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# Interspecific variation in the physiological and reproductive parameters of porcelain crabs from the Southeastern Pacific coast: potential adaptation in contrasting marine environments

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## ABSTRACT

Porcelain crabs inhabit from upper intertidal to subtidal habitats. These environments are characterized by highly variable environmental conditions, which subject species found in these habitats to stress. In this study, we compared reproductive traits of mothers [i.e. fecundity, reproductive output (RO), dry weight, organic matter] and physiological parameters of their offspring (i.e. wet weight, water content, dry weight, organic matter, lactate content of embryos) of three species of porcelain crabs that inhabit the Southeastern Pacific: *Petrolisthes laevigatus* (upper intertidal); *P. violaceus* (low intertidal); *Allopetrolisthes punctatus* (subtidal). Overall, female *P. laevigatus* had lower fecundity ( $802 \pm 115$  vs.  $4181 \pm 1097$  embryos) and amount of organic matter in their embryo masses ( $0.053 \pm 0.006$  vs.  $0.27 \pm 0.025$  g) but higher RO values ( $1.34 \pm 0.34$  vs.  $0.20 \pm 0.07$ ) than *Allopetrolisthes punctatus*. In addition, *P. laevigatus* embryos had higher organic matter content ( $81.09 \pm 28.8$  vs.  $64.54 \pm 6.1$   $\mu$ g), higher water content ( $188.6 \pm 91.9$  vs.  $152.4 \pm 30.8$   $\mu$ L) and higher lactate content ( $0.26 \pm 0.04\%$  vs.  $0.07 \pm 0.01\%$  dry weight) than that found in *A. punctatus* embryos. Furthermore, females and embryos of *P. violaceus* showed low values and similar to those observed in *P. laevigatus*. As a potential strategy to increase survival of the offspring, *P. laevigatus* seems to invest a large portion of its energy in production of high quality embryos, despite costs to fecundity. This study reveals that porcelain crabs have physiological adaptations during their ontogeny that allow them to survive in fluctuating environments.

## 1. Introduction

Invertebrates that inhabit coastal marine environments, including intertidal areas, are exposed to constant changes in key physical-chemical features (e.g. temperature, salinity, humidity, oxygen availability, and ultraviolet light) (Somero, 2002). In coastal areas, environmental variability is mainly related with the periodicity of tidal cycles (Stillman, 2004). This environmental variability generates changes in ionic balance, acid-base regulation, as well as variations in fundamental physiological processes such as respiration and excretion, and in this way affecting globally the energy budget of the organisms that inhabit these environments (McMahon, 2001; Somero, 2002; Urzúa and Urbina, 2017; Whiteley and Taylor, 2015). One example of organisms

that have managed to colonize intertidal areas, despite their environmental variability, are decapod crustaceans (Anger, 1995; Cumberlidge et al., 2009; Yeo et al., 2008) which have developed diverse strategies to survive and grow in these environments. In this context, different strategies have been described in crustaceans for overcoming the stress of environmental fluctuation; these include morphological (Helmuth et al., 2006), biochemical (Hochachka and Somero, 2002), physiological (Felten et al., 2008) and behavioral (Martins De Lima et al., 2015) adaptations. Overall, given that are key components of intertidal communities, decapods are considered excellent model organisms for evaluating physiological adaptations in intertidal habitats (Bartholomew, 1987; Helmuth et al., 2006; Stillman and Somero, 1996). For example, they show a wide metabolic capacity (i.e. aerobic

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and anaerobic) to face these fluctuating environments (Somero et al., 2017).

Decapod crustaceans that inhabit intertidal environments of the Pacific Ocean have various adaptive physiological responses depending on prevailing environmental conditions (e.g. temperature, dissolved oxygen, salinity, pH). Specifically, intertidal crabs during emersion time (i.e. when occurs periods of low tide) have been shown: a) changes in the  $\text{Ca}^+$  content of their legs (Stillman, 2000), b) reduction of the gills surface (Gray, 1957; Hawkins and Jones, 1982), c) increased recirculation of water in the abdomen (Hawkins and Jones, 1982), d) increased levels of respiratory pigment (hemocyanin) (Defur, 1988), as well as e) active mechanisms of osmoregulation (Lagos and Cáceres, 2008). Additionally, female decapod crustaceans that inhabit intertidal zones have been shown to have lower fecundity but produce larger embryos than species of the subtidal (Anger, 1995; Simmoni et al., 2011). As such, recent studies indicate that intertidal crabs would provide greater nutritional resources for the embryogenesis than subtidal crabs (Simmoni et al., 2011), as well as the ability to perform passive gas exchange in aerial environments during low tide (Cannicci et al., 2011).

Among decapods of coastal environments in the South Pacific, species of the Porcellanidae family have notably wide geographic distributions spanning temperate to tropical regions (Carvacho, 1980). Porcelain crabs are also highly species diverse and have a wide range of body sizes and colors (Baeza, 2016; McLaughlin et al., 2010). Additionally, the evolutionary phylogenetic history of this group is closely related (Baeza, 2016). For example, in a temperate zone of the Southeastern Pacific coast (San Vicente bay, Biobío region, Chile) three porcelain crab species coexist: *Petrolisthes laevigatus* (Guerin, 1835), *P. violaceus* (Guerin, 1835), and *Allopetrolisthes punctatus* (Stillman, 2007). While coexisting, the distributions of these species differ by depth and/or by degree of aerial exposure during tidal cycles (Somero, 2002). Specifically, in the Southeastern Pacific along the Chilean coast, *P. laevigatus* inhabits the upper intertidal, *P. violaceus* is found in the low intertidal, and *A. punctatus* inhabits the subtidal (Viviani, 1969; Zuñiga, 2002). These three species reproduce from late autumn to early summer (Gebauer et al., 2007), are omnivores, filter small planktonic organisms (phyto and zooplankton), and are also detritivores (Valdivia and Stotz, 2006). These species are highly abundant under and between boulders found along the coast of Chile (Vargas et al., 2010; Viviani, 1969; Zuñiga, 2002). In addition, given their trophic roles as prey of intertidal fish and as predators of intertidal microorganisms, these porcelain crabs are considered as key species of coastal marine ecosystems (Castilla and Paine, 1987; Gebauer et al., 2007; Rivadeneira et al., 2010).

Comparing the reproductive and physiological features of the early ontogeny of closely related species can help elucidate mechanisms, physiological responses, and adaptations that species have developed to colonize dynamic coastal habitats from less variable marine environments (Baeza, 2016). In this context, the reproductive parameters (Anger, 1995) and physiology of intertidal crustaceans have been shown to differ depending on local environmental conditions and at temporal scales of hours (tidal cycles) to seasons (winter vs. summer) (Gebauer et al., 2007; Simmoni et al., 2011). In this way, these crabs are able to tolerate, survive, and reproduce in a variety of environmental conditions.

