The Panama Canal and the transoceanic dispersal of marine invertebrates: Evaluation of the introduced amphipod *Paracaprella pusilla* Mayer, 1890 in the Pacific Ocean

Macarena Rosa, Gail V. Ashton, Mariana B. Lacerda, James T. Carlton, Maite Vázquez-Luís, José M. Guerra-García, Gregory M. Ruiz

Laboratorio de Biología Marina, Departamento de Zoología, Facultad de Biología, Universidad de Sevilla, Avda. Reina Mercedes 6, 41012, Sevilla, Spain

Smithsonian Environmental Research Center, Romberg Tiburon Center, 3152 Paradise Drive, Tiburon, CA 94920, USA

Departamento de Zoología, Universidad Federal do Paraná, Caixa Postal 19023, Curitiba, Paraná, Brazil

Maritime Studies Program, Williams College-Mystic Seaport, Mystic, CT 06355 USA

Instituto Español de Oceanografía, Centre Oceanogràfic de les Balears, Moll de Ponent s/n, 07015 Palma de Mallorca, Spain

Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA

**Abstract**

Although the Panama Canal is one of the major corridors for shipping and potential dispersal of marine invaders in the tropics, little is known about the effect that the Canal has had on the distribution of marine biota. In this study, we (a) document the existence of established populations of the Western Atlantic caprellid amphipod *Paracaprella pusilla*, Mayer, 1890 for the first time at the Pacific entrance to the Canal, (b) review its distribution in the Pacific Ocean, and (c) evaluate possible mechanisms of introduction. The confirmed distribution of *P. pusilla* in the Pacific Ocean is limited to Australia, Hawaii, and Panama, despite earlier published reports from Chile and China. Laboratory experiments demonstrated intolerance of *P. pusilla* to freshwater, causing 100% mortality, and suggest invasion of the Pacific coast of Panama occurred through the Canal via ships’ ballast water or by secondary spread via ships (ballast water or hull fouling) from another Pacific region.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Despite the increased interest in the study of marine biological invasions, little is known about patterns and processes of marine invasions in the tropics relative to temperate regions (Ruiz et al., 2009a; Freestone et al., 2013). The Panama Canal, which created a new shipping route between the Atlantic and Pacific Oceans in 1914, provides an especially important model system for testing predictions about marine invasions in the tropics (Ruiz et al., 2009a). Surprisingly, little is known about the effect that the Canal has had on the distribution of marine biota (Cohen, 2006). Despite being a significant shipping corridor for 100 years, there are relatively few invasions known from Panama compared to many other global regions, and most of these are recent discoveries (Carman et al., 2011; Schlöder et al., 2013). In general, the effectiveness of the freshwater Gatun Lake as a barrier to passage of marine organisms through the Canal has been questioned and summarized by various authors (see McCosker and Dawson, 1975; Cohen, 2006 and references therein). Past records and studies have documented the successful transport of living marine organisms through the freshwaters of the Panama Canal, indicating that such passage occurs, especially associated with commercial ships. Bishop (1947), for example, found that three species of Pacific barnacles had survived the Canal transit, arriving alive on the hull of a vessel in Liverpool, England. Turner (1966) discovered that the Pacific Ocean shipworm *Teredo furcifera* survived the fresh waters of the Canal, arriving alive in 1962 in New York. Menzies (1968), in a short but notable experiment, towed a random mixture of subtidal and intertidal marine invertebrates through the Canal and found generally high survival. Davidson et al. (2008) found surviving barnacles, bryozoans, and isopods, among other hull fouling.
species, on two ships that passed through the Panama Canal, having originated from low salinity waters of San Francisco Bay. Chapman et al. (2013) found two species of barnacles and an oyster from the Atlantic that survived passage on a vessel hull bound for Oregon. Crustaceans are among the most successful aquatic invaders around the world (Carlton, 2011). Among marine crustaceans, there are an increasing number of reports of introduced caprellids, suggesting this group has a relatively high potential for invasions and detection across many global regions. Caprellids, commonly known as skeleton shrimps, constitute an important trophic link between primary producers and higher trophic levels (Woods, 2009). Their reduced abdominal appendages, which in other amphipods are used for swimming (Takeuchi and Sawamoto, 1998), as well as a lack of a planktonic larval stage, suggest that the wide distribution of some littoral caprellids may largely be due to rafting (Thiel et al., 2003; Astudillo et al., 2009) or to transport associated with ships’ ballast water (Carlton, 1985; Gollasch et al., 2002) or hull fouling (Montelli and Lewis, 2008; Carlton and Eldredge, 2009). Established populations of introduced caprellids have been documented from subpolar waters to the tropics. However, the reported number of non-indigenous species almost certainly represent a small fraction of the actual number of introductions (Ruiz et al., 2009a; Zenetos et al., 2010), as is typical of other smaller-bodied and taxonomically challenging taxa (Carlton, 2009). At higher latitudes the Asian species Caprella mutica Schurin, 1935 was the first non-native caprellid clearly established in coastal waters of Alaska, representing an indicator of the vulnerability of the area to marine introductions (Ashton et al., 2008). In the case of tropical introductions, five non-native caprellids were reported by Carlton and Eldredge (2009) in the Hawaiian Islands. Taking into account that most documented marine invasions are sessile invertebrates on hard substrate (Ruiz et al., 2009b), marine amphipods represent an important model group to understand marine invasions of mobile biota associated with hard substrate.

