



Tesis de Maestría

**La salinidad como factor estresante:
Análisis fisiológico en dos especies
de arañas lobo (Araneae, Lycosidae)
de hábitats costeros**

Lic. Lucía Rabinovich Larrechea

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Título

**La salinidad como factor estresante: Análisis fisiológico en dos especies de
arañas lobo (Araneae, Lycosidae) de hábitats costeros**

Title

Salinity as a Stress Factor: A Physiological Analysis of Two Wolf Spider Species
(Araneae, Lycosidae) from Coastal Habitats

Autora/Author

Lic. Lucía Rabinovich Larrechea

Orientadora/Academic director

Dra. Sabrina Clavijo Baquet

Sección Etología, Facultad de Ciencias, Universidad de la República, Uruguay

Co-orientadora/Academic co-director

Dra. Andrea Albín

Departamento de Etología, Ecología y Evolución, Instituto de Investigaciones Biológicas
Clemente Estable, Uruguay

Sección Entomología, Facultad de Ciencias, Universidad de la República, Uruguay

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Tribunal

Presidente

Dr. Matías Arim

Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este,
Universidad de la República, Uruguay

Vocales

Dra. Grisel Cavieres

Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad
de Concepción, Chile

Dr. Miguel Simó

Sección Entomología, Facultad de Ciencias, Universidad de la República, Uruguay

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- **Rabinovich-Larrechea, L., Albín, A., Dauvergne, X., Pétilion, J., & Clavijo-Baquet, S.** Intraspecific variation in physiological mechanisms under combined salinity and temperature stress in a coastal wolf spider. *Functional Ecology*.

El envío de este artículo depende de la incorporación de resultados del análisis de la composición de la cutícula realizada en los mismos individuos del Capítulo 1. Dicho análisis se realizó en colaboración con la Université de Rennes, Francia.
- **Rabinovich-Larrechea, L., Albín, A., Hagopían, D., Aguilar, A. B., & Clavijo-Baquet, S.** Salinity tolerance and its ecological consequences: intra- and interspecific variation in two wolf spider species from coastal ecosystems. *Journal of Animal Ecology*.
- **Rabinovich-Larrechea, L., Albín, A., Hagopían, D., Laborda, Á., & Clavijo-Baquet, S.** Salinity effects on body size, condition and sexual dimorphism across time and space in a coastal wolf spider. *Journal of Zoology*.
- **Rabinovich-Larrechea, L., Albín A., & Clavijo-Baquet, S.** Discontinuous respiratory patterns under salinity conditions support the hygric hypothesis in sand-dwelling wolf spiders (Araneae, Lycosidae). *Comparative Biochemistry and Physiology Part A*.

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Resumen general

Las variaciones ambientales imponen desafíos constantes a la homeostasis de los organismos, condicionando su desempeño y su interacción con el entorno. Para mantener sus funciones vitales, estos recurren a respuestas comportamentales (como la selección de hábitat), fisiológicas y morfológicas, que interactúan entre sí y se evidencian a distintas escalas espacio-temporales. En este contexto, la flexibilidad fisiológica (reversible e intraindividual) es un mecanismo clave para determinar su viabilidad en ambientes fluctuantes.

Esta tesis aborda los mecanismos fisiológicos que median la respuesta y tolerancia a la salinidad en artrópodos terrestres de ecosistemas costeros, contemplando dos escalas espaciales y niveles de organización. Para ello, se estudiaron dos especies de arañas lobo del género *Allocosa*. A pesar de que la osmorregulación, que regula la concentración de agua y solutos, y la termorregulación, que regula la temperatura corporal, son procesos clave para el funcionamiento de los organismos, se conoce poco sobre su interacción, especialmente en ectotermos terrestres, donde la deshidratación y el sobrecalentamiento imponen fuertes presiones selectivas. Bajo este contexto, el primer capítulo evalúa cómo la exposición simultánea a salinidad y temperatura en condiciones de laboratorio afecta la fisiología de hembras de *Allocosa senex* provenientes de dos playas localizadas cada una en un extremo del gradiente natural de salinidad del estuario del Río de la Plata. Para ello se evaluó la Tasa Metabólica Estándar (SMR), la Pérdida Total de Agua por Evaporación (TEWL) y la pérdida de peso en individuos expuestos a tratamientos combinados de salinidad (0‰, 35‰ y 70‰) y temperatura (25 °C y 32 °C). Las hembras difirieron en sus respuestas fisiológicas (SMR, TEWL, pérdida de peso) en función de su playa de origen y la interacción entre salinidad y temperatura varió según el rasgo considerado. Los resultados resaltan la importancia de integrar esta complejidad para comprender mejor los mecanismos que utilizan los artrópodos terrestres para enfrentar cambios ambientales.

El segundo capítulo evalúa la relación entre la tolerancia fisiológica y la distribución local de *A. senex* (machos y hembras) y *A. marindia* (hembras), en una playa estuarina externa con influencia salina. Comprender cómo la tolerancia fisiológica influye en la selección de hábitat resulta clave para entender de qué manera distintas variables determinan la distribución de las especies y sus interacciones. En este sentido, se evaluó la tolerancia a la salinidad en laboratorio, utilizando tres tratamientos de salinidad (0‰, 35‰, 70‰) y midiendo SMR, TEWL, pérdida de peso y sobrevivencia. Estos resultados se compararon con los patrones de distribución a mesoescala, estimados a través de trampas de caída en campo. Los resultados mostraron diferencias intra e interespecíficas en la tolerancia a la salinidad, vinculadas a rasgos fisiológicos (SMR, TEWL, sobrevivencia) que inciden en la selección de hábitat. Asimismo, sugieren que mientras que para una especie su distribución está más vinculada a su tolerancia fisiológica, la otra podría verse más condicionada por factores ecológicos, como interacciones interespecíficas.

En su conjunto, esta tesis a través de un enfoque mecanicista aporta evidencia sobre las capacidades y limitaciones de la respuesta fisiológica en artrópodos terrestres de ambientes costeros, y contribuye al marco teórico acerca de los factores que influyen sobre la distribución de los organismos en ambientes fluctuantes.

General abstract

Environmental variations impose constant challenges to organismal homeostasis, shaping their performance and interactions with the environment. To maintain vital functions, organisms rely on behavioral responses (such as habitat selection), as well as physiological and morphological adjustments, which interact with each other and manifest across multiple spatial and temporal scales. In this context, physiological flexibility (reversible and intra-individual) is a key mechanism for determining organismal viability in fluctuating environments.

This thesis addresses the physiological mechanisms underlying the response and tolerance to salinity in terrestrial arthropods from coastal ecosystems, considering two spatial scales and organizational levels. For this purpose, two wolf spider species of the genus *Allocosa* were studied. Although osmoregulation, which regulates water and solute concentrations, and thermoregulation, which controls body temperature, are key processes for organismal functioning, little is known about their interaction, particularly in terrestrial ectotherms, where dehydration and overheating impose strong selective pressures. In this context, the first chapter evaluates how simultaneous exposure to salinity and temperature under laboratory conditions affects the physiology of female *Allocosa senex* from two beaches located at opposite ends of the natural salinity gradient of the Río de la Plata estuary. Standard Metabolic Rate (SMR), Total Evaporative Water Loss (TEWL), and weight loss were measured in individuals exposed to combined treatments of salinity (0‰, 35‰, and 70‰) and temperature (25 °C and 32 °C). Females differed in their physiological responses (SMR, TEWL, weight loss) depending on their beach of origin. Moreover, the interaction between salinity and temperature varied according to the trait considered. These results highlight the importance of integrating this complexity to better understand the mechanisms terrestrial arthropods use to cope with environmental changes.

The second chapter evaluates the relationship between physiological tolerance and the local distribution of *A. senex* (males and females) and *A. marindia* (females) on an external estuarine beach influenced by salinity. Understanding how physiological tolerance affects habitat selection is crucial to elucidate how different variables shape species distribution and interactions. Salinity tolerance was assessed in the laboratory using three salinity treatments (0‰, 35‰, 70‰), measuring SMR, TEWL, weight loss, and survival. These results were compared with mesoscale distribution patterns estimated through pitfall traps in the field. The findings revealed intra- and interspecific differences in salinity tolerance, linked to physiological traits (SMR, TEWL, survival) that influence habitat selection. They also suggest that while the distribution of one species is more closely associated with its physiological tolerance, the other may be more constrained by ecological factors, such as interspecific interactions.

Overall, through a mechanistic approach, this thesis provides evidence on the capabilities and limitations of physiological responses in terrestrial arthropods from coastal environments and contributes to the theoretical framework regarding the factors shaping organismal distribution in fluctuating habitats.

Introducción general

Los organismos están constantemente expuestos a variaciones ambientales que desafían su homeostasis. Cuando dichas variaciones ocurren en escalas temporales menores que su tiempo generacional, la plasticidad fenotípica, y en particular, la flexibilidad fenotípica (reversible e intraindividual), se convierte en un factor clave para su viabilidad en ambientes fluctuantes (Pfennig, 2021). En este sentido, la flexibilidad fenotípica permite a los organismos ajustar sus fenotipos y potencialmente aumentar su probabilidad de supervivencia y reproducción (Piersma & Drent, 2003). En el último tiempo, numerosos estudios han avanzado en comprender cómo los factores ambientales influyen en los rasgos de los organismos (fisiológicos y conductuales) y que rol tiene la plasticidad en su respuesta. Sin embargo, aún existen contextos menos explorados como los ecosistemas costeros y los organismos terrestres (Lorrain-Soligon et al., 2024; Rozen-Rechels et al., 2019).

Uno de los principales desafíos fisiológicos que enfrentan los organismos es la osmorregulación, es decir, la capacidad de mantener un balance adecuado de agua y solutos en sus fluidos corporales (Larsen et al., 2014). Este proceso cobra especial relevancia en especies que habitan ecosistemas con marcados gradientes de humedad y salinidad, como los ambientes costeros, donde el dinamismo ambiental impone presiones constantes (Lorrain-Soligon et al., 2024). En dichos ambientes, la tolerancia y la flexibilidad fisiológica resultan determinantes para su selección de hábitat y las relaciones interespecíficas. Asimismo, en la naturaleza los organismos suelen enfrentar de forma simultánea cambios ambientales que afectan distintos procesos fisiológicos, como la osmo y la termorregulación (es decir, la regulación de la temperatura corporal). Sin embargo, son pocos los estudios que han evaluado cómo interactúan estos procesos, lo cual limita una comprensión más realista de cómo y hasta qué punto las especies pueden responder a ciertos cambios en su ambiente. Hasta donde sabemos, las investigaciones que han abordado esta pregunta se realizaron en organismos acuáticos (ej. artrópodos: Torres et al., 2020, 2021; peces: Vargas-Chacoff et al., 2018, 2020). Por ende, a pesar de que los

organismos terrestres también enfrentan importantes desafíos osmorreguladores, han recibido considerablemente menos atención (Lorrain-Soligon et al., 2024; Rozen-Rechels et al., 2019).

En este contexto, los ectotermos terrestres y en concreto, los artrópodos, constituyen un buen modelo de estudio para evaluar capacidades osmorreguladoras ya que enfrentan dos grandes desafíos: ser terrestres y pequeños. Su elevada relación superficie-volumen los hace particularmente vulnerables a altas tasas de pérdida de agua y, en consecuencia, a la deshidratación, un riesgo que se intensifica por su constante contacto con el aire (Chown & Nicolson, 2004). En ecosistemas costeros, donde la variabilidad ambiental impone presiones selectivas sobre rasgos como el metabolismo y la sobrevivencia, el desafío fisiológico es aún mayor (Lorrain-Soligon et al., 2024). Estas especies suelen presentar elevada plasticidad fenotípica y/o adaptación local en rasgos fisiológicos y comportamentales, que les permite desempeñarse y habitar bajo tales condiciones (Lorrain-Soligon et al., 2024; McLachlan & Defeo, 2017). Sin embargo, los mecanismos que subyacen las respuestas fisiológicas de estas especies aún no son comprendidos en detalle.

Los artrópodos presentan diversos mecanismos osmorreguladores, incluyendo modificaciones en la composición de la cutícula, en la respiración y la excreción (Butt & Taylor, 1986; Chown & Nicolson, 2004; Halberg & Denholm, 2023; Pulz, 1987). En algunos organismos, la tolerancia a la pérdida de agua constituye un mecanismo complementario (Chown & Nicolson, 2004). El grado de plasticidad fenotípica y presencia de adaptación local en rasgos asociados a la osmoregulación, varía tanto entre poblaciones de una misma especie como entre especies distintas (ej. Renault et al., 2014, 2016). Por otra parte, en artrópodos acuáticos se ha observado que la interacción entre los procesos de osmo y termorregulación es compleja y difícil de predecir, ya que ambos pueden incrementar la pérdida de agua y modificar el gasto energético (es decir, la tasa metabólica), pero afectan diferentes sistemas fisiológicos (Torres et al., 2021). Sin embargo, esta relación aún no se ha evaluado en artrópodos terrestres (Rozen-Rechels et al., 2019).