Numerous studies have been conducted on the reproductive biology (Gebauer et al., 2007; Gebauer et al., 2010; Hernández and Palma, 2003; Lardies and Wehrmann, 1996;), population dynamics (Gebauer et al., 2007; Lardies et al., 2004), adult ecophysiology (Lagos and Cáceres, 2008; Pardo et al., 1997; Stillman and Somero, 1996; Stillman, 2000; Vargas et al., 2010), and ontogenetic ecophysiology (Alter et al., 2015) of *P. laevigatus* and *P. violaceus* in the Southeastern Pacific. For the species *A. punctatus*, to our knowledge there only exist studies regarding settlement strategies and phylogenetics (Baeza, 2016; Viviani et al., 2010) while the reproduction and physiology of this species are still unknown. Despite the work conducted on these three species, there

is currently no work comparing the reproductive strategies of mothers and the physiological traits of their offspring. Therefore, we herein compare the reproductive parameters of females and physiological traits of embryos of these three porcellanid crab species, *P. laevigatus*, *P. violaceus*, and *A. punctatus* that inhabit contrasting coastal environments of the Southeastern Pacific. From this comparative physiological study, we hope to better understand what reproductive and bioenergetic characteristics have allowed these crabs to adapt to and survive in their highly variable intertidal and subtidal habitats. This information may shed light on the processes and mechanisms that enabled the success of adaptive radiation of these porcellanids in these highly-dynamic coastal environments.

## 2. Methods

### 2.1. Animal collection

Female porcelain crabs were captured by hand net from the upper intertidal (*P. laevigatus*), low intertidal (*P. violaceus*), and subtidal (*A. punctatus*) of the rocky beach in front of the Abate Molina Marine Biology Station (EBAM) of the Universidad Católica de la Santísima Concepción (UCSC) in Lenga, Bahía de San Vicente, Chile (36°46'01"S, 73°10'17"O). Collections were undertaken in May of 2016 (end of autumn), and after collection these crabs were grouped according to "species" and placed in three independent cooling boxes (7 L each) containing seawater and transported for a brief period (ca. 10 min) to EBAM. In the EBAM, ovigerous females of each species were separated by identifying individuals with stage I embryo masses. By means of a stereomicroscope (Euromex-Holland NexiusZoom) equipped with a calibrated eyepiece, micrometer, and a digital camera (CMEX 5 Pro), stage I embryo masses were identified by the uniform distribution of yolk and the absence of visible ocular pigments (Wehrmann, 1990). Subsequently, the selected females were transferred in a cooler with dry ice to the Hydrobiological Resources Lab (LRH) of the Faculty of Sciences of the UCSC where they were frozen in a  $-80\text{ }^{\circ}\text{C}$  freezer for later analysis of reproductive and physiological parameters.

At the LRH, the carapace lengths (CL) of crabs was measured as the distance between the anterior end and posterior margin of carapace using a Vernier caliper with 0.001 mm of precision. *P. laevigatus* had CLs from 14 to 17 mm ( $n = 30$ ). *P. violaceus* had CLs from 15 to 17 mm ( $n = 30$ ), and *A. punctatus* had CLs from 14 to 18 mm ( $n = 30$ ). Subsequently, the total embryo masses of these individuals were extracted for the determination of reproductive parameters (i.e. fecundity, dry weight, organic matter, and reproductive output). A part of the embryo masses ( $n = 100$  embryos) was separated and used to quantify the embryo physiological parameters (i.e. wet weight, dry weight, water content, organic matter content, and lactate content).

### 2.2. Reproductive parameters of the ovigerous females

#### 2.2.1. Fecundity

Using needle-nose forceps and dissection needles, all of the embryos were carefully extracted from the pleopods of all female of each species (total  $n = 90$ , 30 females of each species). The total number of embryos of each ovigerous female (i.e. fecundity) was counted using a manual counter and a stereomicroscope (Motic BA-310).

#### 2.2.2. Dry weight and organic matter

After fecundity measurements, the females of each species and their respective embryo masses were separately dried at  $-80\text{ }^{\circ}\text{C}$  for 48 h in a lyophilizer (Operon FDU-7012). The dried samples were then weighed on a 0.01 mg precision balance (Precisa 120A). Subsequently, the dried samples were incinerated for four hours at  $450\text{ }^{\circ}\text{C}$  in a muffle furnace (WiseTherm, FHP-03). After, the incinerated samples (i.e. with ashes) were weighed on the previously mentioned precision balance. Finally, the organic matter content (OMC) of the females and of the embryo

masses was obtained by subtracting the weight of the ashes (WA) from the dry weight of the sample (DW) ( $OMC = DW - WA$ ).

### 2.2.3. Reproductive output as a function of biomass and organic matter content

The reproductive output based on biomass (ROB) was calculated following Havenhand and Todd (1989). The dry weight of the total embryo mass was divided by the dry weight of the female without its embryos. Then, this was multiplied by 100 ( $ROB = [\text{embryo biomass} / \text{biomass of the female}] \times 100$ ). Additionally, reproductive output was calculated as a function of organic matter (ROM), where the organic matter of the total mass of embryos was divided by the organic matter of the female without her embryos; this was then multiplied by 100 ( $ROM = [\text{organic matter of embryos} / \text{organic matter of female}] \times 100$ ).

## 2.3. Embryo parameters

### 2.3.1. Wet weight, water content, and dry weight of embryos

In order to quantify the embryo parameters, embryos ( $n = 100$ ) were separated from the embryo mass of each female of the three porcellanid species. After separation, the embryos were soaked for a few seconds in distilled water to remove salt crystals. Then, they were briefly dried on lint-free paper (Klenex) and transferred to 1.5 mL Eppendorf tubes that had been previously weighed on a precision balance (Precisa 120A). The wet weight of each embryo (WWE) was determined on the same balance mentioned previously. Specifically, the wet weight of the embryos (WWS) was subtracted from the weight of the Eppendorf tube (WET), and this was divided by 100 ( $WWE = [WET - WWS] / 100$ ). Subsequently, the embryos were dried at  $-80^\circ\text{C}$  for 48 h in a lyophilizer (Operon FDU-7012). After drying, the dry weight of each embryo (DWE) was determined on the same precision balance by subtracting the dry weight of the sample (DWS) from the weight of the Eppendorf tube (WET) and dividing by 100 ( $DWE = [WET - DWS] / 100$ ). Subsequently, the water content of each embryo (WCE) was calculated following Roonwal (1967); specifically, the dry weight of the embryos (DWS) was subtracted from the wet weight of the embryos (WWS) and divided by 100 ( $WCE = [WWS - DWS] / 100$ ).

### 2.3.2. Embryo organic matter content

The ash weight of the embryos sample (AWS) was obtained by incinerating 100 embryos for four hours at  $450^\circ\text{C}$  (WiseTherm, FHP-03) and then weighing them on a precision balance with a sensitivity of 0.001 mg (Sartorius SC2). The organic matter content of each embryo (OME) was then calculated following the methods of Bascur et al. (2017); briefly, the ash weight of the embryos sample (AWS) was subtracted from the dry weight of the embryos sample (DWS), and then this value was divided by 100 ( $OME = [DWS - AWS] / 100$ ).