Here, we report the invasion of the Atlantic caprellid Paracaprella pusilla to the Pacific coast of Panama, at the entrance to the Canal. This tropical/subtropical species, originally described from Rio de Janeiro, Brazil (Mayer, 1890) is native to the Western Atlantic Ocean (Mayer, 1903; McCain, 1968; Carlton and Eldredge, 2009, who describe it as a “strongly Caribbean species”) and is one of the most abundant caprellids along the Caribbean coast of Venezuela and Colombia (Díaz et al., 2005; Guerra-García 2006a). In this study, we: (1) Document the occurrence and distribution of P. pusilla at the Pacific and Atlantic entrances to the Panama Canal; (2) Review and clarify the distribution of previous records of P. pusilla in the Pacific Ocean, and (3) Test experimentally the salinity tolerances of P. pusilla under laboratory conditions, to evaluate its potential to survive the freshwater immersion during passage through the Canal and assess the possible vectors of introduction to Panama. To our knowledge, this is the first report of salinity tolerance of a Paracaprella species, and the first report of an introduced caprellid on the Panamanian coasts.

2. Materials and methods

2.1. Panama survey: study area and sampling collections

Panama Canal is a potential hotspot for tropical marine invasions, receiving more ship arrivals than any of the largest ports in the United States (Ruiz et al., 2006; Miller et al., 2007). Surveys were conducted in summers 2004 and 2008 at several sites within the Pacific and Atlantic entrances to the Panama Canal (Fig. 1, Table 1). A standard fouling panel method was used to collect the samples of the sublittoral invertebrate community. Within each
coast, 10 sites of human activity, including marina and harbor pontoons, private and public docks, and shipping terminals were surveyed. At each site, 5 PVC panels (14 × 14 cm) were deployed horizontally, surface-down at 1 m depth below low tide (mean lower low water). The panels were deployed for 3 months to allow colonization of local marine biota. Upon retrieval, panels and associated biota were preserved in 75% ethanol. In the laboratory, the mobile component (organisms not firmly attached to the panels) of the fouling samples was analyzed for the presence of caprellids. Although Atlantic and Pacific Panama surveys were carried out in different years, identical methods were used on each coast, and panels were retrieved in the same season, allowing comparison between the two areas.

*P. pusilla* were identified using the following characteristics: (1) the large anterolateral projection of pereonite 2, (2) small dorsal tubercle on pereonite 2, (3) proximal knob on the basis of gnathopod 2, and (4) lateral pleura in pereonites 3 and 4, especially the large anterolateral projection of pereonite 2, (2) small dorsal caprellids. Although Atlantic and Pacific Panama surveys were carried out in different years, identical methods were used on each coast, and panels were retrieved in the same season, allowing comparison between the two areas.

*P. pusilla* were identified using the following characteristics: (1) the large anterolateral projection of pereonite 2, (2) small dorsal tubercle on pereonite 2, (3) proximal knob on the basis of gnathopod 2, and (4) lateral pleura in pereonites 3 and 4, especially developed in pereonite 3 (see plate 2, figures 36 and 37 in Mayer (1903)). Although *P. pusilla* is relatively similar to *Paracaprella tenuis* Mayer, 1903 (Laubitz, 1972) and can coexist in the northern Gulf of Mexico (Foster et al., 2004), males of the latter can be distinguished from those of *P. pusilla* by the small and triangular anteroventral margin of pereonite 2 and the absence of characteristics 2, 3 and 4 (McCain, 1968; Guerra-Garcia, 2006a, 2010; Winfield and Ortiz, 2013).