Bajo este contexto, en esta tesis se evaluaron los efectos de la salinidad sobre la fisiología (ej. tasa metabólica, pérdida de agua) y la selección de hábitat en artrópodos terrestres de ambientes costeros. Para ello, se estudiaron dos especies de arañas lobo neotropicales del género *Allocosa*: *A. senex* (Mello-Leitão, 1945) y *A. marindia* (Simó, Lise, Pompozzi & Laborda, 2017) (Figura 1). Estas especies sincrónicas y simpátricas habitan en playas arenosas de Uruguay, distribuyéndose a lo largo de un marcado, pero dinámico, gradiente de salinidad que abarca desde el Río Uruguay, pasando por el estuario del Río de la Plata, hasta el Océano Atlántico (Costa, 1995; Costa et al., 2006; Jackson et al., 2021; Simó et al., 2017). Ambas especies habitan zonas de dunas y son completamente terrestres. A pesar de sus semejanzas, *A. senex* y *A. marindia* exhiben marcadas diferencias: *A. senex* es significativamente más grande (ancho de prosoma: *A. senex*, hembras: 5.4–4.6 mm, machos: 7.0–5.1; *A. marindia*, hembras: 3.2–2.9, machos: 3.7–2.8; Simó et al., 2017) y depreda sobre *A. marindia*. Además, en playas con baja influencia salina, *A. senex* ocupa zonas con poca vegetación y cercanas a la costa, mientras que *A. marindia* ocupa áreas con mayor cobertura vegetal y lejanía a la costa (Aisenberg et al., 2009, 2023; Costa, 1995; Costa et al., 2006). Estas características las convierten en buenos modelos biológicos para estudiar cómo los artrópodos terrestres toleran fisiológicamente los cambios ambientales.

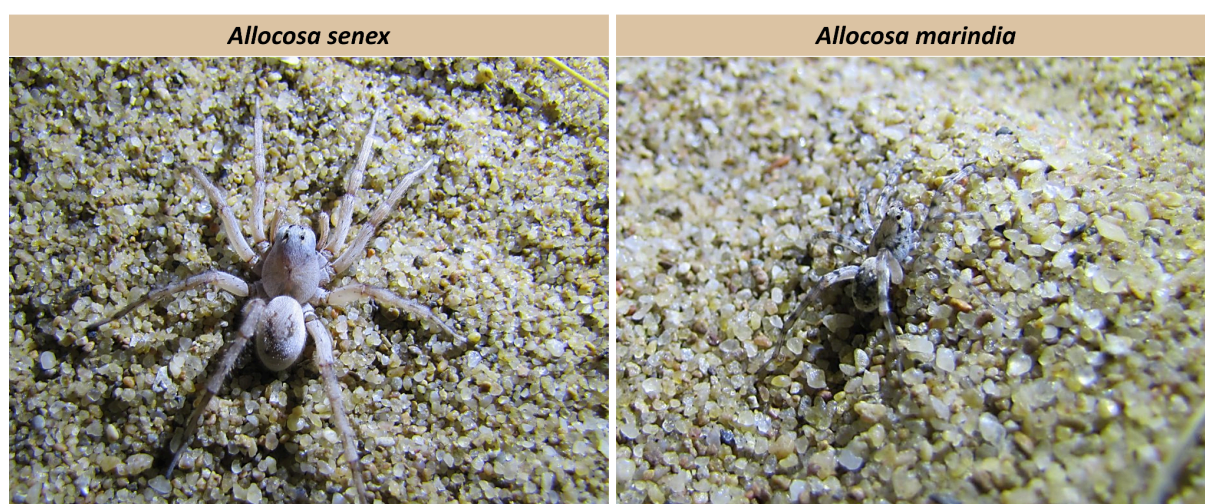


Figura 1. Especies de arañas lobo del género *Allocosa*. Fotos: Sabrina Clavijo Baquet.

La tesis se organizó en 2 capítulos. En el primer capítulo se investiga el efecto simultáneo de la salinidad y la temperatura sobre la fisiología de hembras de *A. senex* provenientes de dos localidades con condiciones contrastantes de salinidad. El segundo capítulo evalúa la tolerancia fisiológica a la salinidad en *A. senex* y *A. marindia* y su relación con los patrones de distribución en una playa estuarina-externa con influencia salina. Estos dos capítulos se escribieron en inglés y con el formato para su posterior publicación. La introducción general, las conclusiones, las perspectivas y los anexos se escribieron en español. En los anexos se presenta información complementaria y novedosa obtenida durante la realización de los capítulos principales, pero que no son relevantes para responder las preguntas de investigación de cada uno.

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Chapter 1



Intraspecific variation in physiological mechanisms under combined salinity and temperature stress in a coastal wolf spider.

Abstract

Organisms frequently face stressful conditions arising from multiple environmental factors. However, most studies on phenotypic plasticity have focused on single-variable effects. In this sense, temperature and salinity have major influences on individual responses, and despite osmo- and thermoregulation being two key physiological processes on organismal functioning, how they interact remains poorly understood. This is especially critical for terrestrial ectotherms, where dehydration and overheating can impose strong selective pressures. Here, we assessed how the simultaneous exposure to salinity and temperature under laboratory conditions affects the physiology of female *Allocosa senex*, a wolf spider inhabiting beaches distributed along a marked salinity gradient in the Río de la Plata estuary, Uruguay. We estimated Standard Metabolic Rate (SMR), Total Evaporative Water Loss (TEWL) and weight loss (WL) of females from two beaches with naturally contrasting salinity regimes, combining salinity (0 ‰, 35 ‰, 70 ‰) and temperature (25 °C, 32 °C) treatments. Oceanic and riparian females displayed different physiological responses: while oceanic females showed no SMR plasticity, riparian females exhibited metabolic depression under high salinity and temperature. TEWL was influenced by salinity-temperature interaction, but its variation differed depending on females' beach of origin, revealing differential sensibility. While all females lost more weight at high temperature, oceanic females exhibited greater WL regardless of temperature. Our study reveals intraspecific variation in physiological responses to multiple stressors and highlights the need to integrate such complexity to further understand the physiological mechanisms that underlie terrestrial arthropods responses to the environment.

Introduction

Water availability and temperature are two key determinants of a species' presence in a given environment, especially in coastal ecosystems (Chown & Nicolson, 2004; Lorrain-Soligon et al., 2024). Yet, most studies have examined them separately, with environmental temperature being the most extensively studied (e.g., González-Tokman et al., 2020; Malmos et al., 2021; Rezende & Bozinovic, 2019; Segura-Hernández et al., 2024). Studying how these factors interact (synergistically, antagonistically, or independently) is a key step for understanding their influence over organismal performance and fitness. Recent research on aquatic ectotherms (e.g., arthropods: Torres et al., 2020, 2021; fish: Vargas-Chacoff et al., 2018, 2020) has begun to unravel the combined effects of salinity and temperature on physiology, as salinity influences water uptake and loss. However, this interaction has been largely unexplored in terrestrial organisms (Lahondère, 2023; Rozen-Rechels et al., 2019). Given the susceptibility of terrestrial ectotherms, particularly arthropods, to water loss (Benoit et al., 2024) and overheating (Lahondère, 2023), addressing this knowledge gap is crucial for understanding how these species cope with combined environmental pressures and what physiological mechanisms underlie their responses (Lorrain-Soligon et al., 2024).

Osmoregulation is a key physiological process that regulates salt concentration and water loss (Larsen et al., 2014). Due to their high surface to volume ratio, arthropods face high rates of water loss (Hadley, 1994; Pulz, 1987), which can be further increased by ambient salinity (Elnitsky et al., 2009). To maintain osmolarity and manage water loss they employ a range of physiological strategies, including modifying their cuticle wax layer composition, respiratory patterns, and excretion (Butt & Taylor, 1986; Chown & Nicolson, 2004; Halberg & Denholm, 2023; Pulz, 1987). Moreover, in terrestrial and coastal arthropods, where living in air already imposes a high risk of dehydration, environmental salinity can further affect survival and reproduction (Foucreau et al., 2012; Leggett et al., 2024; Pétilion et al., 2011). Foucreau et al., (2012) showed that survival and egg-laying were significantly

reduced when females of the salt marsh specialist species *Arctosa fulvolineata* were exposed to hypersaline conditions (70 ‰). In some species, tolerance to salinity reflects the habitats they occupy (e.g., Moloney & Nicolson, 1984; Pétilion et al., 2008, 2011; Renault et al., 2016), and in some cases, it can vary intraspecifically, reflecting different degrees of plasticity or adaptation to local conditions (Gao et al., 2018; Ghalambor et al., 2007; Pfennig & West-Eberhard, 2021). In this sense, Renault et al., (2014) showed that individuals of the Kerguelen Islands fly, *C. moseleyi*, inhabiting a saline zone survived longer and lost less water at high salinity than those in a non-saline zone. While salinity impacts water loss, temperature presents another significant challenge to osmoregulation.

Temperature plays a key role in arthropod physiology, as their body temperature fluctuates with their environment (i.e., ectotherms). Rising temperatures increase metabolic rates (the rate at which organisms consume energy) and water loss through evaporation (Pulz, 1987). Consequently, it challenges homeostasis by reducing energy available for maintenance and risking dehydration. Arthropods may display behavioral and physiological responses to cope with high temperatures (e.g., Brandt et al., 2020; Malmos et al., 2021). For example, metabolic depression (the decrease of metabolic rates) is a common strategy to mitigate the risk of overheating, as it reduces metabolic heat production and respiratory water loss (Anderson, 1970; Teets et al., 2012). Moreover, the cuticle wax layer composition can be modified to reduce water loss (social spider *Stegodyphus dumicola*, Malmos et al., 2021), as well as heat shock protein genes can be up-regulated and antioxidative enzymes levels increased under both heat and drought stress (wolf spider *Pardosa pseudoannulata*, Chen et al., 2023). In addition, as evidenced with salinity, heat tolerance can vary at the intraspecific level, with individuals from warmer locations exhibiting greater tolerance and different cuticle wax layer composition (Malmos et al., 2021). However, it remains unknown how the interaction between high temperature and salinity challenges these responses.

The joint effect of different stressors can be synergistic (one factor enhances the effect of the other), antagonistic (one factor mitigates the effect of the other) or additive (the

effects add up and are independent). Moreover, this can vary depending on numerous factors, such as the level of organization and the ecosystem under consideration (Jackson et al., 2016; Torres et al., 2021). At the organismal level, recent research shows that high temperature and other environmental stressors like pH, salinity and oxygen, can decrease organisms' thermal tolerance (Litchman & Thomas, 2023). Specifically for salinity, Torres et al., (2020, 2021) evidenced that in marine crab larvae, there is an antagonistic effect of low salinity and high temperature (“thermal mitigation of low salinity stress”) on their survival and developmental time. The underpinning mechanism is an increase in the osmoregulatory capacity (i.e., difference between the osmolarity of the haemolymph and the osmolarity of the external medium) at high temperature, as in these organisms it increases ATP production and cellular repair. Kondo et al., (2021) found that, in larvae of aquatic shrimp, salinity and temperature exert additive effects on survival, developmental time, and growth, with relevant species-specific differences that reflect their distribution patterns. Vereshchagina et al., (2016) evidenced in an amphipod species that the salinity level of the developmental environment can influence thermal tolerance, with organisms from non-saline populations being more susceptible to high temperature. This evident complexity in aquatic organisms highlights the importance of understanding how these two factors interact and affect terrestrial arthropods physiology (Rozen-Rechels et al., 2019).

In terrestrial arthropods, salinity–temperature interactions are likely mediated by several physiological mechanisms. Under increased water loss triggered by these drivers, individuals might allocate energy and resources to modify their cuticle wax composition (Malmos et al., 2021) or adjust haemolymph osmolarity (Chown & Nicolson, 2004), potentially increasing metabolic rates due to increased energy expenditure. The physiological outcome of such interactions could depend on factors like body size, given its influence on surface to volume ratios, and species-specific physiological tolerances. However, it is also possible that the simultaneous risks of dehydration and overheating induce metabolic depression, reducing water loss through respiration as an adaptive strategy (Benoit et al.,

2024; Chown et al., 2011; Rozen-Rechels et al., 2019). Understanding the underpinning mechanisms is particularly important in cases where species inhabit fluctuating environmental conditions, such as coastal-estuarine ecosystems (Lorrain-Soligon et al., 2024). In these systems, transitional zones like dunes may expose arthropods to overlapping stressors, reflecting in adaptive traits or high plasticity to cope with changes in salinity and temperature.

Allocosa senex (Mello-Leitão 1945), is a Neotropical wolf spider species that inhabits rivers and coasts of Uruguay, Argentina and Brazil (Simó et al., 2017). This nocturnal species spends the day in burrows it builds in the sand (Costa, 1995; Costa et al., 2006) avoiding high temperatures and the risk of desiccation (Foelix, 2011). Within Uruguay, this species inhabits along an important salinity gradient given by the estuary of the Río de la Plata (Lercari & Defeo, 2006, 2015), being found on riverine, estuarine and oceanic beaches (Simó et al., 2017). This makes it an excellent model for studying the effects of environmental variables on its physiology, as this gradient shows high spatial and temporal variability in water salinity values, which imposes dynamic environmental conditions on the beaches, especially those located in estuarine zones (Jackson et al., 2021; Ministerio de Ambiente, 2023). Moreover, sandy beaches are highly dynamic environments defined by physical variables such as waves, tides and sand grain, with frequent temperature, humidity and salinity changes (McLachlan et al., 2018; Short, 1996). Hence, they impose different selective pressures on the organisms that inhabit them, which may be reflected in plastic traits or the presence of local adaptation (Cavassa et al., 2022; Defeo et al., 2009; McLachlan & Defeo, 2017; Scapini et al., 2019). Interestingly, intraspecific variation in size has been reported in *A. senex* along the estuarine salinity gradient, as individuals from riparian zones are larger than individuals from oceanic zones (Aisenberg et al., 2023). This could be related to the higher osmoregulatory costs of oceanic individuals (e.g., see Gutiérrez et al., 2011), but this has not yet been assessed.