### 2.3.3. Embryo lactate content

The embryo lactate content (ELC) was quantified using the method proposed by Tietz (1995). For this, 100 embryos were separated from the embryo mass of each female from each species. The embryo masses were previously rinsed for a few seconds with distilled water. The separated embryos were transferred to 1.5 mL Eppendorf tubes to which 500  $\mu\text{L}$  of distilled water was added. The contents of the tubes were then homogenized with a homogenizer (Scilogex D160) at 3000 rpm for two minutes. The samples were analyzed with a lactate kit (Spinreact lactate Kit); the kit contained a lactate standard and the working reagent (RT) that reacts with the organic compound lactate. With a micropipette (AxyPet® pipette), 10  $\mu\text{L}$  of standard was pipetted into a 1.5 mL Eppendorf containing 1 mL of RT. Likewise, 10  $\mu\text{L}$  of each sample was combined with 1 mL of RT in individual tubes (a total of 30 samples were analyzed). Then, the Eppendorf tubes were incubated at room temperature ( $18^\circ\text{C}$ ) for 10 min. After incubation, 250  $\mu\text{L}$  of each sample was transferred to a multiplate reader using 32 wells. The multiplate

spectrophotometer (Biotek Elx808) was used to determine absorbance at a wavelength of 490 nm.

### 2.3.4. Statistical analysis

The statistical analyses were performed using standard methods (Sokal and Rohlf, 1995; Zuur et al., 2007) in the software STATISTICA 8.0. All analyses were carried out using 95% confidence levels ( $P < .05$ ). First, to determine the effect of female size, we evaluated the carapace length (CL) of ovigerous females of the three-species using a one-way ANOVA with “crab species” as a fixed factor and CL as response variable. Given that no significant differences were found among female size, comparisons of female and embryo parameters were performed. These female parameters included: fecundity, dry weight, organic matter content, and RO (based on dry weight and organic matter content). Also, the embryo parameters included: wet weight, water content, dry weight, organic matter content, and lactate content. All these parameters were compared separately using “crab species” as fixed factor by one-way ANOVA. The normality and homogeneity of the variances were analyzed using Kolmogorov-Smirnov and Levene tests, respectively. In turn, significant differences among species were analyzed with a HSD Tukey multiple comparison test. Finally, all graphics were made using the program SigmaPlot 12 (SystatSoftware Inc., Chicago, USA).

## 3. Results

### 3.1. Reproductive parameters of the ovigerous females

#### 3.1.1. Female size and fecundity

The mean size of ovigerous females (carapace length, CL) was not significantly different among species ( $P. laevigatus = 17.11 \pm 1.52$  mm;  $P. violaceus = 16.72 \pm 1.63$  mm;  $A. punctatus = 17.29 \pm 2.55$  mm) ( $F_{2,87} = 0.91$ ;  $P = .62$ ).

In turn, significant differences in fecundity were found among the species  $P. laevigatus$  (upper intertidal),  $P. violaceus$  (low intertidal) and  $A. punctatus$  (subtidal) ( $F_{2,87} = 282.86$ ;  $P < 0.001$ ) (Table 1). The females of  $A. punctatus$  had the highest number of embryos ( $4181 \pm 193$  embryos), while the fewest number of embryos were found in the female of  $P. laevigatus$  ( $802 \pm 20$  embryos) and  $P. violaceus$  ( $794 \pm 52$  embryos) (Fig. 1).

#### 3.1.2. Dry weight of ovigerous females and of embryo masses

Dry weight of the females significantly differed among species ( $F_{2,87} = 220.86$ ;  $P < 0.001$ ) (Table 1). The females of  $A. punctatus$  had the highest dry weight ( $6.98 \pm 2.07$  g), while females of  $P. violaceus$  ( $1.48 \pm 0.33$  g) and  $P. laevigatus$  ( $1.42 \pm 0.34$  g) had the lowest dry weight values (Fig. 2A). Similar to the female dry weight results, the dry weight of the total embryo masses significantly differed among species ( $F_{2,87} = 175.37$ ;  $P < 0.001$ ) (Table 1).  $A. punctatus$  had the highest values ( $0.28 \pm 0.027$  g) followed by  $P. violaceus$  ( $0.072 \pm 0.006$  g), and the lowest dry weights were observed in  $P. laevigatus$  ( $0.055 \pm 0.006$  g) (Fig. 2B).

#### 3.1.3. Organic matter of ovigerous females and of embryo masses

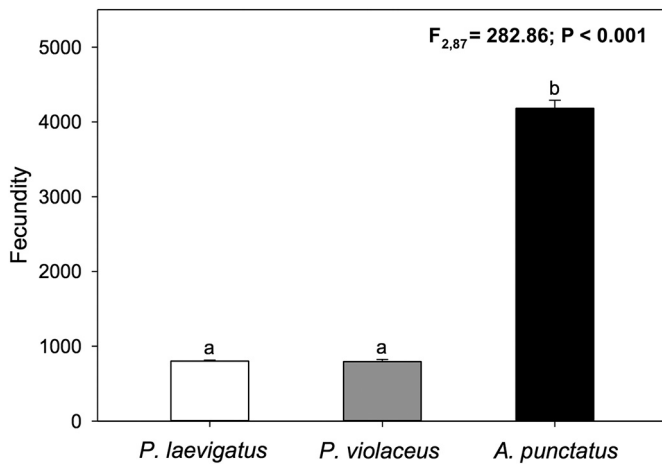
Significant differences in the organic matter content of females were found among the three species ( $F_{2,87} = 263.44$ ;  $P < 0.001$ ) (Table 1). The highest organic matter content was found in  $A. punctatus$  females ( $3.38 \pm 0.29$  g), while the lowest organic matter content was recorded in females of  $P. laevigatus$  ( $0.66 \pm 0.06$  g) and  $P. violaceus$  ( $0.58 \pm 0.08$  g) (Fig. 3A). Consistently, significant differences in female organic matter content were observed related to total sample dry weight (%DW). Organic matter content represented  $49.1 \pm 1.51\%$  of the total dry weight of  $A. punctatus$ ,  $44.41 \pm 2.31\%$  of the total dry weight of  $P. violaceus$ , and  $40.16 \pm 4.61\%$  of the total dry weight of  $P. laevigatus$  ( $F_{2,87} = 6.68$ ;  $P < 0.05$ ) (Fig. 3B) (Table 1).

Among species, significant differences were found in the organic

**Table 1**  
*Petrolisthes laevigatus*, *P. violaceus* and *Allopetrolisthes punctatus*. Interspecific variation in ovigerous female parameters.