All individuals of *P. pusilla* collected from the plates were photographed with a stereomicroscope Motic K-400L with a Nikon D90 digital camera. Body length was measured from the front of the head to the end of pereonite 7, using the PC-based digitizing software Scion Image Alpha 4.0.3.2 (2000–2001 Scion Corporation). Voucher specimens are housed at Smithsonian Environmental Research Center.

### 2.2. Abundance and Distribution Range (ADR) of *P. pusilla* in Panamá

The assessment of the ADR is based on the method of Olenin et al. (2007) and provides a combination of the abundance and frequency of *P. pusilla* in each station (assessment units) examined. This method was modified to adapt it to the fouling panel method in this study. The abundance of *P. pusilla* was ranked in relation to the abundance of the relevant ecological group to which the alien species belongs. In this case, we consider all the mobile fauna present on the fouling panels as the relevant ecological group, including crustaceans (caprellids, gammarids, isopods, tanaids and decapods), annelids, pycnogonids, molluscs and equinoderms. Due to the difficulty of obtaining quantitative data of mobile fauna from fouling panels, since there can be some loss of animals during removal from the water, the units of abundance for each mobile component were based on a semiquantitative scale with 5 levels: 0 (absent), 1 (1–10 individuals per panel), 2 (11–100 individuals per panel), 3 (101–1000 individuals per panel) and 4 (more than 1000 individuals per panel). Based on the ADR method, abundance of *P. pusilla* in each station was then categorized in three levels: 'low' if it represented only a small portion of the mobile community; 'moderate' when it constitutes less than half of the abundance of the mobile community, and 'high' if it exceeded half the abundance and dominated. The distribution scale for *P. pusilla* was evaluated as I, where it appeared only in one panel; II, where it was present in less than half of the panels (2 panels); III, where it was found in more than half of the panels (3–4 panels) and IV, where it was found in the total of panels deployed (5 panels). Combinations of abundance and distribution provide a scale that ranges from 'A' few individuals in one panel to 'E' where a species occurs in high numbers in all panels (Table 2).

<table>
<thead>
<tr>
<th>Coast</th>
<th>Stations</th>
<th>Coordinates</th>
<th>Date of retrieval</th>
<th>Caprellids present</th>
<th>Frequency of occurrence of <em>P. pusilla</em> (%)</th>
<th>Abundance of <em>P. pusilla</em> per plate (semiquantitative scale)</th>
<th>ADR abundance (see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATLANTIC</td>
<td>1</td>
<td>9° 22' 13&quot;N, 79° 56' 57&quot;W</td>
<td>September 2004</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9° 22' 6&quot;N, 79° 57' 3&quot;W</td>
<td>September 2004</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9° 21' 14&quot;N, 79° 54' 44&quot;W</td>
<td>September 2004</td>
<td>P. pus.</td>
<td>40</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9° 20' 57&quot;N, 79° 54' 10&quot;W</td>
<td>September 2004</td>
<td>P. pus.</td>
<td>20</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9° 20' 32&quot;N, 79° 54' 4&quot;W</td>
<td>September 2004</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9° 12' 53&quot;N, 79° 32' 2&quot;W</td>
<td>September 2004</td>
<td>P. pus.</td>
<td>60</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>9° 22' 24&quot;N, 79° 53' 2&quot;W</td>
<td>September 2004</td>
<td>P. pus. C. eq.</td>
<td>80</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9° 22' 14&quot;N, 79° 53' 1&quot;W</td>
<td>September 2004</td>
<td>P. pus.</td>
<td>40</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>9° 21' 40&quot;N, 79° 53' 4&quot;W</td>
<td>September 2004</td>
<td>P. pus. C. eq.</td>
<td>100</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9° 17' 23&quot;N, 79° 55' 9&quot;W</td>
<td>September 2004</td>
<td>P. pus.</td>
<td>100</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>PACIFIC</td>
<td>11</td>
<td>8° 57' 56&quot;N, 79° 34' 24&quot;</td>
<td>September 2008</td>
<td>P. pus.</td>
<td>60</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>8° 57' 10&quot;N, 79° 34' 30&quot;</td>
<td>September 2008</td>
<td>P. pus. C. eq.</td>
<td>60</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>8° 57' 19&quot;N, 79° 33' 57&quot;</td>
<td>September 2008</td>
<td>P. pus.</td>
<td>20</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>8° 57' 31&quot;N, 79° 33' 20&quot;</td>
<td>September 2008</td>
<td>P. pus.</td>
<td>40</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8° 55' 2&quot;N, 79° 32' 6&quot;</td>
<td>September 2008</td>
<td>P. pus.</td>
<td>40</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>8° 55' 3&quot;N, 79° 31' 58&quot;</td>
<td>August 2008</td>
<td>P. pus.</td>
<td>20</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>8° 54' 47&quot;N, 79° 31' 15&quot;</td>
<td>August 2008</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>8° 58' 14&quot;N, 79° 31' 41&quot;</td>
<td>August 2008</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>8° 51' 47&quot;N, 79° 40' 17&quot;</td>
<td>September 2008</td>
<td>P. pus. C. eq.</td>
<td>20</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8° 48' 27&quot;N, 79° 31' 22&quot;</td>
<td>September 2008</td>
<td>P. pus.</td>
<td>60</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