Here, we evaluate the effect of salinity and temperature on the physiology of females of *A. senex* from two beaches with contrasting salinity regimes. To do so, we exposed them to combined salinity (0, 35, 70 ‰) and temperature (25, 32 °C) treatments for 15 days, and estimated Standard Metabolic Rate (SMR), Total Evaporative Water Loss (TEWL) and weight loss. We hypothesised that, (1) under high salinity conditions *A. senex* females display physiological mechanisms to reduce respiratory and/or cuticular water loss. Thus, *A. senex* females under high salinity treatments will exhibit lower TEWL, and show a decrease in their SMR. (2) As riparian females are not naturally exposed to high salinity, they lack adaptations to salinity and therefore face higher energetic costs of osmoregulation under such conditions. Therefore, we predicted that under high salinity conditions, riparian females will exhibit higher SMR (reflecting higher energetic costs) than oceanic females. (3) Combined exposure to high salinity and temperature increases osmoregulation costs, as it increases water loss (both respiratory and cuticular) and metabolic demands, making it energetically costly to adjust several traits simultaneously. Therefore, females exposed to both stressors will exhibit higher TEWL and SMR compared to females under single-stressor treatments.

Methods

Collection and housing

Adult *Allocosa senex* females were collected from two beaches with contrasting natural salinities but similar granulometry (fine sand; Departamento de Evolución de Cuencas, Facultad de Ciencias, UDELAR, Uruguay), along the Río de la Plata estuary in Uruguay (Figure 1). Las Grutas beach (hereafter, “Oceanic beach”, 34°54'33.4"S 55°02'11.4"W) is located on the external section of the estuary, adjacent to the Atlantic Ocean, with a water salinity similar to the sea (25–30 ‰; Ministerio de Ambiente, 2023). Zagarzazú beach (hereafter, “Riparian beach”, 34°58'43"S, 54°55'59"W) is located on the riverine section of the estuary with near to zero water salinity values (0–5 ‰; Ministerio de Ambiente, 2023), mainly influenced by precipitation and freshwater discharges from the Uruguay and Paraná rivers.

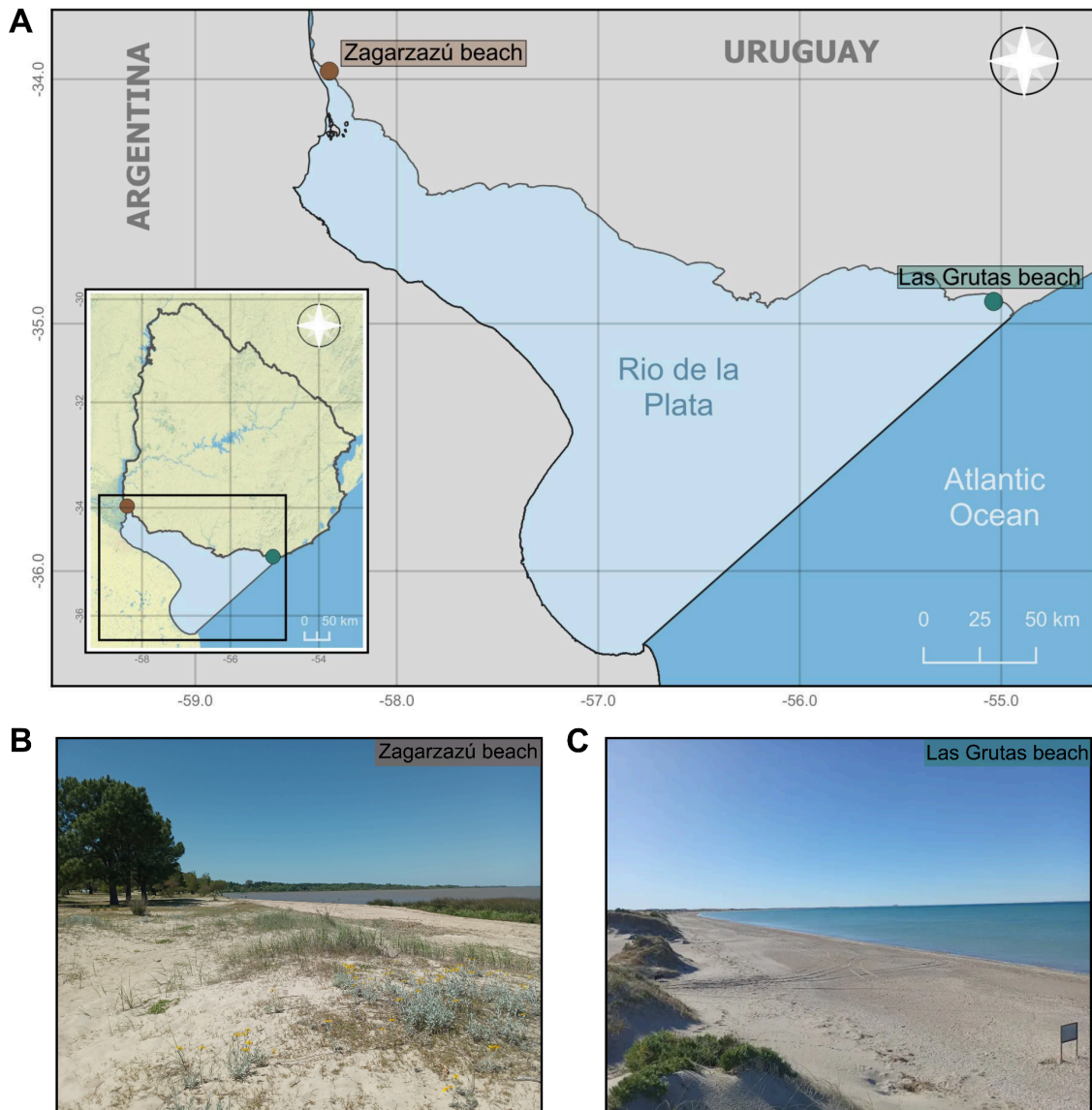


Figure 1. Location of the sampling beaches (A), Zagarzazú beach (i.e., riparian beach, B) and Las Grutas beach (i.e., oceanic beach, C). In A, the area encompassed by the Río de la Plata estuary is highlighted. At its inner end, it borders the Uruguay River, and at its outer end, the Atlantic Ocean. The map was created using QGIS software.

A. senex shows sex role inversion and inverted size dimorphism: the female is the mobile and smaller sex, whereas the male is more sedentary and larger (Aisenberg & Costa, 2008). Consequently, females are more frequently exposed to ambient salinity and are also more easily found when sampling. Females were manually collected at night using headlamps, during the species' reproductive period (October–November 2022), when they are more active and abundant (Aisenberg et al., 2007; Aisenberg & Costa, 2008; Costa,

1995). A total of 133 females were collected: 69 females from the oceanic beach and 64 females from the riparian beach. Females were transported to the laboratory and conditioned in plastic containers with sand and moist cotton.

Once in the laboratory, we measured morphologic traits to assess differences between females. We measured prosoma width (stereoscopic Premiere binocular magnifying glass, SMZ-05), a representative trait of body size in spiders (Moya-Laraño et al., 2003), and body mass (precision balance, RADWAG WTC 200, 1 mg). Both morphological measurements were taken prior to feeding the individuals. During the inter-experimental period, spiders were maintained individually in Petri dishes (9.5 × 1.5 cm, diameter × height), containing moist cotton and sand from the collection site as substrate. Females were offered a larva of *Tenebrio molitor* (Coleoptera) weekly and kept at room temperature.

Experimental design

To evaluate the effect of salinity and temperature on the physiology of *A. senex* females, they were randomly assigned to one of this six treatments which differed in temperature (25 ± 1 °C or 32 ± 1 °C) and salinity concentration (0, 35 or 70 ‰): 25 °C - 0 ‰ (Oceanic $n = 11$, Riparian $n = 11$), 25 °C - 35 ‰ (Oceanic $n = 10$, Riparian $n = 10$), 25 °C - 70 ‰ (Oceanic $n = 11$, Riparian $n = 10$), 32 °C - 0 ‰ (Oceanic $n = 13$, Riparian $n = 10$), 32 °C - 35 ‰ (Oceanic $n = 12$, Riparian $n = 13$), 32 °C - 70 ‰ (Oceanic $n = 12$, Riparian $n = 10$). The temperature levels correspond to the average temperature of the female burrows during their reproductive period (25 °C, A. Albin unpublished information) and a potentially challenging temperature (32 °C). For salinity levels, we selected three standardized salinity treatments based on previous experimental studies on spiders (Foucreau et al., 2012; Lambret et al., 2021; Renault et al., 2016; Siaussat et al., 2013): 0 ‰ (null salinity treatment, similar to the Riparian beach water), 35 ‰ (average ocean salinity, similar to the Oceanic beach water) and 70 ‰ (maximum salinity in salt marshes, highly challenging for this species).

During the experiment, females were placed in plastic containers (7×5 cm, diameter $\times$ height) containing 110 g of sand saturated with the corresponding salt solution (35 ‰ or 70 ‰) or distilled water (0 ‰). The containers were placed in incubators with controlled temperature (25 ± 1 °C or 32 ± 1 °C) and a 12L:12D photoperiod for 15 days. The sand was previously cleaned to remove any organic matter (modified from Gross, 1971), and salinity treatments were achieved by dissolving artificial marine salt (Sera Sea Salt, Sera, Germany) in distilled water until reaching the desired concentration (measured with a YSI EC300 salinometer). To ensure a constant salinity concentration over time, the plastic containers were tightly sealed. In a similar setup, Pétillon et al., (2011) showed that salinity concentration remained constant over a two-month period. Besides, females were food-deprived for a minimum of 24 hours prior to the start of the experiment and throughout its duration. This prevented them from obtaining water from their prey, which could have introduced confounding factors. As wolf spiders can tolerate starvation periods of up to 200–300 days (Nentwig, 1987) and Pétillon et al., (2011), showed that salinity treatments did not alter the contents of body composition in other wolf spider species (*Pardosa agrestis* and *P. purbeckensis*), we assumed that a starvation period of between 16–22 days would not influence the results of the experiment.

Respirometry Measurements

Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) and Total Evaporative Water Loss (TEWL, $\text{mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) were estimated on day 0 (before-treatment) and on day 15 (after-treatment) of the experiment. To do so, we followed the experimental setup, calibration and analysis procedure described in Clavijo-Baquet et al., (2020), but using a 0.05 % CO_2 concentration (Airliquide, USA) for the span of the CO_2 sensor. Females were weighted before each measurement (precision balance, HR-200, 0.1 mg). We registered up to three females simultaneously, placing them in an individual 14 mL miniature glass metabolic chamber (Sable Systems, NV) in a flow-through respirometry system. All measurements were performed at 25 °C with an air flow of 50 mL/min, between 9 a.m. and 2 p.m., which

corresponded to the light phase of the photoperiod, and therefore, the inactivity period of *A. senex*. Measurements were performed in the Ethology Section, Facultad de Ciencias, UDELAR, Uruguay.

For each female, flow rate (mL/min), barometric pressure (Pa), H₂O (kPa), and CO₂ concentration (%) were recorded for 30 minutes at 1-s intervals. The first chamber was recorded twice, to ensure the female was at rest. In cases where possible, the second chamber was also recorded twice. For the estimation of SMR, we used the following equation: $VCO_2 = FR \times 60 \times [(FeCO_2 - FiCO_2) \times \frac{BP}{BP - WVP}]$, where *FR* is the flow rate of the incoming air, *FiCO₂* is the incoming CO₂, *FeCO₂* is the CO₂ leaving the chamber, *BP* is the barometric pressure (kPa), and *WVP* is the water vapor pressure (kPa) (Lighton, 2018). For the estimation of TEWL, we calculated the absolute humidity (kg/m³) entering and leaving the metabolic chamber as $= \frac{P}{T \times R_w}$, where *P* is water vapor pressure of the air (Pa), *T* is the dewpoint temperature (K), and *R_w* is the gas constant for water vapor (Lide, 2012). For SMR, we averaged CO₂ concentration for the 10 min with the lowest values after the steady state was reached. We also averaged the same 10 min of relative humidity (RH) record for TEWL estimation. The output from the respirometry system was digitized using the Universal Interface II and recorded using the ExpeData Acquisition Software, both from Sable Systems. For the analysis, only continuous respiration patterns were taken into consideration. Some females (*n* = 19) displayed other patterns of CO₂ production, equivalent to discontinuous gas exchange and interburst-burst in insects (see Chown & Nicolson, 2004 and the “Anexo 2” of this thesis). As these respiratory patterns involve different mechanisms of gas exchange, they were excluded.

Of the 133 females measured before-treatment (i.e., at day 0), 108 were included in the analysis (57 oceanic, 51 riparian). Data not included were due to non-continuous respiratory patterns or recording failures. For after-treatment analysis (i.e., at day 15), 81 measurements were used (36 oceanic, 44 riparian), because of female mortality during the

experiment, extreme weakness, egg sac presence or laying the day of the measurement, non-continuous respiratory patterns, or recording failures.

It is worth noting that *A. senex* becomes reproductively active after its last molt, when they become adults (Aisenberg, 2006). Thus, it is not possible to decouple the reproductive period from adulthood to measure SMR. When an egg sac was seen on day 15, SMR and TEWL were not measured ($n = 3$ females from the oceanic beach at 32 °C and 0 ‰; $n = 1$ female from the riparian beach at 25 °C and 35 ‰). If an egg sac was seen before the day 15, it was removed from the female and the SMR and TEWL was measured on day 15 ($n = 2$ females from the riparian beach: one at 25 °C and 0 ‰, egg sac removed at day 5; one at 25 °C and 35 ‰, egg sac removed at day 9). Neither of these two cases were outliers in the SMR or TEWL distribution.