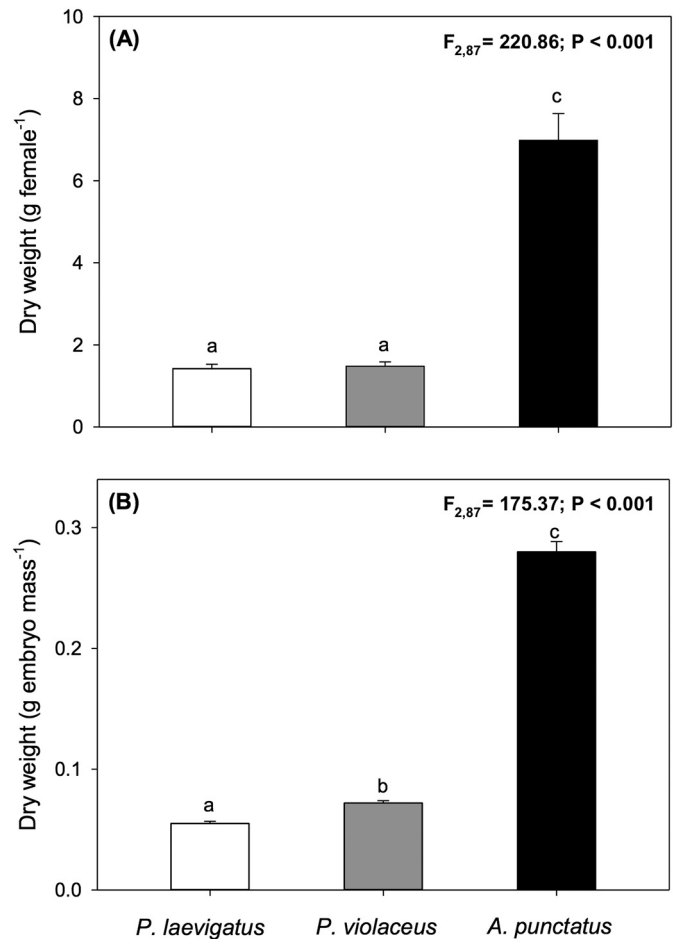
| Female Parameters    | Factor  | df | MS         | F      | P         |
|----------------------|---------|----|------------|--------|-----------|
| Fecundity            | Species | 2  | 1.14E + 08 | 282.86 | < 0.001** |
|                      | Error   | 87 | 4.05E + 05 |        |           |
|                      | Total   | 89 | 1.15E + 08 |        |           |
| Female DW (g)        | Species | 2  | 306.79     | 220.09 | < 0.001** |
|                      | Error   | 87 | 1.39       |        |           |
|                      | Total   | 89 | 308.18     |        |           |
| Embryo mass DW (g)   | Species | 2  | 0.47       | 175.37 | < 0.001** |
|                      | Error   | 87 | 0.03       |        |           |
|                      | Total   | 89 | 0.50       |        |           |
| Female OM (g)        | Species | 2  | 76.34      | 263.44 | < 0.001** |
|                      | Error   | 87 | 0.29       |        |           |
|                      | Total   | 89 | 76.63      |        |           |
| Female OM (%DW)      | Species | 2  | 597.62     | 6.68   | 0.05*     |
|                      | Error   | 87 | 89.53      |        |           |
|                      | Total   | 89 | 687.15     |        |           |
| Embryo mass OM (g)   | Species | 2  | 0.44       | 192.66 | < 0.001** |
|                      | Error   | 87 | 0.02       |        |           |
|                      | Total   | 89 | 0.46       |        |           |
| Embryo mass OM (%DW) | Species | 2  | 176.67     | 4.06   | < 0.05*   |
|                      | Error   | 87 | 43.53      |        |           |
|                      | Total   | 89 | 220.20     |        |           |
| ROB (DW)             | Species | 2  | 9.74       | 101.73 | < 0.001** |
|                      | Error   | 87 | 0.10       |        |           |
|                      | Total   | 89 | 9.84       |        |           |
| ROM (OM)             | Species | 2  | 381.05     | 86.85  | < 0.001** |
|                      | Error   | 87 | 4.39       |        |           |
|                      | Total   | 89 | 385.44     |        |           |

E +, exponent; MS, mean square; df, degrees of freedom; DW, dry weight; ROB, reproductive output related to biomass; ROM, reproductive output related to organic matter content. Differences in all female parameters among species were evaluated using a one-way ANOVA. Significant differences are marked with an asterisk (\* $P < .05$ ; \*\* $P < .001$ ; after HSD Tukey test).



**Fig. 1.** *Petrolisthes laevigatus*, *P. violaceus* and *Allopetrolisthes punctatus*. Interspecific variation in the fecundity (number of embryos) of ovigerous females. Different letters denote significant differences among species. Average values ± DE.  $n = 90$ .

matter content of the embryo masses ( $F_{2,87} = 192.66$ ;  $P < 0.001$ ) (Table 1). Highest organic matter content was found for *A. punctatus* ( $0.27 \pm 0.025$  g) and the lowest values were found for *P. violaceus* ( $0.067 \pm 0.007$  g) and also for *P. laevigatus* ( $0.053 \pm 0.006$  g) (Fig. 3C). When taking into account total dry weight (%DW), significant differences were consistently observed in the organic matter content of the embryo masses among species. Organic matter represented  $96.86 \pm 0.59\%$  of the total dry weight of embryos of *A. punctatus*,  $95.86 \pm 0.28\%$  of the total dry weight of the embryo masses of *P. laevigatus*, and  $94.76 \pm 0.59\%$  of the total dry weight of the embryo



**Fig. 2.** *Petrolisthes laevigatus*, *P. violaceus* and *Allopetrolisthes punctatus*. Interspecific variation in total biomass: (A) Dry weight of ovigerous females ( $\text{g female}^{-1}$ ); (B) Dry weight of embryo masses ( $\text{g embryo mass}^{-1}$ ). Different letters denote significant differences among species. Averages ± DE. In all cases  $n = 90$ .

masses of *P. laevigatus* ( $F_{2,87} = 4.06$ ;  $P < 0.05$ ) (Fig. 3D) (Table 1).