### 2.3. Pacific distribution data

A detailed review of the records of *P. pusilla* in the Pacific Ocean was conducted. Morphology of *P. pusilla* populations from Panama was compared with other *P. pusilla* populations, including drawings of this caprellid species from different world areas (Colombia, Table 2

<table>
<thead>
<tr>
<th>Abundance</th>
<th>Distribution scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>A</td>
</tr>
<tr>
<td>Moderate</td>
<td>B</td>
</tr>
<tr>
<td>High</td>
<td>C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADR classes of abundance and distribution in each station (modified from Olenin et al., 2007, see 2.2 Section)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>High</td>
</tr>
</tbody>
</table>
The survival of *P. pusilla* at various salinities was tested experimentally. Individuals were collected from *Eudendrium sp.* hydroids attached to pонтoon structures in Brazil at Paranaguá harbor (25° 30' S, 48° 30' W) in November 2012 (late dry season), at a temperature of 25 °C and a salinity of 35. Paranaguá harbor is among the most important handling sites for grain and fertilizers in South America (Mizerkowski et al., 2012). It is located in the middle section of Paranaguá Bay, a partially mixed estuary which experienced a large seasonal variation in freshwater discharge (Mantovanelli et al., 2004). During the rainy season, salinity ranges from 0 to 35 and temperature from 23 to 30 °C; during the dry season, salinity ranges from 3 to 35 and temperature from 18 to 25 °C (Marone et al., 2005).

The collection site, close to the type locality of *P. pusilla* (Rio de Janeiro, Brazil), was selected based on the abundance of *P. pusilla* due to high number of individuals needed for the experiments (a total of 570 individuals). The animals were transported in sea water (without food), still attached to the hydroid, in a 20 L container that received constant aeration with several air pumps. Animals were maintained at 25 °C ± 1 °C with a photoperiod regime of 12 D:12 L throughout the tolerance experiments. Temperature was selected based on the average temperature in the collection site at the moment of collection.

Five adults of each sex were placed separately without food in plastic cylindrical containers (9 cm diameter, 12 cm height) open to the air, with 400 ml of water at different salinities. A 1 mm plastic mesh was used as substrate for attachment. Salinities were achieved by addition of artificial sea salt (Tropical Marin) to freshwater treated by reverse osmosis. Evaporation was checked, and the container was topped up with distilled water if necessary. The salinity was measured with a conductivity meter CRISON MM40 and monitored daily to confirm its stability. Survival was checked at 24 and 48 h after the start of the experiment, and dead animals were removed. Additional measures at 1 h and 12 h after the start of the range-finding test were also checked. Death was assumed if an individual did not move when stimulated with a fine needle (Ashton et al., 2007). The first range-finding experiment was conducted at a coarse scale with 7 treatments, using salinities of 0, 5, 15, 25, 30, 45 and 55. A control treatment was included using a similar salinity to the site of collection, 35. These salinities were selected in an effort to include mortality rates ranging from 0% to 100%.

The second experiment employed a narrower range of salinities to detect both the lower and upper lethal limit. The first was estimated to lie at around 15 at 48 h, employing salinities of 13, 15, 17, 19 and 21 in the narrower test, and the second one was estimated to lie at around 45 at 48 h, using salinities of 41, 43, 45, 47, 49 and 51. Consequently, each group of five individuals of the same sex was subjected to 11 treatments with three replicates by treatments and sex.

