarez, 2009), we modified this index by controlling for the significant effect of size. The modified W/CL index of pueruli was higher than average in winter 2011 and lower than average in summer 2013, yet compared with values for first juveniles, values for pueruli changed relatively less between seasons but exhibited greater within-season variability. This result would be consistent with variations in nutritional condition of late stage phyllosomata prior to metamorphosis in oceanic waters and in the distances potentially swum by individual pueruli to the settlement habitats (Espinosa-Magaña et al., 2018). Genetic variability could also account for some of this variation, as the northern portion of the Mexican Caribbean has been identified as a sink area potentially receiving postlarvae of *P. argus* from multiple Caribbean locations (Briones-Fourzán, Candela & Lozano-Álvarez, 2008; Kough, Paris & Buter, 2013). Another factor possibly involved in the variability in the W/CL index of benthic pueruli is the seasonal variation in the strength of the Yucatan current, which runs very close to the eastern coast of the Yucatan peninsula. This current is one of the swiftest western boundary currents in the world, but exhibits greater velocities in spring-summer than in autumn-winter (Cetina et al., 2006; Carrillo et al., 2015). As the nektonic pueruli would need to cross this current in order to arrive to our sampling locations, this may result in a greater expense of energy resources when the current is stronger (Espinosa-Magaña et al., 2018).

In contrast with pueruli, the modified W/CL index of first juveniles exhibited more between- and less within-season variability. Because first juveniles need to resume feeding after the post-settlement molt to restore energy reserves, these results suggest that their nutritional condition depends to some extent on variation of local conditions of the settlement habitat, such as food availability and predation mortality. In particular, although small juveniles invest more in defensive structures, including disruptive coloration, than larger conspecifics (Anderson et al., 2013), the risk of predation is ever present and may reduce foraging activity of small juveniles (Smith & Herrnkind, 1992; Weiss, Lozano-Álvarez & Briones-Fourzán, 2008) even if food is readily available, as it is in pueruli collectors. Yet, first juveniles of *P. argus* can starve for up to

842 12 days and still be able to molt if they can resume feeding after this period (Espinosa-Magaña,
843 Lozano-Álvarez & Briones-Fourzán, 2017). In addition to the potential effect of local conditions,
844 there might be a meaningful carry-over effect of the previous condition after the post-settlement
845 molt. This is an important issue to consider because conspecific individuals can differ widely in
846 their metabolic phenotype (i.e. their rate of energy metabolism) (Burton et al., 2011), and this
847 phenotypic variation can be more important than variation in larval supply in regulating marine
848 populations (Marshall & Morgan, 2011).

850 **CONCLUSIONS**
851 Pueruli and first juveniles of *P. argus* had a similar range in CL, but the latter were heavier and
852 had a greater CH than the former. As predicted, there was significant seasonal variation in CL,
853 W, and the W/CL index (a morphometric condition factor) of both stages. However, within-
854 season variation in the modified W/CL index was greater for pueruli than for first juveniles,
855 potentially reflecting variability in the condition of larvae prior to metamorphosis and in the
856 distances swum by individual pueruli to the settlement habitat, whereas between-season variation
857 was greater for first juveniles than for pueruli, suggesting a combination of carry-over effects and
858 variation in the effects of local factors (e.g. predation risk and food availability). Whether such
859 differences have any impact on survival of these stages of *P. argus* remains to be determined. For
860 example, validating the results of W/CL index of pueruli and first juveniles with biochemical
861 analyses (lipid and protein contents) would support their continued use to monitor nutritional
862 condition of these stages over the long term, whereas experimental studies could help determine
863 how the condition factor of first juveniles varies in the presence/absence of predation risk at
864 different levels of food availability. These and other investigations are necessary to unravel the
865 complexity of linkages between factors potentially decoupling settlement from recruitment
866 processes.

Deleted: The potential carry-over effects of puerulus condition on fitness of early benthic juveniles and hence on their recruitment to the adult populations warrants further investigation to increase insight into the factors potentially decoupling settlement from recruitment processes.