### 3.1.4. Reproductive output as a function of biomass (ROB) and of organic matter content (ROM)

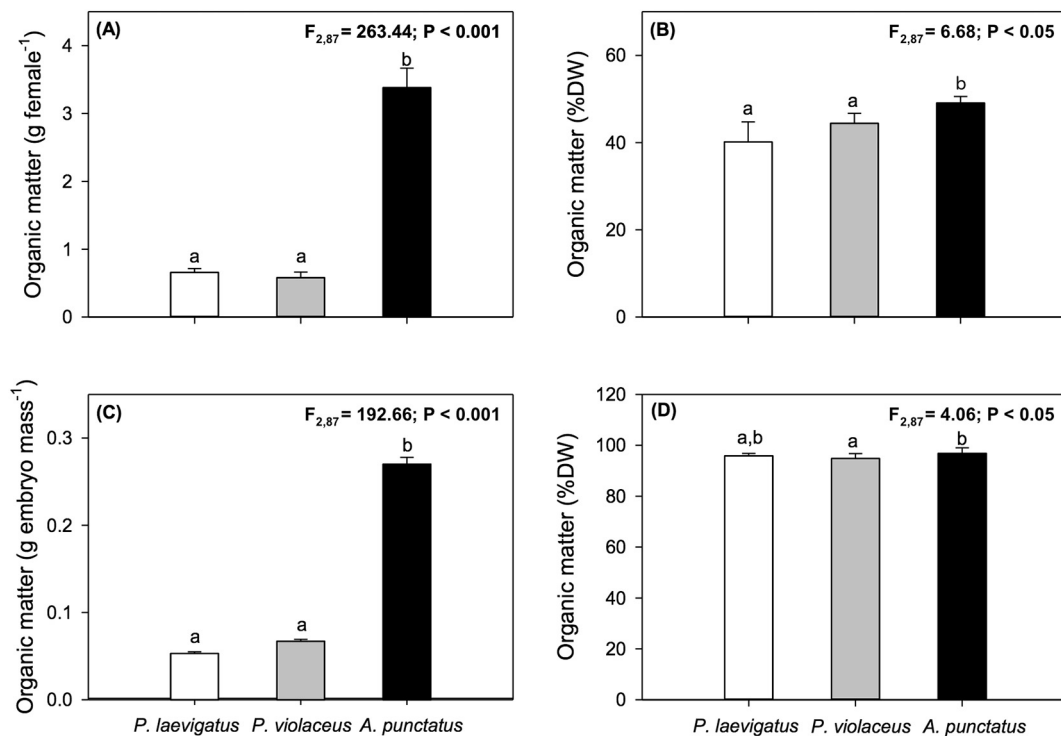
The ROB index values differed significantly among species, but the trend observed was opposite that found for the fecundity results. The highest ROB values were found for *P. laevigatus* ( $1.34 \pm 0.34$ ) and *P. violaceus* ( $0.87 \pm 0.4$ ), and the lowest values were found for *A. punctatus* ( $0.20 \pm 0.07$ ) ( $F_{2,87} = 101.73$ ;  $P < 0.001$ ) (Fig. 4A) (Table 1). Consistently, the ROM index values differed significantly among species and followed the same trend as that found for the ROB index. The highest ROM values were found for *P. laevigatus* ( $11.11 \pm 1.02$ ) and *P. violaceus* ( $8.06 \pm 0.60$ ), and the lowest values were found for *A. punctatus* ( $4.01 \pm 0.29$ ) ( $F_{2,87} = 86.85$ ;  $P < 0.001$ ) (Fig. 4B) (Table 1).

## 3.2. Embryo parameters

### 3.2.1. Embryo wet weight, water content, dry weight, and organic matter content

Embryo wet weight differed significantly among species. The highest wet weight values were found for *P. laevigatus* ( $279.81 \pm 24.62$   $\mu\text{g}$ ), followed by *P. violaceus* ( $244.41 \pm 13.09$   $\mu\text{g}$ ), and the lowest values were obtained for *A. punctatus* ( $219.01 \pm 11.69$   $\mu\text{g}$ ) ( $F_{2,87} = 9.86$ ;  $P < 0.001$ ) (Fig. 5A) (Table 2). Significant differences were also found in the water content among species ( $F_{2,87} = 5.32$ ;  $P < 0.01$ ) (Table 2). *P. laevigatus* had the highest





**Fig. 3.** *Petrolisthes laevigatus*, *P. violaceus* and *Allopetrolisthes punctatus*. Interspecific variation in female and embryo parameters: (A) Organic matter content of females (g female<sup>-1</sup>); (B) Organic matter content of females (% dry weight, DW); (C) Organic matter content of embryos (g mass of embryo<sup>-1</sup>); (D) Organic matter content of embryo masses (% dry weight, DW). Different letters denote significant differences among species. Averages  $\pm$  DE. In all cases  $n = 90$ .

water content ( $188.6 \pm 61.9 \mu\text{L}$ ) while *P. violaceus* ( $168.97 \pm 62.44 \mu\text{L}$ ) and *A. punctatus* ( $152.4 \pm 30.8 \mu\text{L}$ ) had the lowest water content (Fig. 5B). Additionally, the dry weight of the embryos differed significantly among species. *P. laevigatus* embryos had the highest dry weight ( $88.8 \pm 19.3 \mu\text{g}$ ), followed by *P. violaceus* embryos ( $70.1 \pm 3.37 \mu\text{g}$ ), and *A. punctatus* embryos had the lowest dry weight ( $66.5 \pm 7.36 \mu\text{g}$ ) ( $F_{2,87} = 31.52$ ;  $P < 0.001$ ) (Fig. 5C) (Table 2). Finally, significant differences were found in the organic matter content of embryos among species ( $F_{2,87} = 31.26$ ;  $P < 0.001$ ). The highest embryo organic matter content was found for *P. laevigatus* ( $81.09 \pm 28.82 \mu\text{g}$ ) while *P. violaceus* embryos ( $66.80 \pm 2.88 \mu\text{g}$ ) and *A. punctatus* embryos ( $64.5 \pm 6.13 \mu\text{g}$ ) had the lowest organic matter content (Fig. 5D) (Table 2).

### 3.2.2. Embryo lactate content

Lactate content differed significantly among species ( $F_{2,87} = 40.02$ ;  $P < 0.001$ ) (Table 2). *P. laevigatus* embryos had the highest lactate content ( $0.23 \pm 0.02 \mu\text{g}$ ), followed by *P. violaceus* embryos ( $0.20 \pm 0.03 \mu\text{g}$ ), and *A. punctatus* embryos had the lowest lactate content ( $0.07 \pm 0.01 \mu\text{g}$ ) (Fig. 6A). When dry weight was accounted for, the differences in lactate content among species were also significant ( $F_{2,87} = 48.98$ ;  $P < 0.001$ ). Embryos of *P. laevigatus* had the highest percentage of lactate per dry weight ( $0.26 \pm 0.04\%$  dry weight), followed by *P. violaceus* embryos ( $0.10 \pm 0.01\%$  dry weight), and *A. punctatus* embryos had the lowest percent lactate ( $0.07 \pm 0.01\%$  dry weight) (Fig. 6B) (Table 2).