### 2.5. Statistical analysis

The 24 h and 48 h median lethal concentration (LC50), the concentration of salinity causing 50% mortality for each species, and associated 95% confidence limits were calculated by use of Probit analysis (Finney, 1971). To test whether the data fits the Probit model, Chi-squared analysis were used. A pairwise Student’s-*t*-test was used to compare differences in mortality of male and female *P. pusilla* at each salinity. All analyses were performed using the statistical package SPSS 15.0.

### 3. Results

#### 3.1. Panama survey

The mobile component associate with the panels from the Atlantic coast was dominated by annelids (mainly polychaetes), followed closely by amphipods (caprellids and gammarids), decapods, tanaids and pycnogonids. In minor proportion were molluscs, isopods and equinoiderms (ophiurids). On the Panamanian Pacific coast the dominant group was amphipods (mainly gammarids), followed by annelids (mainly polychaetes), decapods, isopods and tanaids. Echinoderms (ophiurids), pycnogonids and molluscs were also found, but in very low proportion. *P. pusilla* was the dominant caprellid being found for the first time on both Panamanian coasts. Another caprellid species, *Caprella equilibra* Say, 1818 (the variant with ventral projection between gnathopods 2, see McCain, 1968) was also found, being present in two stations in the Atlantic coast and two stations in the Pacific coast (Table 1), where it co-occurred with *P. pusilla*.

*P. pusilla* was found in 70% of stations sampled in the Atlantic coast and 80% of stations sampled in the Pacific coast. However, ADR classes obtained by combination of ADR abundance and frequency of occurrence in each station (Table 1) were different on the two sides of the Panama Canal (Fig. 1). On the Atlantic coast, stations were characterized in general terms by an ADR = B–C with moderate abundance of *P. pusilla* in several or many panels (Table 2). Only in the case of station 10, the closest station to the channel entrance, was *P. pusilla* the dominant species of the mobile fauna associated with all of the panels deployed (ADR = E). On the Pacific coast stations, *P. pusilla* also occurred in several or many panels but always in low numbers (ADR = A – B).

Ovigerous females were found on both sides of the Panama Canal. Males and females on both sides were similar in size (Fig. 2). No morphological differences were found between populations of the Atlantic and Pacific coast of Panama, nor between *P. pusilla* from Panama and populations from other world sites.

#### 3.2. Distribution of *P. pusilla* in the Pacific Ocean

*P. pusilla* presents a disjunct and reduced distribution in the Pacific Ocean. The first occurrence in the Pacific was a female reported by Mayer (1890) from Amoy, China. While this record has been referenced through modern times in almost all caprellid literature, Mayer (1903) in fact later named the same material as a new species, *Paracaprella crassa*. There are thus no verified records of *P. pusilla* that we can locate from the Western Pacific.

The second record of *P. pusilla* in the Pacific Ocean is in Honolulu harbor in Oahu in the Hawaiian Islands (Edmondson and Mansfield, 1948). Although we could not examine specimens from this area, their drawings show the diagnostic features of *P. pusilla*, confirming its presence there.

The next record is a juvenile caprellid reported from Coquimbo, Chile by Guerra-García and Thiel (2001). Their drawings are of a juvenile *Paracaprella*, but the specimen still lacks the diagnostic characteristics of *P. pusilla* and may, therefore, be another species of this genus. A second record of *P. pusilla* from Chile is from aquaculture buoys (Astudillo et al., 2009). These specimens proved to be juvenile *Deutella* sp.

The last record of *P. pusilla* in the Pacific Ocean is in Australia, on hull fouling communities of local vessels (Montelli and Lewis, 2006a; India, Guerra-García et al., 2010; Venezuela, Díaz et al., 2005 and Hawaii, Edmondson and Mansfield, 1948) and our own examination of specimens from Chile, Australia, the Gulf of Mexico, Brazil, Southern Spain, the Balearic Islands and India, in order to establish possible morphological differences.
208. The pictures of adult specimens provided together with our own examination of specimens from this area confirm the identification.

In summary, the presence of *P. pusilla* in the Pacific Ocean is confirmed for Hawaii and Australia (and, now, Panama). Adult *Paracaprella* will be required to confirm if *P. pusilla* is present in Chile.