Statistical analyses

Morphologic analysis

Prosoma width and body mass differences between *A. senex* females from the riparian and oceanic beach were evaluated fitting linear models (LM) with normal or lognormal (i.e., log() in the response variable) distribution. Moreover, we calculated a Body Condition Index (BCI) as it has been reported to vary between locations in this species (see Postiglioni et al., 2025). To do so, we followed the *residual index* methodology from Jakob et al., (1996), which controls for body size by regressing log (body mass) on log (prosoma width), and uses the residuals as the response variable. These residuals represent the deviation of an individual's body mass from the expected for its size, reflecting variation in fat, water, or other components. To control if females from different beaches have a different body mass-body size relationship, we fitted the regression including the interaction of log (prosoma width) with beach of origin. As this interaction was statistically significant ($t(129) = -8.65$, $p < 0.001$), we confirmed that females from different beaches have different allometric relationships. Therefore, we fitted one regression for each beach of origin and obtained residuals independently. The models for body mass, prosoma width and BCI included beach of origin

and day of collection as explanatory variables. The variable day of collection reflects the advance of the reproductive period; it was calculated taking October 1 as day zero, the day of the beginning of the reproductive period of *A. senex* (Aisenberg et al., 2007; Aisenberg & Costa, 2008; Costa, 1995).

Before-treatment analysis (day 0)

We assessed SMR and TEWL differences between the two locations before-treatment (day 0) fitting LM with lognormal distribution. We included body mass and days in the laboratory in interaction with the beach of origin as explanatory variables. The variable days in the laboratory was calculated considering day 0 as the day the individual was brought to the laboratory. We included this variable to control the effect of habituation to laboratory conditions and the change of diet during the inter-experimental period. In the models where SMR was the response variable, we log-transformed SMR and body mass given the known logarithmic relationship between metabolism and body mass (Angilletta Jr., 2009; McNab, 2002).

We corroborated that body mass differences between females from the two locations (see Results) were held until the start of the experiment, by fitting a LM with body mass as the response variable and beach of origin and days in the laboratory as explanatory variables. Females from the riparian beach were bigger than females from the oceanic beach ($t(105) = 3.53$, $p = 0.001$) and this was not affected by the days in the inter-experimental period ($t(105) = 0.89$, $p = 0.376$).

After-treatment analysis (day 15)

To assess whether beach and treatment affected the probability of death of the individuals we fitted a logistic regression model. The response variable was death (0 or 1), and the paired interaction between beach, salinity, and temperature was included. We also calculated the percentage of survival by beach and treatment.

To evaluate if salinity and temperature treatments had different effects on SMR and TEWL of females from the two beaches, we fitted LM with lognormal distribution of residuals. Given that in some treatments the number of registers was low due to the reasons previously exposed, we could not fit a model that included beach, temperature and salinity and their interaction. Therefore, for each beach, we initially fitted a model including the interaction between temperature and salinity treatments, as well as the additive effect of body mass (log-transformed in the SMR models). If the interaction was non statistically significant, the model was simplified to include only the additive effects of salinity and temperature.

We assessed if the experimental treatments influenced body mass loss and if this differed between beaches, as a complementary variable. To do this, we fitted a LM model with normal distribution and included the percentage of weight loss (WL) between day 0 and 15 as the response variable. Beach of origin, salinity, temperature and days in the laboratory were included as explanatory variables. The variable days in the lab was included to control if the females that spent more days in the inter-experimental period had a different pattern of body mass change. We performed post-hoc comparisons using the False Discovery Rate method (Benjamini & Hochberg, 1995) to assess differences between multiple groups (predictmeans R package).

All statistical analyses and graphics were performed using R software (R Core Team, 2023) with the packages ggplot2, fitdistrplus, predictmeans, DHARMA. We checked for correlation between numeric variables to avoid collinearity in the models. In all models, continuous variables were standardized and centered and residual analysis was performed in all models to ensure normality and homoscedasticity (Inchausti, 2022). Model selection followed a hypothesis-driven approach and was done using Likelihood Ratio Test (LRT), only in cases where interactions or control variables, such as days in the laboratory and body mass, were not statistically significant. Overall effects of explanatory variables were assessed using ANOVA for variables with three or more levels and post-hoc comparisons were performed only when significant global effects were detected for these variables.

Results

Our results reveal that the physiological responses of *A. senex* females exposed to combined temperature and salinity treatments differ regarding their beach of origin (oceanic or riparian). While oceanic females exhibited a stable SMR across treatments, riparian females decreased SMR under high temperature and high salinity conditions (Figure 2). In females from both locations TEWL variations were explained by the interaction between temperature and salinity, but the response differed between them (Figure 3). Lastly, body weight loss was greater at high temperature in females from both locations. However, oceanic females exhibited greater weight loss at both temperatures compared to riparian females (Figure 4).

Morphologic analysis

When collected from the field, females from the riparian beach had higher body mass than females from the oceanic beach ($t(131) = 6.81$, $p < 0.001$; $R^2 = 26\%$), being only retained in the final model the variable beach of origin (Figure S1, A). Regarding prosoma width, the final model included beach of origin, day of collection and their interaction ($R^2 = 14\%$). Females did not differ in their prosoma width by beach ($t(129) = 1.17$, $p = 0.244$) (Figure S1, B). While riparian females decreased their prosoma width as the day of collection increased ($t(129) = -3.16$, $p = 0.002$), oceanic females did not ($t(129) = 1.70$, $p = 0.092$). Finally, the model for BCI retained only the beach of origin. However, no differences were found between *A. senex* females ($t(131) = 0.00$, $p = 1.00$) and the R^2 of the model was 0 % (Figure S1, C).

Before-treatment analysis (day 0)

Females did not differ in their SMR nor their TEWL before-treatment (Figure S2, Table 1). While SMR decreased with the number of days in the laboratory, TEWL increased. The table for model selection for the before-treatment analysis can be found in the Supplementary Material (Table S1).

Table 1. Summary of the best model for Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) and Total Evaporative Water Loss (TEWL, $\text{mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) of *Allocosa senex* females before-treatment (day 0). Females belong from two beaches with naturally contrasting salinity exposure (Oceanic and Riparian). Values are expressed in log-scale. Body mass was log-transformed for the SMR model. B) Beach of origin, D) Days in the laboratory (standardized), Mb) Body mass (g, standardized).

Standard Metabolic Rate				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	3.87	0.06	62.89	<0.001
B (Riparian)	-0.18	0.09	-1.92	0.058
D	-0.15	0.04	-3.26	0.002
Mb	0.24	0.05	5.24	<0.001
Observations	108			
<i>df</i>	104			
<i>R</i> ²	0.25			
Total Evaporative Water Loss				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-1.51	0.06	-27.08	<0.001
B (Riparian)	0.12	0.08	1.43	0.155
D	0.23	0.04	5.65	<0.001
Mb	0.08	0.04	2.06	0.042
Observations	108			
<i>df</i>	104			
<i>R</i> ²	0.28			

After-treatment analysis (day 15)

Females from both beaches exhibited the lowest survival under the most challenging treatment, 32 °C - 70 ‰, regardless of their beach of origin, with only 41.7 % of oceanic females and 50.0 % of riparian females surviving until day 15 (Table S2). Moreover, survival of riparian females was similar between the 32 °C - 35 ‰ (53.8 %) and 32 °C - 70 ‰ (50.0 %) treatment, whereas, almost 70 % of oceanic females survived until day 15 under the 32 °C - 35 ‰ treatment. The logistic regression confirmed a significant interaction between salinity and temperature ($\chi^2_2 = 8.81$, $p = 0.01$) on female survival probability; beach was not retained in the model. At 32°C, post-hoc comparisons showed a marginally significant difference between survival at 0 ‰ and 35 ‰ ($z = -2.30$, $p = 0.055$) and a significant difference between 0 ‰ and 70 ‰ ($z = -2.96$, $p = 0.01$) (Figure S3).

Salinity ($F_{2, 31} = 0.42$, $p = 0.66$) and temperature did not significantly affect the SMR of oceanic females, whereas females from the riparian beach had lower SMR at high-temperature (i.e., 32 °C) (Figure 2, Table 2). Besides, riparian females acclimated to 70 ‰ exhibited lower SMR values than females acclimated to 0 ‰. However, this effect was weak as the overall effect of salinity was non-significant ($F_{2,39} = 0.87$, $p = 0.43$).

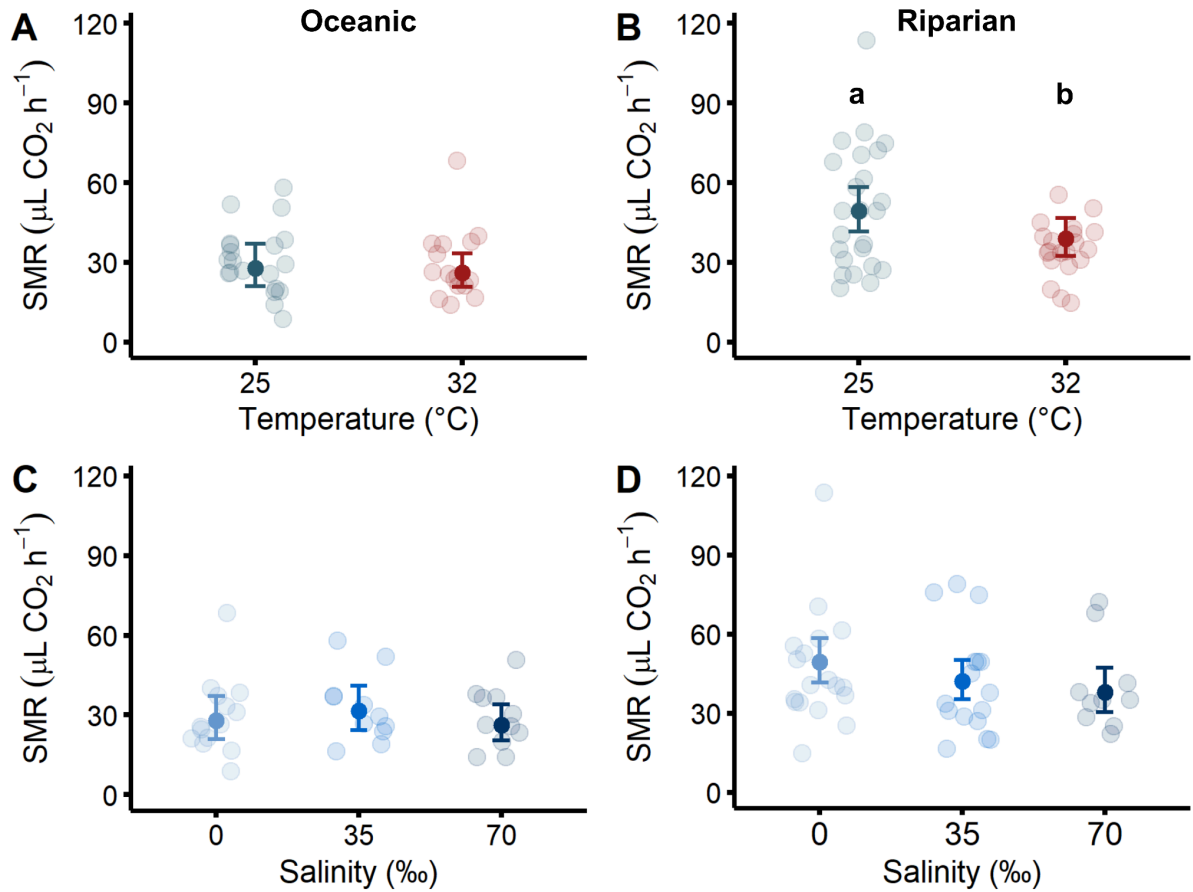


Figure 2. Standard Metabolic Rate (SMR) values of adult *Allocosa senex* females from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian), after being exposed to temperature (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. A) and C) show SMR values for females from the oceanic beach; B) and D) show SMR values for females from the riparian beach. The upper panels show SMR with respect to temperature and the lower panels to salinity. Mean and 95 % CI are shown for an individual with average standardized mass for each beach. Different letters indicate statistically significant differences between groups; letters are shown only where differences were detected. Salinity did not have an overall significant effect in SMR.

Table 2. Summary of the best model for Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) of *Allochoca senex* females exposed to different temperatures (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. Females belong from two beaches with naturally contrasting salinity exposure (Oceanic and Riparian). SMR and body mass were log-transformed. T) Temperature, S) Salinity, Mb) Body mass (g, standardized).

Oceanic beach				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	3.33	0.14	23.98	<0.001
T (32 °C)	-0.06	0.14	-0.44	0.661
S (35 ‰)	0.12	0.16	0.74	0.463
S (70 ‰)	-0.06	0.17	-0.34	0.734
Mb	0.20	0.07	2.98	0.006
Observations	36			
<i>df</i>	31			
<i>R</i> ²	0.24			
Riparian beach				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	3.90	0.08	46.58	<0.001
T (32°C)	-0.23	0.09	-2.50	0.017
S (35‰)	-0.16	0.11	-1.50	0.142
S (70‰)	-0.26	0.12	-2.13	0.039
Mb	0.29	0.05	6.01	<0.001
Observations	44			
<i>df</i>	39			
<i>R</i> ²	0.55			

Regarding TEWL, the interaction between temperature and salinity was significant for riparian females ($F_{2,37} = 4.39$, $p = 0.02$) and marginally significant for oceanic females ($F_{2,30} = 3.07$, $p = 0.06$) (Table 3). While oceanic females acclimated at 25 °C did not exhibit different TEWL between salinity treatments (Post-hoc test: $df = 30$, $p = 0.86$ for all three comparisons), those acclimated at 32 °C did. At 32 °C, TEWL was lower at 70 ‰ than at 0 ‰ (Post-hoc: $df = 30$, $p = 0.02$), but did not differ from 35 ‰ (Post-hoc: $df = 30$, $p = 0.07$). Moreover, there were no differences between TEWL at 0 ‰ and 35 ‰ (Post-hoc: $df = 30$, $p = 0.58$) (Figure 3).