## 4. Discussion

In this comparative physiological study, biochemical and adaptive features of the main reproductive and embryonic parameters of three porcellanid crab species (*Petrolisthes laevigatus*, *P. violaceus* and *Allopetrolisthes punctatus*) were measured. The studied species are phylogenetically closely related (Baeza, 2016) and inhabit close yet contrasting coastal marine environments, i.e. upper intertidal (*P.*

*laevigatus*), lower intertidal (*P. violaceus*) and coastal subtidal (*A. punctatus*). Hence, these species are exposed to frequent variation in environmental conditions (e.g. temperature, oxygen, hydrodynamics). The results gathered here reveal that these species differ in terms of reproductive and physio-energetic parameters. Significant differences were found among species/habitat in terms of female reproductive parameters (e.g. fecundity, RO = reproductive output) and in offspring biomass and energy characteristics (water content, dry weight and organic matter content) as well as, physiological features (lactate content). This variability is mainly driven by the coastal habitat, and possibly may also lead the process of adaptive radiation of the studied three species. Hence, the present approach is expected to be a feasible tool disentangling the role of environmental conditions in determining the evolution of morphological, ecological and behavioral traits of these porcelain crabs in the marine realm (Baeza and Thiel, 2003; Baeza and Asorey, 2012), as it has been formerly shown in phylogenetic studies conducted in the decapod genera *Petrolisthes*, *Allopetrolisthes* and *Lio-petrolisthes* (Baeza and Thiel, 2003; Baeza and Asorey, 2012; Gebauer et al., 2010). These genera have been extensively studied because they showed a high variety of body sizes, microhabitats, color patterns and life cycles (Haig, 1960; Antezana et al., 1965; Baeza and Thiel, 2003, 2007).

The family Porcellanidae has a subtidal marine origin, but possibly due to selective pressure, i.e. competition for food (Firstater et al., 2012; Seibel and Drazen, 2007) and predator avoidance (Wilkinson et al., 2015), these crabs have migrated and diversified in new coastal habitats. The porcelain crabs can be found in intertidal environments where they are exposed to highly variable environmental conditions (e.g. temperature, pH, oxygen) that fluctuate with tidal cycles (Somero et al., 2017). Thus, during their evolutionary history porcellanid crabs have developed adaptive physiological strategies at all ontogenic stages to ensure survival and offspring success (Lima and Bednekoff, 1999; Trussell et al., 2006; Werner and Peacor, 2003; Wilkinson et al., 2015). For example, adult semiterrestrial crabs, namely *Grapsus tenicratus* and *Hemigrapsus sanguineus*, have been shown to avoid predation by

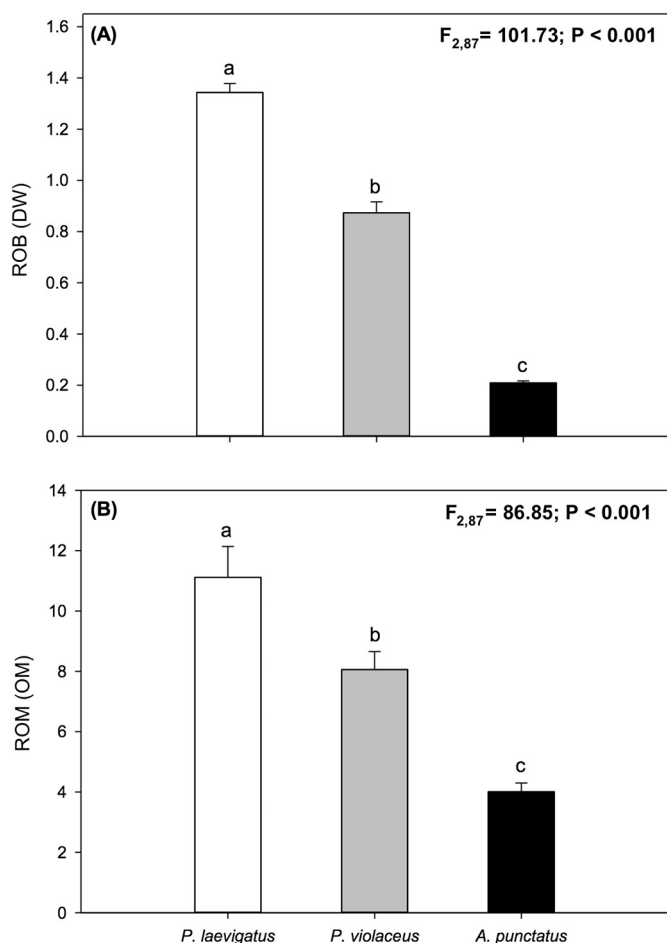


Fig. 4. *Petrolisthes laevigatus*, *P. violaceus* and *Allopetrolisthes punctatus*. Interspecific variation in female parameters: (A) Reproductive output as a function of biomass (ROB); (B) Reproductive output as a function of organic matter content (ROM). Different letters denote significant differences among species. Averages  $\pm$  DE. In both cases  $n = 90$ .

gradual moving to new intertidal zones (Graham et al., 2009; Pushchina and Panchenko, 2002). This displacement to unexploited ecological niches may be integrated as an example of adaptive radiation in response to natural selection and/or ecological opportunity (Glor, 2010). Integrative physiological analyses, as the present study, can identify patterns of diversification that provide clues about the drivers and expected outcomes of adaptive radiation (Rabosky, 2009), as resulted herein with the three-studied species from the upper intertidal (*Petrolisthes laevigatus*), low intertidal (*P. violaceus*) and subtidal (*Allopetrolisthes punctatus*).

Overall, there was a gradual increase in reproductive output of these female porcelain crabs (taking into consideration both dry weight and organic matter content) from the subtidal to the upper intertidal. Hence, these species are adapted to the environmental variability experienced in the intertidal zones they inhabit. Specifically, females in the upper intertidal must invest a large amount of organic matter in their embryos to ensure survival in this highly variable environment (in terms of temperature and oxygen). In this context, the reproductive output of these porcelain crabs could be triggered by the period of emersion. An increase of reproductive output of semi-terrestrial crabs (*Armases miersii* (Rathbun, 1987); *Cryptodromia pileifera* (Alcock, 1900)) and terrestrial crabs (*Sesarma cookei* (Hartnoll, 1971); *Armases ricordi* (Milne-Edwards, 1853); *Metopaulias depressus* (Rathbun, 1896)) has been previously shown to be positively correlated with variability of intertidal zones, and consistently with a higher reproductive output

relative to subtidal crab species (Anger, 1995; Diesel et al., 2000; Diesel and Schubart, 2007; Litulo, 2004; Hines, 2011; Vogt, 2013).

While *P. laevigatus* and *P. violaceus* embryos (“intertidal crabs”) had higher dry weights and more organic matter than the embryos of *A. punctatus* (“subtidal crab”), *A. punctatus* females had higher fecundity (i.e. total number of embryos) than *P. laevigatus* and *P. violaceus*. Likely these characteristics of *P. laevigatus* and *P. violaceus* embryos should allow them to resist the highly variable environmental conditions of their habitat. Conversely, the *A. punctatus* had high fecundity but low values of biomass and organic matter in its embryos. These results could indicate that the less variable conditions of the subtidal favor successful embryo and larval development. In general, this study supports the idea that crab species within the same family (i.e. “phylogenetically related”) allocate energy based on one of two reproductive strategies; either few embryos with high biomass and organic matter content are produced or many embryos with less biomass and less organic matter are produced. Therefore, despite the fact that the total number of offspring and total biomass of offspring differed among the studied species, total biomass was compensated with the amount of organic matter invested in each embryo (ca. 93–96% DW). This bioenergetic trait (i.e. organic matter of embryos) could be considered as an adaptation when there is selective pressure on offspring survival (Firstater et al., 2012; Hartnoll et al., 2007, 2010; Jivoff et al., 2007). Both life strategies, i.e. the subtidal species (*Allopetrolisthes punctatus*) and both intertidal species (*Petrolisthes laevigatus* and *P. violaceus*) are in concordance with r and k-selection, respectively. R-species are characterized by a large number of embryos but low reproductive effort while K-species have a small number of embryos but high reproductive effort (Parry, 1981).