3.3. Salinity tolerance experiments

In the first range-finding test, *P. pusilla* stops all activity, measured as body movement, just after submerged in freshwater. After 1 h, a mortality rate of 100% was confirmed for this treatment. In the case of caprellids exposed to a salinity of 5, a high mortality (86%) was observed after 1 h. Although some individuals were still alive, their movements were very slow. After 12 h, a mortality rate of 100% was confirmed for caprellids exposed to salinities of 5 and 55. In the second experiment, which employed a narrower range of salinities, the low salinity 24 h LC50 (Fig. 3A) was significantly lower (*P* < 0.01) for females, estimated as 10.4, than that for males, estimated as 13.3. The low salinity 24 h LC50 for the total population was estimated in 11.7. Confidence intervals were unreliable at 24 h because of low salinity 24 h LC50 was beyond the experimental conditions and was 17.8 (16.7–19.3) for the total population. The lower LC50 for females, which was 16.7 (15.1–18.4), was significantly below (*P* < 0.05) that for males, estimated as 18.7 (17.2–22.2).

In experiments of high salinity tolerances, no significant differences were found between males and females. The high salinity 24 h LC50 (95% confidence limits) for females was 46.4 (45.4–47.4), for males was 46.3 (44.8–47.8) and for the total population was 46.3 (45.5–47.1) (Fig. 3A). After 48 h, the high salinity LC 50 (95% confidence limits) for females was 43.7 (42.3–44.8), for males was 42.8 (41.0–44.0) and for the total population was 43.3 (42.2–44.1) (Fig. 3B).

4. Discussion

4.1. Relative abundance of *P. pusilla* in Panama and taxonomic remarks

Our analyses indicate that the frequency of occurrence of *P. pusilla* was very high on both Panamanian coasts. However, the ADR method highlighted important differences in the abundance and local distribution range of *P. pusilla* between the Atlantic and Pacific entrances to the Panama Canal. *P. pusilla* was less abundant and its distribution per station was more limited on the Pacific side than on the Atlantic side of the Panama Canal.

The ADR method provides baseline information about the status of an alien species at a certain point in time that is independent of previous surveys (Minchin and White, 2014), but it does not inform us about the potential effects of the invasion. While all alien species may have some level of impact (Carlton, 2002) following colonization (e.g., through occupying space, consuming native prey, or other processes), the ecological effects of *P. pusilla* has not yet been evaluated in Panama and are poorly understood in other geographic regions.

*P. pusilla* from different biogeographic areas, including populations found in Panama, showed little intraspecific variation in morphology. This contrast with other caprellid species, such as *Caprella acanthifera*, *C. equilibra*, *Caprella penantis* or *Caprella scabra*, which belong to a complex of several morphological forms (e.g. McCain, 1968; Krapp-Schickel and Vader, 1998; Krapp et al., 2006; Cabezas et al., 2013). In the case of *P. pusilla*, there are no different forms described or morphological variations that remain constant among populations. Furthermore, Cabezas (2014) found low levels of mitochondrial cytochrome c oxidase subunit I (COI) genetic divergence between geographically distant populations, confirming the absence of cryptic species and clearly separating those *P. pusilla* populations from the closely-related species *P. tenuis*. As expected, preliminary genetic analyses indicate that Pacific Panam, Gulf of Mexico, and Brazilian populations are the same species (M. Ros and P. Cabezas, unpublished data). Thus, morphological and molecular evidences support the usefulness of Mayer’s diagnostic characters (see Material and Methods) to properly identify specimens belonging to *P. pusilla* and to differentiate them from similar species like *P. tenuis*.

4.2. On the introduction of *P. pusilla* in the Pacific Ocean

The first confirmed record of *P. pusilla* in the Pacific Ocean was about two decades after the opening of the Panama Canal, in 1937, in Honolulu harbor (Edmondson and Mansfield, 1948). Curiously, there are no additional verified reports of the species in the Pacific until 2003, when it was found on the hulls of local vessels in Australia (Montelli and Lewis, 2008). In 2008, as noted here, it was found at the Pacific entrance to the Panama Canal. In all of these locations — Hawaii, Australia, and Panama — *P. pusilla* may have been present years or decades earlier. No caprellid studies preceded those of Edmondson and Mansfield in Hawaii, and *P. pusilla* may have been overlooked as well in Australia. However, in the last case, none of the previous extensive studies focused on the caprellid fauna of Australia, including Tasmania (Guerra-García, 2004; 2008b: Guerra-García and Takeuchi, 2004) reported the presence of *P. pusilla* in the area. In contrast, previous caprellid studies in Panama are limited to isolated records of a few species, such as *C. equilibra*, which was found at Taboga Island (McCain and Steinberg, 1970) and *Paracaprella barnardi* McCain, 1967 from Culebra Island.
More extensive surveys along the Pacific coasts of Costa Rica, Panama, Colombia, and other Central and South American shores will be of great value in establishing the full range of *P. pusilla* in the tropical Eastern Pacific. If combined with detailed genetic analyses, this may also reveal the invasion history of the region.