Females from the riparian beach responded differently to temperature x salinity treatments (Figure 3, Table 3). Riparian females acclimated at 25 °C decreased TEWL at 70 ‰ relative to 0 ‰ (Post-hoc: $df = 30$, $p = 0.04$) and 35 ‰ (Post-hoc: $df = 30$, $p = 0.03$), with no differences between 0 ‰ and 35 ‰ (Post-hoc: $df = 30$, $p = 0.58$). However, for females acclimated at 32 °C, TEWL decreased at lower salinity concentrations, as TEWL at 35 ‰ (Post-hoc: $df = 30$, $p = 0.005$) and 70 ‰ (Post-hoc: $df = 30$, $p = 0.01$) were both lower than 0 ‰. No differences were found between TEWL at 35 ‰ and 70 ‰ (Post-hoc: $df = 30$, $p = 0.85$).

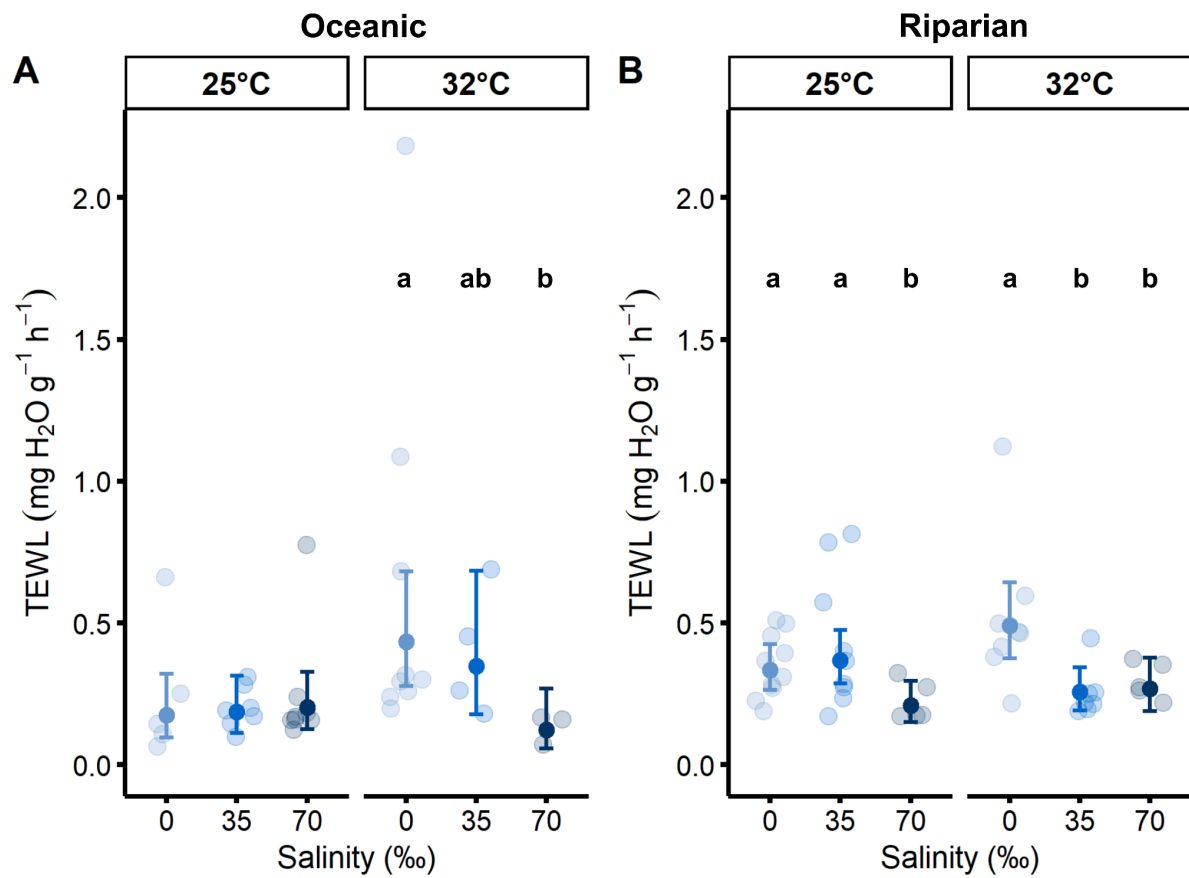


Figure 3. Total Evaporative Water Loss (TEWL) values of adult *Allocosa senex* females from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian), after being exposed to temperature (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. A) shows TEWL values for females from the oceanic beach, whereas B) shows TEWL values for females from the riparian beach. Mean and 95% CI are shown for an individual with average standardized mass. Different letters are shown only where significant post-hoc differences were detected between groups.

Table 3. Summary of the best model for Total Evaporative Water Loss (TEWL, mg H₂O g⁻¹ h⁻¹) of *Allocosa senex* females exposed to different temperature (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. Females belong from two beaches with naturally contrasting salinity exposure (Oceanic and Riparian). TEWL was log-transformed. T) Temperature, S) Salinity, Mb) Body mass (g, standardized).

Oceanic beach				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-1.74	0.30	-5.88	<0.001
T (32 °C)	0.91	0.37	2.46	0.020
S (35 ‰)	0.07	0.39	0.18	0.859
S (70 ‰)	0.14	0.38	0.38	0.706
T (32 °C) × S (35 ‰)	-0.29	0.56	-0.53	0.602
T (32 °C) × S (70 ‰)	-1.40	0.58	-2.42	0.022
Observations	36			
<i>df</i>	30			
<i>R</i> ²	0.32			
Riparian beach				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-1.09	0.12	-9.26	<0.001
T (32 °C)	0.38	0.18	2.14	0.039
S (35 ‰)	0.10	0.17	0.56	0.578
S (70 ‰)	-0.47	0.20	-2.29	0.028
Mb	0.14	0.06	2.34	0.025
T (32 °C) × S (35 ‰)	-0.74	0.26	-2.87	0.007
T (32 °C) × S (70 ‰)	-0.14	0.30	-0.46	0.645
Observations	44			
<i>df</i>	37			
<i>R</i> ²	0.41			

Lastly, females from both locations showed higher WL at high temperature (32 °C), but females from the oceanic beach lost more weight than females from the riparian beach, at both temperatures (Figure 4, Table S3). Salinity treatment did not have a significant effect on WL ($F_{2,87} = 0.11$, $p = 0.90$). The table for model selection of all the physiological after-treatment analysis can be found in the Supplementary Material (Table S4).

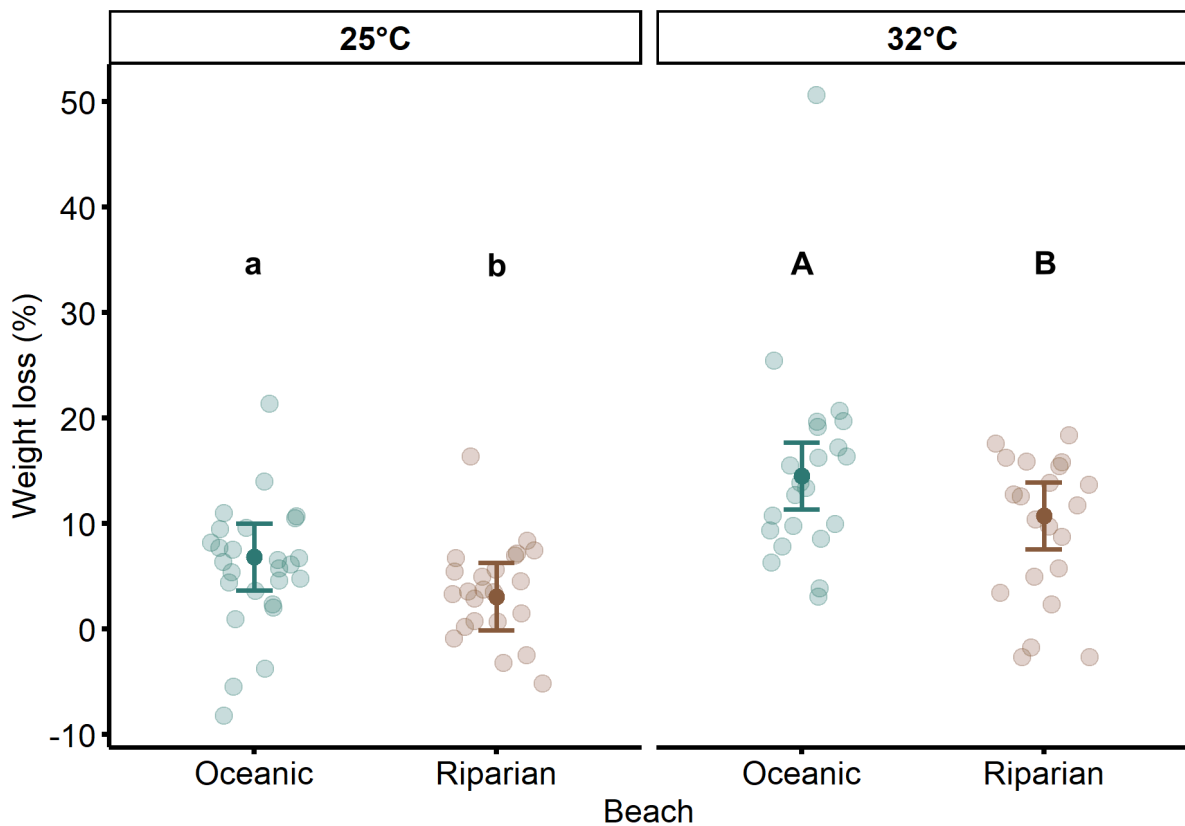


Figure 4. Percentage of weight loss (WL) of adult *Allocosa senex* females from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian), after being exposed to temperature (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. Mean and 95 % CI are shown. Different letters indicate statistically significant differences between groups: lowercase letters (a,b) correspond to comparisons at 25°C, and uppercase (A,B) correspond to comparisons at 32°C. Additionally, each letter pair (a-A, b-B) indicates differences within each beach between temperatures.

Discussion

We found that the simultaneous exposure to salinity and temperature differentially affects *A. senex* females from beaches with contrasting salinity regimes, and that the outcome also depends on the physiological trait considered. Oceanic females showed no response in SMR to either factor, whereas riparian females exhibited metabolic depression at high temperature and salinity through an additive effect. In contrast, the effect over TEWL was synergistic, as females from both beaches decreased water loss when exposed to combined high salinity and temperature conditions. However, they differed in their osmotic sensitivity to salinity, reflecting a different plastic response depending on previous salinity exposure. Our results highlight the complexity of physiological responses under multiple environmental factors, and reveal intraspecific differences in tolerance to osmotic and thermal stress. Overall, this study provides compelling information about the mechanisms underlying the response of coastal terrestrial ectotherms to environmental stress.

Starting point: morphology and physiological baseline

Contrary to what was previously reported by Aisenberg et al., (2023), in this work we did not find differences in prosoma width (i.e., body size) between riparian and oceanic females. In this sense, a group of riparian females collected in November 2022 had a notoriously smaller prosoma width than the other females from this beach, which probably influenced this lack of difference. In any case, it is interesting to highlight the variability in body size that this species exhibits, since prosoma differences between beaches may vary depending on the reproductive season or year, probably associated with changes in environmental variables such as salinity (see “*Anexo 1*” of this thesis). Moreover, despite females’ differences in body mass, they did not differ in their body condition when brought from the field, suggesting overall similar energetic reserves.

Before-treatment, SMR and TEWL were explained by body mass and by the number of days in the inter-experimental period. This relationship with body mass has been widely

supported in literature (Angilletta Jr., 2009; Chown & Nicolson, 2004; McNab, 2002). The effect of the inter-experimental period could be due to the differences between field and inter-experimental conditions. SMR decreased with the number of days in the laboratory, which is consistent with the expected decrease in activity levels of individuals under housing conditions. Moreover, as previous studies on the proper fasting periods for spiders before metabolic measurements are lacking, CO₂ production could have reflected both fat oxidation for ATP (our target) and glucose oxidation from food (Lighton, 2018). However, all females were food-deprived for at least 24 hours before the experiment, and there was little variation in fasting periods between them, plus a randomized selection for experimental treatments. Thus, if there were an effect, it was the same for all after-treatment measurements. For TEWL, an increase in water loss with the inter-experimental period can be attributed to the *ad libitum* access to fresh water, and probably the acquisition of water through non-salty food (*T. monitor* larvae). Thus, the osmoregulatory pressure of their home environments may have been relaxed. Given this inter-experimental period, the before-treatment measurements do not reflect the SMR or TEWL in the field, just a starting point of the experiment.

Coping with multiple environmental stressors

The after-treatment analysis revealed that females from both beaches exhibited a synergistic effect of temperature-salinity on survival, as they survived less when exposed to both high salinity and temperature. Interestingly, despite *A. senex* females differed in their physiological response regarding their beach of origin, they had the same survival rates, implying that neither had an advantage in terms of survival over the time here analysed (i.e., 15 days). In that sense, our results do not align with other Lycosidae studies where survival reflects the species habitat of origin (e.g., Renault et al., 2016). However, unpublished data from Albín et al., showed that when exposed to the same salinity treatments for a longer period (30 days at 25 °C), *A. senex* females from the oceanic beach survive longer than those from the riparian beach, although survival decreased for all at 70 ‰. Moreover, females from both beaches had survival rates similar to salt marshes species (Pétillon et al., 2011). Therefore,

the effect of the environmental conditions of origin over survival could be evidenced after a longer period of exposure.