The higher values of biomass and organic matter content in the embryos produced by intertidal females could indicate that during mating and oogenesis females anticipate (e.g. from changes in temperature, humidity, and oxygen) the stressful environmental conditions that their embryos will face. Additionally, mothers that carried embryos could preferentially select microhabitats with more constant environmental conditions so that they can devote more energy to their embryos, which would result in larger embryos with higher water content, biomass, and organic matter content. Similar response of lifestyle has been described in decapod crustaceans inhabit complex and extreme environments such as intertidal habitat (Anger et al., 2015; Benvenuto et al., 2015). Consequently, the newly hatched larvae from larger embryos would be larger and have greater energy reserves to allow them to tolerate periods of low or unpredictable food availability (Lardies and Wehrmann, 1996; Lee and Strathmann, 1998; Mantelatto and Christofolletti, 2001), and in this way the probability of their survival in the plankton would be increased (Leone and Mantelatto, 2015).

Given that porcellanid females house their embryos under their abdomens (Baeza and Fernández, 2002; Dick et al., 2002; Pavanelli et al., 2010; Ruiz-Tagle et al., 2002), mother and embryos alike are exposed to the same environmental conditions. Here, our results showed that adults and embryos are physiologically well prepared to face the stressful environmental conditions of the intertidal (e.g. physiological strategies: Felten et al., 2008; behavioral strategies: Martins De Lima et al., 2015). Among the physiological adaptations that have been documented in the early ontogeny of coastal species, embryos in the intertidal often have higher water contents than embryos in the subtidal so that they avoid desiccation during aerial exposure (Greenaway, 2003). Additionally, embryos with higher water content are able to prevent low oxygen tension in the interstices, facilitating the diffusion of oxygen from the external environment (Cannicci et al., 2011; Strathmann and Chaffee, 1984). Oxygen has been shown to be a limiting factor during the embryonic development of decapods (Lardies and Fernández, 2002); organisms that inhabit intertidal zones often face periods of desiccation and/or become trapped in small bodies of water that quickly become hypoxic (Holman and Hand, 2009). However, some marine invertebrates have been shown to maintain their metabolic rates by activating alternative physiological pathways such

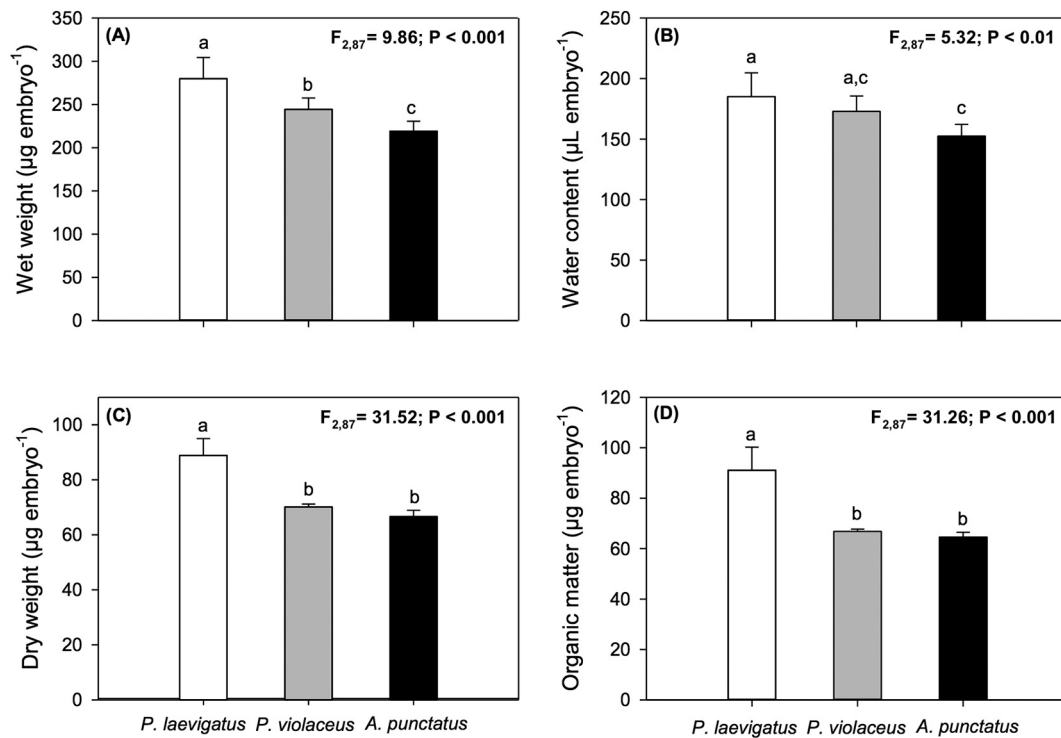


Fig. 5. *Petrolisthes laevigatus*, *P. violaceus* and *Allopandalis punctatus*. Interspecific variation in embryo parameters: (A) Wet weight ( $\mu\text{g embryo}^{-1}$ ); (B) Water content ( $\mu\text{L embryo}^{-1}$ ); (C) Dry weight ( $\mu\text{g embryo}^{-1}$ ); (D) Organic matter ( $\mu\text{g embryo}^{-1}$ ). Different letters denote significant differences among species. Averages  $\pm$  DE. In all cases  $n = 90$ .