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Deleted: Benthic transparent and pigmented pueruli of *P. argus* did not differ significantly in any of the body dimensions that we measured (CL, CH, CW, TL, W). After the molt from puerulus to first juvenile, the more marked morphological changes were a significant increase in weight and in carapace height, with a less marked (but significant) increase in total length and carapace width, and virtually no change in carapace length. These morphological changes are reflected in different slopes in the regressions of all body dimensions against CL except for the TL vs CL regression, in which the slopes did not differ but the elevations did. There was significant variation in the CL, W, and W/CL index, a morphometric index of nutritional condition, in both developmental stages. For benthic pueruli, the mean corrected W/CL index showed great within-season variation, potentially reflecting variability in nutritional condition of larvae prior to metamorphosis and in the distances swum by individual pueruli. For first juveniles, the mean corrected W/CL index showed great between-season variation, suggesting a combination of carry-over effects of puerulus condition and effects of local conditions (e.g. food availability and predation risk). ¶

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1221 **FIGURE LEGENDS**

1222 Figure 1. Developmental stages of *Panulirus argus* considered in the present study.

1223 From left to right: transparent puerulus, pigmented puerulus, and first-stage juvenile of the

1224 Caribbean spiny lobster *Panulirus argus*. The term "pueruli" in the text refers to transparent +

1225 pigmented pueruli. (Photo: F Negrete-Soto, with permission).

1226

1227 Figure 2. Size distribution of organisms.

1228 Size (carapace length) distribution of the sample of pueruli (red columns, N = 380) and first-stage

1229 juveniles (blue columns, N = 226) of *Panulirus argus* collected at Puerto Morelos and Bahía de

1230 la Ascensión, Mexico, between August 2012 and September 2013.

1231

1232 Figure 3. Morphometric relationships of pueruli and first juveniles.

1233 Relationships between (A) total length and carapace length (CL), (B) weight and CL, (C)

1234 carapace height and CL, and (D) carapace width and CL, with ordinary least-squares regression

1235 lines, in pueruli (red dots and continuous lines, N = 380) and first juveniles (blue dots and dashed

1236 lines, N = 226) of *Panulirus argus* collected at Puerto Morelos and Bahía de la Ascensión,

1237 Mexico, between August 2012 and September 2013.

1238

1239 Figure 3. Seasonal variation in size (CL), weight (W), and W/CL.

1240 Seasonal variation in (A) size (carapace length, CL), (B) total weight (W) and (C) the W/CL ratio

1241 of pueruli (red dots and lines) and first juveniles (blue dots and lines) of *Panulirus argus*

1242 collected at Puerto Morelos, Mexico, between autumn 2010 and summer 2013. Error bars are

1243 95% confidence intervals. Seasonal Ns for benthic pueruli: 20–45; for first juveniles: 12–35.

1244

1245 Figure 4. Seasonal variation of modified condition factor (W/CL index).

1246 Seasonal variation of the modified condition factor (W/CL index) after controlling for the

1247 significant effect of size (CL, covariate) in (A) pueruli and (B) first juveniles of *Panulirus argus*

1248 collected at Puerto Morelos, Mexico, between autumn 2010 and summer 2013. Error bars are

1249 95% confidence intervals (CI). In each panel, the overall mean modified W/CL index and its 95%

1250 CI are denoted by the black continuous and dashed lines, respectively. Seasonal Ns for pueruli:

1251 20–45; for first juveniles: 12–35.

To examine seasonal variation in size, weight, and the W/CL index, we used exclusively the samples from Puerto Morelos, which amounted to 12 consecutive calendar seasons. For these individuals, we measured the carapace length (CL, from between the rostral horns to the posterior edge of the cephalothorax) with digital Vernier calipers (± 0.1 mm) and the total wet weight (W) with a digital scale (± 1 mg) after blotting excess moist. These dimensions allowed for the estimation of the W/CL index. For the morphometric relationships, we used all the samples taken between August 2012 and September 2013, from both Puerto Morelos and Bahía de la Ascensión. For these individuals, in addition to CL and W, we measured carapace height (CH, across the mid-point between the bases of the first pair of pereopods and the highest point in the cephalothorax), carapace width (CW, across the widest part of the cephalothorax), and total length (TL, from between the rostral horns to the posterior edge of the telson). All length dimensions were measured under a stereoscopic microscope to reduce measurement error.

Statistical analyses

Because the main monthly settlement pulse occurs during the dark phase of the moon but the collectors are checked the day after the third quarter lunar phase, the monthly catch is typically dominated by pigmented pueruli and first juveniles, whereas transparent pueruli are scarce or altogether absent (Briones-Fourzán, 1994). However, as exploratory analyses revealed no significant differences in body dimensions between transparent and pigmented pueruli (see Results), the data from all pueruli were pooled as "benthic pueruli" for further comparisons.