Regarding physiological measurements (SMR and TEWL), some of our initial hypotheses were partially supported by our findings. Consistent with Hypothesis 1, riparian females showed a decrease in SMR and TEWL with increasing salinity. However, contrary to this hypothesis, salinity had no effect on SMR in oceanic females, and TEWL was influenced by salinity only under combined high salinity and temperature treatments. For Hypothesis 2, we could not directly compare SMR between beaches due to the reasons exposed in the Methods section, but riparian females did exhibit higher SMR than oceanic females under most salinity and temperature treatments. Regarding Hypothesis 3, the simultaneous effect of salinity and temperature on SMR was additive in riparian females but null in oceanic females, rather than interactive as we had predicted. However, we did find an interaction on TEWL, but contrary to our hypothesis, females exposed to high salinity and temperature managed to decrease TEWL as an osmoregulatory response to the same level as females under low temperature and salinity treatments.

Our results revealed that *A. senex* females differed in their degree of phenotypic plasticity in metabolic rate (SMR) depending on their beach of origin. Riparian females exhibited metabolic depression at both high temperature and high salinity, though the effect of salinity was weak (overall effect was not significant). This confirms that metabolic depression could function as a strategy to avoid overheating and dehydration in this species (Benoit et al., 2024; Chown et al., 2011; Rozen-Rechels et al., 2019). Unlike arthropods with efficient tracheal respiratory systems and therefore low respiratory water loss (Chown & Nicolson, 2004), Lycosidae spiders have both book lungs and tracheae. Therefore, reducing metabolic rate could be adaptively significant when facing desiccation conditions.

The lack of metabolic variation from females from the oceanic beach could be explained by local adaptation, developmental plasticity, or a high level of physiological

tolerance. However, Postiglioni et al., (2019) revealed an extremely low genetic diversity among individuals from different beaches, reducing the possibility of local adaptation. Likewise, the fact that the metabolic rate variability of the oceanic females before-treatment differed from the variability after-treatment (see Figure S2 and Figure 2), reinforces that this is not a case of local adaptation or developmental plasticity, as we would expect no changes between them (Piersma & Drent, 2003). Therefore, our results probably reflect a high tolerance to high salinity and high temperature conditions, associated with these females natural environmental conditions (Lorrain-Soligon et al., 2024).

Regarding water loss (TEWL), we found a synergic effect between temperature and salinity for individuals from both beaches. In riparian females, high salinity triggers a decrease in water loss, but when high salinity is combined with high temperature, this response is triggered earlier. In contrast, oceanic females have higher thermal and osmotic tolerance since their response was only triggered by the combination of high salinity and high temperature. Consistent with the findings of Vereshchagina et al., (2016) for amphipods, *A. senex* females developed in a non-saline environment exhibited greater susceptibility to temperature, which was reflected both by their earlier decrease in water loss and in their changes in metabolic rate. In this regard, the mechanism underlying riparian females TEWL reduction could be both respiratory (via reduced metabolism) and cutaneous (modifying cuticle permeability) water loss reduction (Chown & Nicolson, 2004); however, in the case of oceanic females, it would appear that only cuticular water loss reduction is involved. These results contribute to previous evidence in marine organisms (e.g., Torres et al., 2020, 2021). They highlight the relevance of understanding the combined effect of environmental variables, as we show how environmental conditions of origin (i.e., salinity) may affect the physiological response to other variables (i.e., temperature).

Temperature and beach of origin, but not salinity, influenced weight loss. Previous studies in other Lycosidae (Pétillon et al., 2011; Renault et al., 2016) showed that salinity significantly influenced body water content. Moreover, the magnitude of this effect depended on the species' environment (e.g., salt marshes or forest). However, salinity had no effect in *A. senex* females, despite being the main factor that differed between the beaches of origin. The greater weight loss of *A. senex* females at high temperature is likely due to an increased water loss and a greater use of energy reserves, for example, to modify cuticle composition. However, the fact that oceanic females, who have the lowest body mass, lost more weight regardless of temperature, is interesting. These females, like some insects, may be highly tolerant to body water loss, which is part of the repertoire of mechanisms for coping with dehydration (Chown & Nicolson, 2004).

Conclusions

Overall, this research contributes to understanding how organisms respond to simultaneous environmental challenges and addresses gaps in knowledge regarding the physiology of terrestrial coastal species. Future studies should investigate the effects of salinity and temperature on other important traits of this spider species, such as reproduction, and evaluate how developmental environmental conditions influence their responses and plastic abilities.

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Supplementary Materials

Morphologic analysis

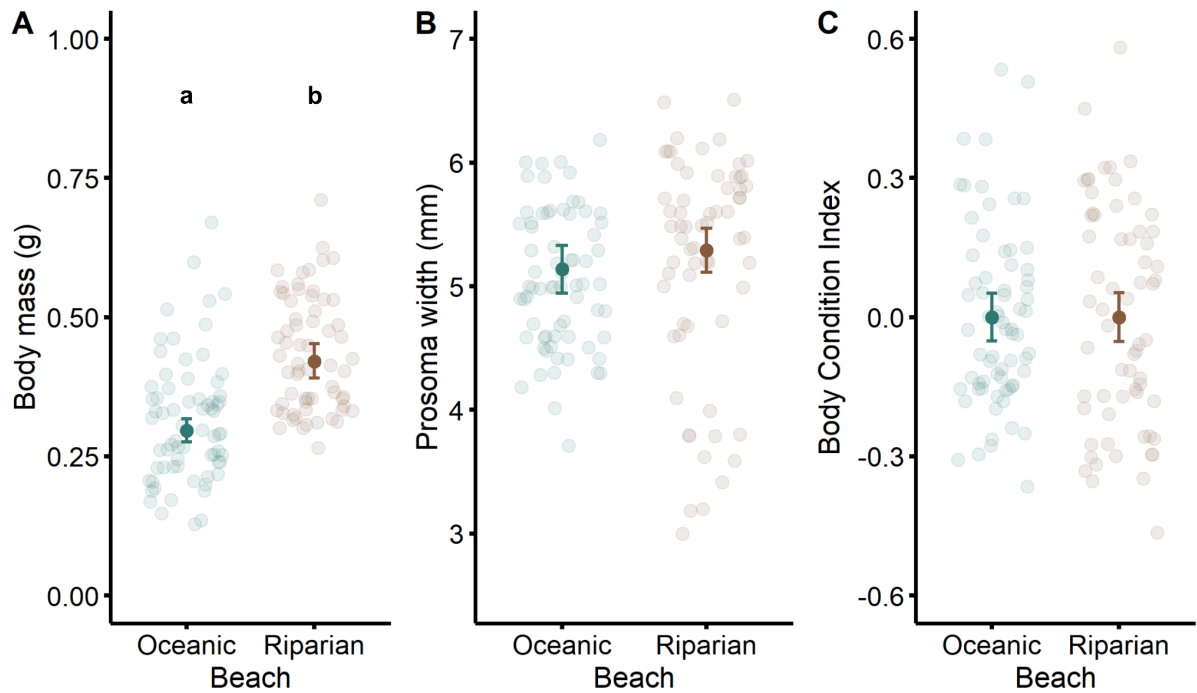


Figure S1. Body mass (A), Prosoma width (B) and Body Condition Index (C) differences between adult *Allocosa senex* females from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian). Different letters are shown only when statistically significant differences between groups were found. Mean and 95 % CI are shown.

Before-treatment analysis

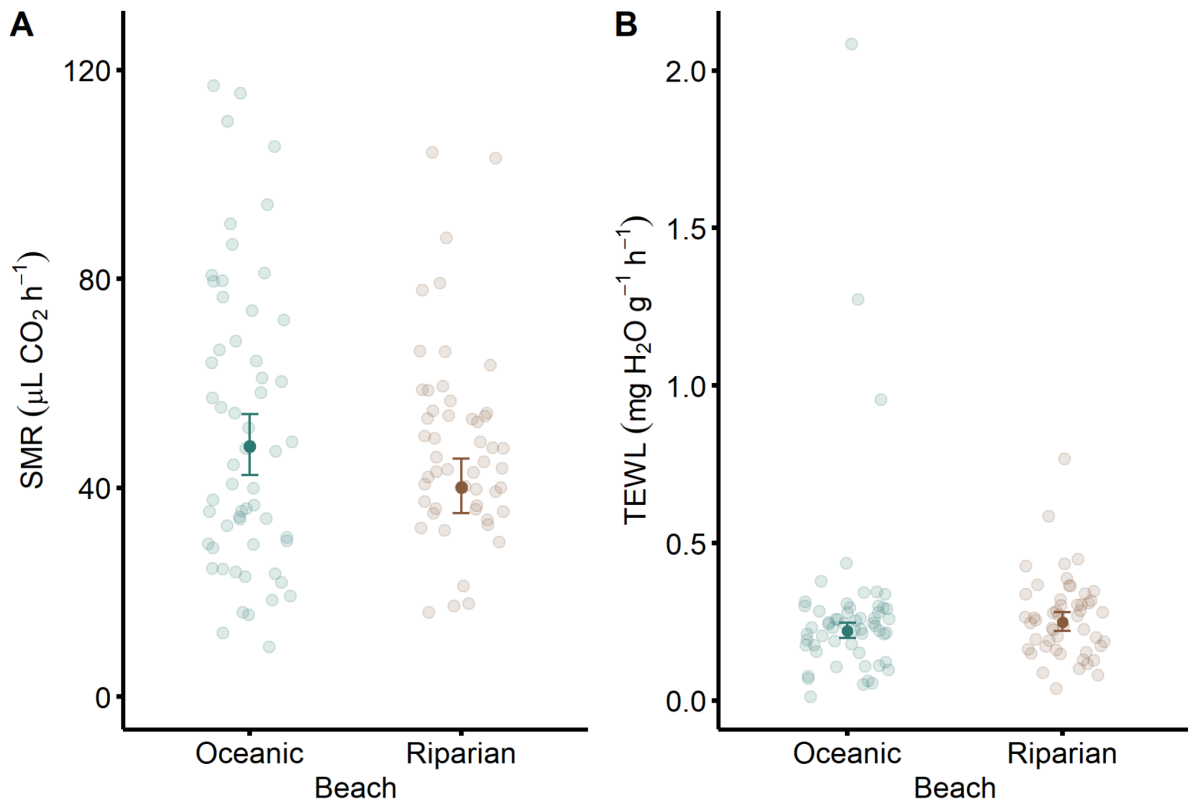


Figure S2. Physiologic measurements of adult *Allocosa senex* females from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian) before the start of the experiment. Females were kept under interexperimental conditions with *ad libitum* access to water and fed with *Tenebrio molitor* once a week. A) Standard Metabolic Rate (SMR), B) Total Evaporative Water Loss (TEWL). Mean and 95 % CI are shown. No significant differences were found between groups.

Table S1. Model selection via Likelihood Ratio Test for physiological variables of *Allocosa senex* females from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian) before the start of the experiment. SMR) Standard Metabolic Rate, TEWL) Total Evaporative Water Loss, B) Beach of origin, D) Days in the laboratory, Mb) Body mass.

SMR	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B × D + B × Mb	102	20.52			
D + B × Mb	103	20.57	-1	-0.05493	0.601
B + D + Mb	104	20.58	-1	-0.006009	0.862
TEWL	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B × D + B × Mb	102	17.09			
D + B × Mb	103	17.15	-1	-0.06082	0.547
B + D + Mb	104	17.16	-1	-0.009252	0.814

After-treatment analysis

Table S2. Percentage of survival of *Allocosa senex* females exposed to different temperature (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days.

Beach of origin	Temperature (°C)	Salinity (‰)	% of survival until day 15
Oceanic	25	0	72.7
		35	91.7
		70	83.3
	32	0	92.3
		35	66.7
		70	41.7
Riparian	25	0	90.9
		35	100.0
		70	70.0
	32	0	90.0
		35	53.8
		70	50.0

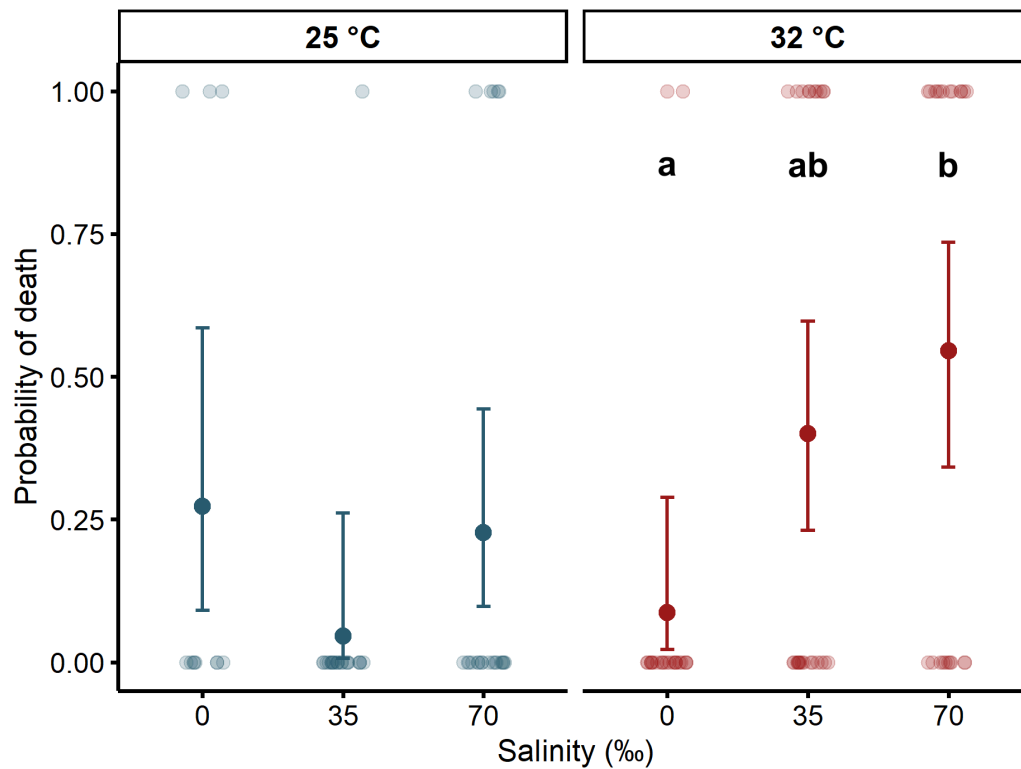


Figure S3. Predicted probability of death of *A. senex* females from two beaches with contrasting salinity regimes, after being exposed to combined salinity and temperature treatments for 15 days. Mean and 95 % CI are shown. Different letters are shown only when statistically significant post-hoc differences between salinity treatments within each temperature treatment were found.