Table 2

*Petrolisthes laevigatus*, *P. violaceus* and *Allopandalis punctatus*. Interspecific variation in early embryo parameters.

| Embryo Parameters                | Factor  | df | MS        | F     | P       |
|----------------------------------|---------|----|-----------|-------|---------|
| Wet weight ( $\mu\text{g}$ )     | Species | 2  | 27,988.23 | 9.86  | 0.001** |
|                                  | Error   | 87 | 2837.99   |       |         |
|                                  | Total   | 89 | 30,826.22 |       |         |
| Water content ( $\mu\text{L}$ )  | Species | 2  | 11,207.33 | 5.32  | 0.01*   |
|                                  | Error   | 87 | 2106.15   |       |         |
|                                  | Total   | 89 | 13,313.48 |       |         |
| Dry weight ( $\mu\text{g}$ )     | Species | 2  | 4297.78   | 31.52 | 0.001** |
|                                  | Error   | 87 | 136.33    |       |         |
|                                  | Total   | 89 | 4434.11   |       |         |
| Organic matter ( $\mu\text{g}$ ) | Species | 2  | 3879.31   | 31.26 | 0.001** |
|                                  | Error   | 87 | 124.09    |       |         |
|                                  | Total   | 89 | 4003.40   |       |         |
| Lactate ( $\mu\text{g}$ )        | Species | 2  | 0.20      | 40.02 | 0.001** |
|                                  | Error   | 87 | 0.01      |       |         |
|                                  | Total   | 89 | 0.21      |       |         |
| Lactate (%DW)                    | Species | 2  | 0.33      | 48.98 | 0.001** |
|                                  | Error   | 87 | 0.01      |       |         |
|                                  | Total   | 89 | 0.34      |       |         |

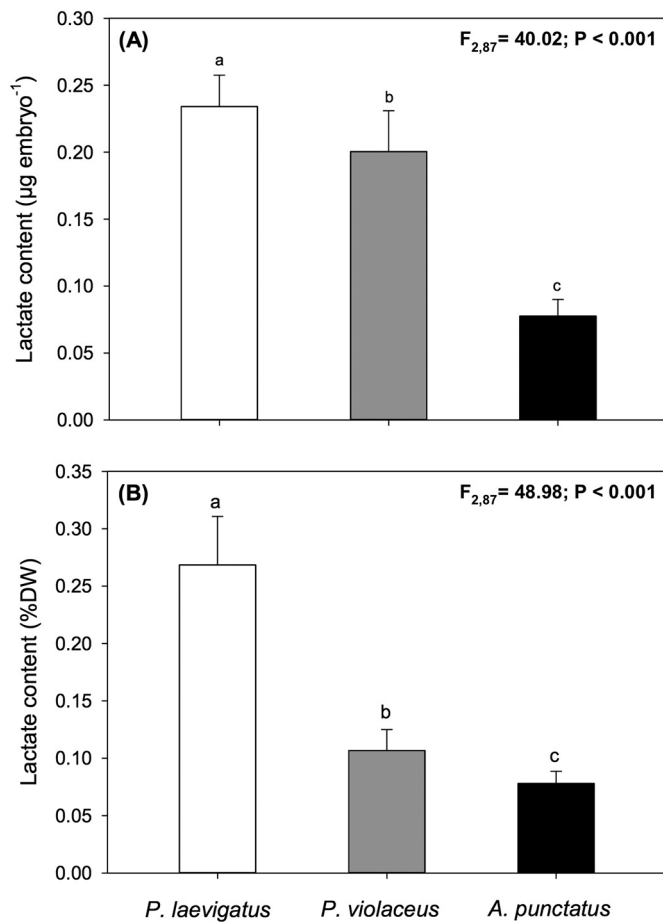
MS, mean square; df, degrees of freedom; DW, dry weight. Differences in all embryo parameters among species were evaluated using one-way ANOVA. Significant differences marked with asterisk (\* $P < .01$ ; \*\* $P < .001$ ; after HSD Tukey test).

as, the anaerobic metabolic pathway (Hourdez and Lallier, 2007; Spicer, 1999) whose final product is the organic compound lactate (Stillman and Somero, 1996).

Interestingly, here we found higher lactate contents in embryos produced by upper intertidal females compared to embryos produced by subtidal females. In decapod crustaceans, the accumulation of this compound occurs when the demand for ATP exceeds the supply available from oxidative metabolism (Hardy et al., 2006; Jimenez et al., 2008; Morris and Adamczewska, 2002). Subsequently, the presence of

lactate activates metabolic pathways that favor the production of the respiratory pigment hemocyanin and the affinity for oxygen molecules (Alter et al., 2015; Bouchet and Truchot, 1985). As such aerobic metabolism, where carbon dioxide ( $\text{CO}_2$ ) is the final product, is maintained. Despite this, if embryos suffer prolonged aerial exposure,  $\text{CO}_2$  and lactate cannot be eliminated and accumulate thus causing acidosis and permanent tissue damage (James et al., 2005; Holman and Hand, 2009; Wheatly and Taylor, 1981). Interestingly, the embryos of intertidal species tolerate the accumulation of these compounds and quickly eliminate them during high tide (Maciel et al., 2008; Marqueze et al., 2006). In support of the above mentioned, the studied intertidal species (*Petrolisthes laevigatus* and *P. violaceus*) showed low fecundity, high reproductive output, and embryos with high organic matter, water, and lactate contents.

In the other hand, the subtidal species (*Allopandalis punctatus*) had high fecundity, low reproductive output, and embryos with less organic matter, water, and lactate content. These strategies would reflect the characteristics of the habitats, such that these species can maintain their population dynamics and the recruitment of juveniles (Drolet et al., 2013; Hines et al., 1987; Tarling, 2003). Hence, our results support the idea that intertidal species develop strategies that allow them to tolerate the environmental fluctuations caused by daily tidal cycles. In turn, these strategies could allow these species to cope with the future consequences of climate change. Despite this, the geographic distribution of these species could be negatively affected by the consequences of climate change, i.e. sea level rises (Devlin et al., 2017) or increased periods of low tide. In contrast, subtidal species are more adapted to constant environmental conditions. Thus, changes in temperature (Warren et al., 2011; Sun et al., 2018), acidification (Sampaio et al., 2018), oxygen availability (Robbins and Lisle, 2017) and nutrients (Westwood et al., 2018) could seriously alter the metabolism of these organisms (Byrne, 2011; Foo and Byrne, 2017). As a consequence, possible extinctions of local populations or massive migrations to deep areas or adjacent coastal regions could occur (Chen et al., 2014). Unfortunately, these scenarios could occur in the near future given the



**Fig. 6.** *Petrolisthes laevigatus*, *P. violaceus* and *Allopetrolisthes punctatus*. Interspecific variation in embryo biochemical parameters: (A) Lactate content ( $\mu\text{g embryo}^{-1}$ ); (B) Lactate content (% dry weight, DW). Different letters denote significant differences among species. Averages  $\pm$  DE. In all cases  $n = 90$ .

current rate of global warming that continues to increase yearly (Hernán et al., 2017; Lotze et al., 2018; Tonmoy et al., 2018). Likely, many of the porcellanid crab species worldwide (ca. 280 spp) will be affected (Osawa and McLaughlin, 2010; Osawa and Uyeno, 2013; Werding and Hiller, 2015) and thus, a high range of coastal marine ecoregions where porcellanid crabs play a pivotal role for ecosystem function (de Mattos et al., 2014; Thiel and Watling, 2015). These crab family has adapted to diverse coastal ecosystems from temperate to tropical regions; thus, their adaptation to new climate scenarios is of utmost importance to understand their consequences on coastal biodiversity. Furthermore, these changes have already been evidenced on southeastern Pacific namely, a steady decrease of oxygen and pH content in oceanic coastal waters (Alegre et al., 2014; Tamaki et al., 2017; Vargas et al., 2017).

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