### 4.3. Possible vectors of its introduction to Pacific Panama

There are two possible shipping-related vectors for the appearance of *P. pusilla* on the Pacific coast of Panama. The first is transport from the Caribbean or Atlantic South America in ballast water through the Canal. Importantly, our results indicate that *P. pusilla* would not survive the time of exposure on a ship’s hull to completely fresh water while passing through Gatun Lake (this study, and as further discussed below). Caprellids are common in harbors and ports drifting on small pieces of debris, seaweed, or seagrasses, and are often found in plankton tows “free” floating in the water as members of the tychoplankton; as such, they could easily be taken up into ballast tanks, where they have in fact been found (Carlton, 1985; Gollasch et al., 2002). No objects are known to raft through the entire Canal, from one ocean to the other; nor would rafting permit a rapid transit of a few hours through Gatun Lake. While we cannot exclude the human-aided transfer of the caprellids associated with construction materials, fishing gear, dredges, or other objects across coasts of Panama, any such transfers would have to remain wet (but not exposed to freshwater of the Canal) and be rapidly immersed into Pacific waters to allow species survival, appearing to be a rare if not unlikely event. Reish and Barnard (1979) commented that while the Panama Canal could be a pathway for amphipod transport, they knew of no species that had yet “transgressed” the Canal. If *P. pusilla* originated from the Caribbean, this caprellid would be the first example of an amphipod that was known to have successfully passed through the Canal.

The second likely source is transport associated with ballast water tanks and/or the biofouling community on ships’ hulls or underwater surfaces arriving from introduced populations in Australia or Hawaii, with caprellids dispersing from hull fouling communities while vessels from either of these regions were stationary at the Canal entrance, as vessels can wait at anchorage for days before transiting the Canal. Genetic studies may assist in identifying the origin of the Pacific Panama *P. pusilla* population, particularly if Western Atlantic, Australian, or Hawaiian populations have either private alleles, unique proportions of haplotypes, or both. Unfortunately, the limited preserved specimens in absolute ethanol from the potential source populations analyzed and the need for further intensive sampling from both more native and non-native populations of this species prevent us to address this question throughout molecular tools.

### 4.4. Salinity tolerance of *P. pusilla* and its implications for its dispersal

The salinity tolerance of *P. pusilla* is similar to that of other caprellid species. The lower 48 h LC50 (17.8) is slightly lower than that of the invasive caprellid *C. mutica* with 48 h LC50 values of 18.7 (Ashton et al., 2007). However, the 24 h LC50 of *P. pusilla* (11.6) is higher than that of another invasive caprellid, *C. scaura* Templeton,
1836, with 24 h LC50 values of 7.5 (Cockman and Albone, 1987). The increased tolerance to lower values of salinity of P. pusilla females in comparison with males is just the opposite case to C. mutica, in which males are more tolerant than females (Ashton et al., 2007). However, a high tolerance in females has been described in other amphipods like Bathyporeia pelagica, Bathyporeia pilosa (Preene, 1970) and Corophium volutator, in which the gravid females have a wider tolerance than non-gravid adult females which in turn are more tolerant than adult males (Mills and Fish, 1980). During freshwater input events, a higher potential to survive by reproductive females, with eggs in its brood pouchs, may help in the survival of their offspring.

In general terms, the wide salinity tolerance of P. pusilla may help in the survival and establishment success of the species in either side of Gatun Lake and in the Panamanian coasts, which support large freshwater inputs in the rainy season (when the species was collected). This is also frequent in anthropogenic habitats, like marinas and harbors, in which euryhaline species may be favored. In the approach channels of the Panama Canal, measurements indicate that salinity ranges between 10 and 30 (Cohen, 2006). If low salinity periods are maintained over time, P. pusilla could reduce its distribution to those places where low salinities are not below 15. However, rapid changes in salinity could be supported if they are not below 5. Therefore, although P. pusilla may be associated with fouling communities of ship hulls, it would not survive the freshwater barrier of the Panama Canal. For this reason, ballast water is suggested as the introduction vector to its inter-oceanic introduction through the Panama Canal, if the Caribbean is the source of this population.