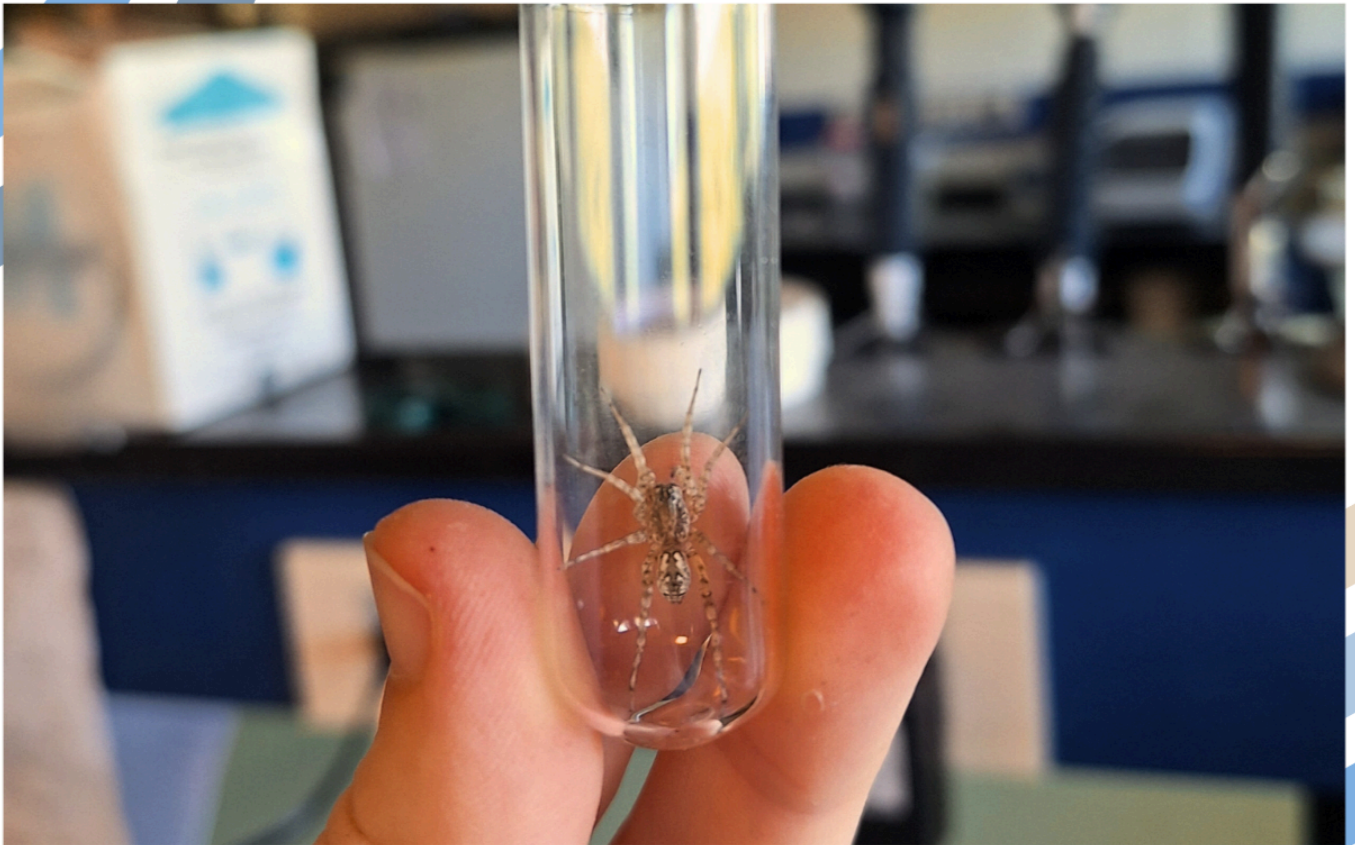
Table S3. Summary of the best model for the percentage of weight loss (WL) of *Allocosa senex* females exposed to different temperature (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. Females belong from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian). B) Beach of origin, T) Temperature, S) Salinity.

Weight loss (%)				
Predictors	Estimates	Std. Error	t value	p
(Intercept)	6.85	1.59	4.30	<0.001
B (Riparian)	-3.76	1.48	-2.55	0.013
T (32 °C)	7.68	1.48	5.19	<0.001
S (35 ‰)	-0.79	1.75	-0.45	0.654
S (70 ‰)	-0.18	1.83	-0.10	0.924
Observations	92			
df	87			
R ²	0.28			

Table S4. Model selection via Likelihood Ratio Test for physiologic and morphometric variables of *Allocosa senex* females exposed to different temperature (25 °C and 32 °C) and salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. Females belong from two beaches with naturally contrasting salinity regimes (Oceanic and Riparian). B) Beach of origin, T) Temperature, S) Salinity, Mb) Body mass (g), D) Days in the laboratory.

SMR	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
Oceanic beach					
T × S + Mb	29	4.525			
T + S + Mb	31	4.821	-2	-0.2957	0.388
Riparian beach					
T × S + Mb	37	3.691			
T + S + Mb	39	3.702	-2	-0.01069	0.948
TEWL	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
Oceanic beach					
T × S + Mb	29	12.36			
T × S	30	13.16	-1	-0.8011	0.17
Weight Loss	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B + T + S + D	86	4318			
B + T + S	87	4337	-1	-18.83	0.54

Chapter 2



**Salinity tolerance and its ecological consequences:
intra- and interspecific variation in two wolf
spider species from coastal ecosystems.**

Abstract

Physiological tolerance is one of the key determinants of habitat selection at the organismal level. In dynamic ecosystems like coastal environments, assessing physiological limits is essential for understanding how physiology shapes patterns of habitat use, species distributions, and ecological interactions. We aimed to assess how the laboratory physiological tolerance to salinity, matches the local distribution of two sympatric *Allocosa* wolf spiders, *A. senex* (males and females) and *A. marindia* (females), on an outer-estuarine beach influenced by saline conditions. Salinity tolerance was evaluated by measuring Standard Metabolic Rate (SMR), Total Evaporative Water Loss (TEWL), weight loss and survival among three experimental treatments: 0 ‰, 35 ‰ and 70 ‰. The distribution pattern was assessed through a pitfall trap protocol in the field. Our results revealed intra- and interspecific differences. Salinity treatments did not affect SMR or TEWL in *A. senex*, but they significantly influenced *A. marindia* females. Interestingly, despite *A. senex* males being larger, they exhibited lower osmotic tolerance than females, reflected in reduced survival under high salinity treatments. *A. marindia* females showed higher mortality with increasing salinity. Relating this to the species' local distribution, *A. marindia* appears to be constrained by its physiological tolerance to salinity, whereas *A. senex*'s distribution likely reflects other ecological factors, such as species interactions.

Introduction

Habitat selection occurs when individuals preferentially occupy certain habitats within a set of available ones, and is therefore a key factor shaping species' distributions (Mayor et al., 2009; Morris, 2003). As it is scale-dependent, habitat selection is influenced by processes and their underlying mechanisms operating from the organism to the ecosystem scale (Dussault et al., 2005; Mayor et al., 2009). At the organismal level, individual traits such as physiology, morphology and behavior interact with biotic and abiotic factors to determine individuals' survival and reproduction (Huey, 1991). In this sense, habitats can be classified as physiologically suitable based on the interaction between organisms' physiological tolerance and plasticity, and the environmental conditions (Huey, 1991). Therefore, understanding the role of physiology in habitat selection across environmental gradients is a relevant aspect to explaining species distributions.

Sometimes, the habitat selected by individuals is restricted by their physiological tolerance (e.g., Hilton et al., 2008; Moloney & Nicolson, 1984; Pétilion et al., 2008, 2011; Somero, 2002). In these cases, abiotic variables such as salinity or temperature likely act as strong environmental filters, sometimes outweighing the effect of biotic interactions. For example, interspecific differences among closely related species in thermal tolerance and metabolism, explain subtidal-intertidal zonations (Somero, 2002). Likewise, Pétilion et al., (2011) showed that osmotic tolerance among three *Pardosa* spiders that inhabit different habitats can explain their distribution pattern, as the salt marsh species shows higher survival rate and lower water loss at higher salinity concentrations. Moreover, intraspecific differences can be found when species inhabit environmental gradients, as populations exposed to the higher end of the gradient may exhibit higher physiological tolerance (e.g., salinity tolerance, Relyea et al., 2024), revealing either evolutionary processes such as adaptation, or phenotypic plasticity.

In this regard, the link between morphology and physiology is another factor that shapes organisms' responses to the environment (Huey, 1991; Pulz, 1987). For example, body size modulates physiological tolerance by influencing metabolic rate, total energetic requirements and water loss. As body size increases, the surface to volume ratio decreases, reducing total water loss as well as energy expenditure per gram. Therefore, body size can impact osmotic and thermal tolerance. This has been revealed in wolf spiders, where females who have higher body volume, better tolerate salinity treatments than males (Pétillon et al., 2011).

On the other hand, the habitat selected by individuals may not be a direct reflection of their physiological tolerance, as this can be strongly influenced by the interaction with other relevant factors such as interspecific competition and food availability. For example, Mauro et al., (2021) evidenced that the euryhaline fish *Poecilia reticulata*, avoids brackish water due to interference competition with the competitively dominant *P. picta*. The underlying mechanism is a trade-off between osmoregulatory costs and competitive ability. Similarly, salt marsh spiders that have maximal survival rate under null salinity treatments, are only found under extreme salinity conditions in nature (Renault et al., 2016). This highlights that the role of physiology in shaping an individual's distribution may be case-specific, and that when possible, laboratory results should be contrasted with the patterns found in nature.

Although distribution patterns and their underlying mechanisms have been extensively studied, particularly in estuarine gradients and among aquatic taxa, they remain largely unexplored in other systems. For example, coastal-terrestrial organisms inhabiting sandy beaches are exposed to highly dynamic conditions, facing changes in ambient salinity through sea spray, temperature, winds and tides that cause sudden floods (McLachlan & Defeo, 2017). These impose selective pressures that affect metabolism, growth and survival (Lorrain-Soligon et al., 2024). Particularly, ectothermic organisms with fully terrestrial habits that live in coastal ecosystems face two major challenges: being terrestrial and being small. Osmotic regulation in these organisms is challenged by limited freshwater availability and by

having a high surface area that increases salt and water fluxes (Lorrain-Soligon et al., 2024). Thus, they may exhibit highly plastic traits or local adaptation (McLachlan & Defeo, 2017), and may be more constrained by abiotic variables than by biotic interactions. This makes them an interesting model to study the scope of physiological tolerance on habitat selection.

In this sense, the wolf spiders *Allocosa senex* and *A. marindia* are two sympatric and synchronic species that inhabit sandy coastal areas of Uruguay, Argentina, and Brazil (Simó et al., 2017). In Uruguay, they inhabit sandy beaches along a pronounced salinity gradient given by the Río de la Plata estuary (Lercari & Defeo, 2006, 2015). There, they construct burrows in sand dunes where they remain during the winter and the daytime in summer, becoming active on summer nights (Costa, 1995). As dunes are transitional zones between land and sea, dune-living organisms experience highly dynamic environmental conditions (McLachlan & Defeo, 2017). Interestingly, both species exhibit sexual size dimorphism and sex-role reversal, with males being larger and females being the mobile, courting sex (Aisenberg & Costa, 2008). Despite their similarities, the two species show some relevant differences: *A. senex* is larger and preys upon *A. marindia* (Aisenberg et al., 2009, 2023). Furthermore, in beaches without saline influence, *A. senex* mainly occupies foredunes with scarce vegetation, whereas *A. marindia* is more frequently found in landward dunes with denser vegetation cover (Costa, 1995; Costa et al., 2006; Simó et al., 2017).

We aimed to assess the physiological tolerance of *A. senex* and *A. marindia* to salinity under laboratory conditions and contrast these results with their mesoscale distribution patterns on an outer-estuarine beach. To do so, we measured Standard Metabolic Rate (the rate at which organisms consume energy, SMR), Total Evaporative Water Loss (TEWL), and survival of both species under controlled salinity treatments (0, 35 and 70 ‰ at 25 °C for 15 days), and assessed species distribution using pitfall trapping in relation to sand salinity, vegetation cover, and prey availability. We hypothesised that, due to its lower body size, and therefore, higher surface to volume ratio, *A. marindia* exhibits higher water loss than *A. senex* under the same level of ambient salinity and thus, has lower

tolerance to salinity exposure. Moreover, we hypothesised this body size-salinity tolerance relationship also operates at the intraspecific level, as *A. senex* males, being larger, lose less water and exhibit higher salinity tolerance than females. Based on these hypotheses, we predicted that: (1) under high salinity treatments, *A. marindia* females will show an increase in SMR (reflecting increased energetic costs of water conservation) and a decrease in TEWL compared to the null salinity treatment, and lower survival rate than *A. senex* females. (2) *A. senex* individuals will be highly tolerant to salinity exposure. They will decrease TEWL under high salinity treatments, with low or null effect on SMR. However, females will be less tolerant than males and exhibit less survival probability; and (3) differences in salinity tolerance under laboratory conditions will be reflected in these species' distribution along the studied beach, with *A. marindia* occupying areas with less salinity concentration, and the opposite pattern being found for *A. senex*. This study provides insight into the role of abiotic variables in shaping the habitat selection of terrestrial organisms in a highly variable habitat.