Seasonal variation in CL, W and the W/CL index. Because the monthly catch from collectors tends to be rather low (e.g., some months the catch can be zero, Briones-Fourzán, Candela & Lozano-Álvarez, 2008), we pooled the monthly data for the entire sampling period from Puerto Morelos by calendar season to examine seasonal variation in CL, W, and the WL index for benthic pueruli and first juveniles. These data were subjected to factorial analyses of variance (ANOVAs) with two (fixed) categorical factors: season (12 levels, from autumn 2010 to summer 2013) and developmental stage (2 levels, benthic pueruli and first juveniles). However, because the W/CL index is affected by size (Briones-Fourzán, Baeza-Martínez & Lozano-Álvarez, 2009), the seasonal variation in the W/CL index was further evaluated with an analysis of covariance (ANCOVA) using CL as a covariant, to control for its significant effect. This variable is hereafter referred as "corrected

W/CL index" In all cases, results were considered significant if p < 0.05.

Morphometric relationships. Regression analyses were used to examine morphometric relationships with data obtained from Puerto Morelos and Bahía de la Ascensión between August 2012 and September 2013. First, we tested for a potential effect of location (with two levels, Puerto Morelos and Bahía de la Ascensión) and developmental stage (with two levels, benthic pueruli and first-stage juveniles) on each variable (CL, CH, CW, TL, and W) with separate factorial analyses of variance (ANOVA). As the effect of location was not significant (see Results), the data from both locations were pooled. Then, for each developmental stage, the variables CH, CW, TL, and W were regressed against CL, as this variable can be measured with less error than TL. For each relationship, the slopes of regressions were compared between developmental stages with Student's *t*-tests; if the slopes did not differ significantly, we then compared the elevations (Zar, 1999). Finally, using the log-transformed data of all dimensions, we also tested departures from isometry ($b = 1$ for log-transformed length-length relationships, and $b = 3$ for length-weight relationships) (Hartnoll, 1982), also with *t* -tests (Zar, 1999).

The analyses of seasonal variation in CL, W, and the W/CL index encompassed 12 consecutive seasons, from November (autumn) 2010 to September (summer) 2013. During this period, we obtained 329 benthic pueruli and 252 first-stage juveniles at Puerto Morelos. Results of ANOVAs showed a significant effect of both main factors (Developmental stage and Season), but not of their interaction, on each of the three variables (Table 1). First juveniles had higher mean values of all three variables than benthic pueruli, with a similar trend for both developmental stages over the sampling period (Fig. 3). For both stages, all three variables were low in summer 2011 and also tended to decrease between spring 2012 and summer 2013 (Fig. 3). However, results of ANCOVA showed that, after controlling for the significant effect of CL (the covariant), the corrected W/CL index of benthic pueruli varied significantly with season, with higher than average values in autumn 2010 and winter of 2011, and lower than average values in summer 2011 and spring-summer 2013 (Fig. 4A). The corrected W/CL index of first juveniles also varied significantly with season after controlling for the significant effect of CL, with higher than average values in autumn 2010, and winter-spring 2011, and lower than average values in autumn 2011 and winter 2012 (Fig. 4B).

Morphometric relationships

Results from the factorial ANOVAs examining the effects of location and

developmental stage on body dimensions (Table 2) indicated that, except for CL, which did not differ significantly between benthic pueruli and first juveniles, all other body dimensions were significantly affected by developmental stage, but not by location, and the interaction term was also not significant. Therefore, the data from both locations were pooled prior to regression analyses.

In general, compared with first juveniles, morphometric relationships of benthic pueruli exhibited a greater dispersion of data around the regression line, resulting in lower coefficients of determination than for first-stage juveniles (Fig. 5). In the TL vs CL relationship, the slopes of the regressions did not differ between benthic pueruli and first juveniles, but the elevations did (Fig. 5A, Table 3). The slopes of all other regressions differed significantly between both developmental stages (Table 3). The most evident morphometric changes between pueruli and first juveniles were an increase in weight (Fig. 5B) and carapace height (Fig. 5C) relative to CL in juveniles, with weight increasing more steeply with increasing CL in juveniles. In contrast, changes in total length (Fig. 5A) and carapace width (Fig. 5D) relative to CL, although significant as well, were less marked. For both developmental stages, all regressions with log-transformed length dimensions exhibited negative allometry (i.e., slope was significantly lower than 1), except for the relationship carapace height vs CL, which exhibited isometry (Table 3). The slopes of the W vs CL relationships also differed significantly from 3 in both stages, showing negative allometry (Table 3).