Relative to its high salinity tolerance to hypersaline waters, with upper salinity tolerances above 46 at 24 h and 43 at 48 h observed in our experiment, it is probable that P. pusilla would be able to survive the hypersaline waters of the Suez Canal within the fouling communities attached to ship hulls that transit Suez. In fact, the presence of P. pusilla in the Suez Canal was recorded by Schellenberg (1928), although since this report, no evidence of established populations of P. pusilla has been found in this area or surroundings (Ros et al., 2013). Different factors other than salinity tolerance may be involved if P. pusilla failed to establish in the Suez Canal.

Acknowledgments

We express our gratitude to the SERC (Smithsonian Environmental Research Center) staff who were involved with sample collection in Panama, and to K. Larson, M. Repetto and S. Havarad for their technical support. We thank all those who kindly provided material for us to study: E. Baeza-Rojano from the Gulf of Mexico, L. Montelli from Australia, and M. Thiel and J.C. Astudillo from Chile. We gratefully thank P. Fofonoff for his support during the stay of the first author at SERC and S. Massunari for her advice and support during the experiments. Three referees provided useful comments that improved the manuscript. This work was funded by a pre-doctoral grant from the Ministerio de Educación de España (Reference AP-2009-3380), and by the Smithsonian Institution.

References

M. Ros et al. / Marine Environmental Research 99 (2014) 204–211

211

Ros, M., Vázquez-Luis, M., Guerra-García, J.M., 2013. The tropical caprellid amphipod
Ruiz, C.M., Torchin, M.E., Grant, K., 2009a. Using the Panama Canal to test predictions
Ruiz, C.M., Freestone, A.F., Fofonoff, P.W., Simkanin, C., 2009b. Habitat distribution and
Schellenberg, A., 1928. Report on the amphipoda. Zoological results of the Cam-
Takeuchi, I., Sawamoto, S., 1998. Distribution of caprellid amphipods (Crustacea) in
littoral caprellids (Crustacea: Amphipoda: Caprellidea) along the Pacific coast of
of Comparative Zoology, Harvard University, Cambridge MA, p. 265.
Winfield, I., Ortiz, M., 2013. The Caprellidea (Crustacea: Peracarida: Amphipoda)
from the Gulf of Mexico with a description of a new species of Paracaprella. Sci.
Mar. 77, 161–168.
resource? Aquaculture 289, 199–211.
Zenetos, A., Gofas, S., Verlaque, M., Cinar, M.E., Garcia Raso, J.E., Bianchi, C.N.,
Morri, C., Azzurro, E., Bilencenoglu, M., Froglia, C., Siokou, I., Violanti, D., Sfriso, A., San Martin, C., Giargiante, A., Karağan, T., Ballesteros, E., Ramos-

Mantovanelli, A., Marone, E., da Silva, E.T., Lautert, L.F., Klingensfuss, M.S., Prata, V.P.,
Noernberg, M.A., Knoppers, B.A., Angulo, R.J., 2004. Combined tidal velocity and
duration asymmetries as a determinant of water transport and residual flow in
Mayer, P., 1903. Die Caprellidae der Siboga Expedition. Siboga-Exped. 34, 1
Mayer, P., 1890. Die Caprelliden des Golfes von Neapel und der angrenzenden
McCain, J.C., 1968. The Caprellidea (Crustacea: Amphipoda) of the western North
Gruner, H.E., Holthuis, L.B. (Eds.), Crustaceorum Catalogus, Pars 2. Dr. W. Junk
McCosker, J.E., Dawson, C.E., 1975. Biotic passage through the Panama Canal with
Menzies, R.J., 1968. Transport of marine life between oceans through the Panama Canal.
Mizerkowski, B.D., Hesse, K.J., Ladwig, N., Machado, E.C., Rosa, R.A., Araújo, T.G.,
Koch, D., 2012. Sources, loads and dispersion of dissolved inorganic nutrients in
Miller, A.W., Lion, K., Minton, M.S., Ruiz, G.M., 2007. Status and Trends of Ballast
Water Management in the United States. U.S. Coast Guard, Washington, D.C.
Mills, A., Fish, J.D., 1980. Effects of salinity and temperature on Corophium volutator
and C. arenarium (Crustacea: Amphipoda), with particular reference to distri-
Minchin, D., White, B., 2014. A rapid assessment method for an invasive mollusc in
Crustacea; Isopoda and Amphipoda; Caprellidea. Defence Science and Tech-
Olenin, S., Minchin, D., Daunys, D., 2007. Assessment of biopollution in aquatic
Preece, G.S., 1970. Salinity and survival in Botryllus schlosseri Linndrum and