Methods

Collect and housing

Allocosa senex males and *A. marindia* females were collected from an oceanic-influenced beach (Las Grutas beach, 34°54'33.4"S 55°02'11.4"W; hereafter, "oceanic beach") located on the external section of the Río de la Plata estuary, Uruguay. This beach has a direct influence from the Atlantic Ocean and a water salinity concentration similar to the sea (25–30 ‰; Ministerio de Ambiente, 2023). The reversed size dimorphism of both species is reflected in their prosoma width (Aisenberg & Costa, 2008): *A. senex* males = 5.77 ± 0.64 mm; *A. senex* females = 5.14 ± 0.54 mm; *A. marindia* males = 2.95 ± 0.35 mm, *A. marindia* females = 2.70 ± 0.20 mm (Authors' data from this population). Moreover, both species exhibit sex role inversion (Aisenberg & Costa, 2008), with females being the mobile and courting sex, while males are more sedentary. This characteristic makes females more easily found when sampling.

Spiders were manually collected at night using headlamps, during the reproductive period of the species, when they are more active and abundant (Aisenberg et al., 2007; Aisenberg & Costa, 2008; Costa, 1995). Sampling took place in 2023 (October–November) for *A. senex* males ($n = 21$) and *A. marindia* females ($n = 64$). Only six *A. marindia* males were collected, thus, we did not include them in the analysis. To compare with *A. senex* females, we used physiology and morphology data from Chapter 1 of this thesis (2022) and survival data from previous studies of our research group (2021).

Individuals were transported to the laboratory and conditioned in plastic containers with sand and moist cotton. Once in the laboratory, they were weighted (precision balance, HR-200, 0.1 mg) and we measured prosoma width (stereoscopic Premiere binocular magnifying glass, SMZ-05), considered a representative trait of body size in spiders (Moya-Laraño et al., 2003). We then used these measurements to calculate a Body

Condition Index (see Statistical analysis). All morphological measurements were taken before feeding the spiders.

During the inter-experimental period, *A. marindia* females were maintained individually in Petri dishes (9.5 × 1.5 cm, diameter × height) and *A. senex* males in bigger plastic containers (6.5 × 8.5 cm), with moist cotton and sand from the beach as substrate. Individuals were offered a larva of *Tenebrio molitor* (Coleoptera) weekly and kept at room temperature.

Experimental design

To evaluate the effect of salinity on the physiology and survival of *A. senex* and *A. marindia*, individuals were randomly assigned to one of three salinity treatments: 0, 35, and 70 ‰ for 30 days. These salinity levels correspond to standardized salinity treatments based on previous experimental studies (Foucreau et al., 2012; Lambret et al., 2021; Renault et al., 2016; Siaussat et al., 2013): 0 ‰ (no salinity treatment), 35 ‰ (average ocean salinity) and 70 ‰ (maximum salinity in salt marshes, highly challenging for these species). Individuals were kept at 25 °C, which corresponds to the average temperature of *A. senex* burrows during their reproductive period (A. Albín unpublished data). The sample sizes for *A. marindia* females were: 0 ‰ ($n = 21$), 35 ‰ ($n = 21$), 70 ‰ ($n = 22$). For *A. senex* males, the sample sizes were: 35 ‰ ($n = 10$) and 70 ‰ ($n = 10$); we did not perform the 0 ‰ treatment given the low number of individuals. We chose the high salinity treatments to evidence its impact over physiology and survival. The sample size of *A. senex* females used for physiology analysis was: 0 ‰ ($n = 11$), 35 ‰ ($n = 10$), 70 ‰ ($n = 11$) (data from the Chapter 1 of this thesis); for survival analysis it was: 0 ‰ ($n = 17$), 35 ‰ ($n = 15$), 70 ‰ ($n = 13$) (A. Albín unpublished data).

During the experiment, individuals were placed in plastic containers (7 × 5 cm) with 110 g of sand saturated with the corresponding salt solution (35 ‰ or 70 ‰) or distilled water (0 ‰). The containers were placed in incubators with controlled temperature (25 ± 1 °C) and

a 12L:12D photoperiod for 30 days. The sand was previously cleaned to remove any organic matter (modified from Gross, 1971). Salinity treatments were achieved by dissolving artificial marine salt (Sera Sea Salt, Sera, Germany) in distilled water until reaching the desired concentration (measured with a YSI EC300 salinometer). To ensure a constant salinity concentration over time, the plastic containers were tightly sealed. In a similar setup, Pétiillon et al., (2011) demonstrated that salinity concentration remained constant over a two-month period.

Individuals were food-deprived for a minimum of 24 hours before the start of the experiment and throughout its duration to prevent them from obtaining water from their prey, and avoid confounding factors. As wolf spiders can tolerate starvation periods of more than 100 days (Nentwig, 1987), and Pétiillon et al., (2011) confirmed salinity treatments did not alter the contents of body composition in other wolf spider species, we assumed that a starvation period of between 31–37 days would not influence the results of the experiment.

Respirometry Measurements

We estimated Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) and Total Evaporative Water Loss (TEWL, $\text{mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) on day 0 (before-treatment) and day 15 (after-treatment) of the experiment. We followed the experimental setup, calibration and analysis procedure described in Clavijo-Baquet et al., (2020). For the span CO_2 calibration, we used air with a 0.05 % CO_2 concentration (Airliquide, USA). Individuals were weighted before each measurement (precision balance, HR-200, 0.1 mg). We measured up to five spiders simultaneously using an open flow system. Individuals were placed in a 14 mL metabolic chamber and measurements were performed at 25 °C with an airflow of 50 mL/min for *A. senex* males and 40 mL/min for *A. marindia* females. Measurements took place during the light phase of the photoperiod, and therefore, the inactivity period of these species. Regarding *A. senex* females, we used a similar set up detailed in Chapter 1 of this thesis. Respirometry measurements were performed in the Ethology Section, Facultad de Ciencias, UDELAR.

For each individual, we recorded flow rate (mL/min), barometric pressure (Pa), H₂O (kPa), and CO₂ concentration (%) for 30 minutes at 1-s intervals. The first chamber was recorded twice, to ensure the individual was at rest. In cases where possible, the second chamber was also recorded twice. For the estimation of SMR, we used the following equation: $VCO_2 = FR \times 60 \times [(FeCO_2 - FiCO_2) \times \frac{BP}{BP - WVP}]$, where FR is the flow rate of the incoming air, $FiCO_2$ is the incoming CO₂, $FeCO_2$ is the CO₂ leaving the chamber, BP is the barometric pressure (kPa), and WVP is the water vapor pressure (kPa) (Lighton, 2018). For the estimation of TEWL, we calculated the absolute humidity (kg/m³) entering and leaving the metabolic chamber as $= \frac{P}{T \times R_w}$, where P is the water vapor pressure of the air (Pa), T is the dewpoint temperature (K), and R_w is the gas constant for water vapor (Lide, 2012). For SMR, we averaged CO₂ concentration for the 10 min with the lowest values after the steady state was reached. We also average the same 10 min of relative humidity (RH) record for TEWL estimation. The output from the respirometry system was digitized using the Universal Interface III and recorded using the Expedata data acquisition software, both from Sable Systems. For the analysis, only continuous respiration patterns were taken into consideration. Some *A. marindia* females ($n = 12$) displayed other patterns of CO₂ production, equivalent to discontinuous gas exchange and interburst-burst in insects (see Chown & Nicolson, 2004 and “Anexo 2” of this thesis). As these respiratory patterns involve different mechanisms of gas exchange, they were excluded.

Of the 64 *A. marindia* females measured before-treatment (day 0), 9 recordings were not included/obtained due to non-continuous respiratory patterns or recording failures. In the case of *A. senex* males, all before-treatment measurements were obtained and included in the analysis. For after-treatment analysis (day 15), 49 measurements were used for *A. marindia* females and 12 for *A. senex* males. Data not included/obtained was due to mortality during the experiment and extreme weakness, egg sac presence or laying the day of the measurement, non-continuous respiratory patterns, or recording failures.

Given that *A. senex* and *A. marindia* individuals become reproductively active after their last molt (Aisenberg, 2006), it is not possible to decouple the reproductive period from adulthood. When *A. marindia* females laid an egg sac on day 15, respirometry measurements were not conducted ($n = 1$). On the other hand, if an egg sac was seen before day 15, it was removed from the female, and SMR and TEWL were measured on day 15 ($n = 12$). This variable was included in the statistical analysis (see below).

Survival

Survival was recorded every day (from day 1 to 30) at the same hour. To do so, we checked the status of individuals without opening the containers. For *A. senex* females, data was obtained using the same protocol (A. Albín unpublished data).

Distribution pattern

To assess the spatial distribution of *A. senex* and *A. marindia* in the study site, we used a systematized pitfall protocol. We defined three zones on the beach, covering all the dune area in each one. In each area, 3–4 transects separated 10 m from each other were placed, with perpendicular orientation to the coast; a total of 10 transects were placed. The first pitfall of each transect was placed 10 m towards the coast starting from the first line of dunes, in order to standardize the beginning of each transect given the diverse morphology of the dunes in the beach. The rest of the pitfalls were placed 10 m from each other (Figure 1A). The length of each transect was determined by the end of the dune zone, so it varied between 8–12 pitfalls. A total of 102 pitfalls were placed. Each pitfall consisted of a plastic cup buried in the sand up to the edge. Over each pitfall we placed a plastic plate at a cautious height functioning as a roof, in order to minimize the probability of the sand covering the pitfall during the night (Figure 1B).

The sampling of individuals was performed in three events, corresponding to three consecutive nights (from 7 p.m. to 8 a.m.) in early November 2023. Prior to 7 p.m. each day, soapy water with salt was placed in each pitfall. The next morning the contents of each pitfall

was collected in airtight plastic containers properly labeled. In the laboratory, the water was replaced with 70 % ethanol to preserve the integrity of the samples. Subsequently, in the Entomology Section, Facultad de Ciencias, UDELAR, the contents of each container was analysed under a stereomicroscope and the taxonomic group, developmental stage and sex of each individual were identified.

Likewise, for each sample station, the environmental conditions were characterized. We quantified the percentage of vegetation cover using a 1×1 m quadrat placed with the pitfall point in the center (Figure 1C). We collected 75 g of sand at each point and placed it in a tightly closed plastic bag, to later quantify the electrical conductivity (EC; a *proxy* of salinity) in the laboratory. To calculate a mean of EC in each point we took sand samples in all 3 sampling days (i.e., 306 samples). To quantify the EC ($\mu\text{S}/\text{cm}$), we used a modified Loveday protocol (Kargas et al., 2020; Loveday, 1974). Briefly, 250 mL of distilled water was added to 50 g of sand sample (EC 1:5). This mixture was shaken for 30 min using a mechanical stirrer and then kept at rest for 10 min before measuring EC with a salinometer (YSI EC300 salinometer). This analysis was carried out at the Soil and Water Laboratory, Department of Soil and Water, Facultad de Agronomía, UDELAR.



Figure 1. Systematized pitfall protocol used to assess the distribution pattern of *Allocosa senex* and *A. marindia* in Las Grutas beach, Maldonado, Uruguay. (A) The three sampling zones are highlighted, with a detail of the transect and pitfall placement on the left. (B) Example of the pitfall design. (C) Quadrant used to estimate vegetation cover.

Statistical analyses

Morphologic analysis

We calculated a Body Condition Index (BCI) to assess if it could be a confounding factor in our results, as it has been reported to vary between sexes and locations in both *Allocosa* species (e.g., Bollatti et al., 2017; Postiglioni et al., 2025). Following the *residual index* methodology from Jakob et al., (1996), we checked for BCI differences at the intraspecific (for both *A. senex* females and males) and the interspecific level (only for females). We fitted linear models (LM) with BCI residuals as the response variable and sex/species, day of collection (i.e., *proxy* of the advance of the reproductive season) and their interaction as fixed factors.

A. senex males brought from the field exhibited a higher body condition than *A. senex* females ($R^2 = 15\%$; Table S1). No differences in body condition were found between *A. senex* and *A. marindia* females; however, the model had a low R^2 ($<10\%$) which implies that other variables better explain BCI variation in females (Table S2). As expected, body condition decreased as the reproductive season progressed for both the intraspecific and the interspecific model. Thus, differences between groups explained little or no BCI variation, so they would not have affected our subsequent results. The tables from these analyses can be consulted in Supplementary Material (Table S1, S2 and S3).

Before-treatment analysis (day 0)

We assessed SMR and TEWL differences before-treatment (day 0) as a baseline value, at the intraspecific (*A. senex* females and males, and *A. marindia* females) and interspecific (between females) level fitting LM with lognormal distribution. We included body mass and days in the laboratory in interaction with species or sex as fixed effects. Days in the laboratory, was calculated as the number of days since the individual was brought to the laboratory (day 0) until the day of the respirometry measurement (day 0 of the experiment). We included it to control for the effect of the inter-experimental period (food, *ad libitum* water, etc.).

Before-treatment, *A. senex* males had lower SMR values than *A. senex* females, but higher TEWL (Figure S1, Table S4). Moreover, females did not differ in their SMR, but the TEWL of *A. senex* females was higher than *A. marindia*'s (Figure S2, Table S5). The tables and figures from this analysis can be consulted in Supplementary Material (Table S4, S5, S6, Figures S1, S2). The before-treatment SMR and TEWL measurements were used to evaluate individuals' response to salinity treatments by comparing their before- and after-treatment values, as detailed in the next section.

After-treatment analysis (day 15)

To evaluate the effect of salinity treatments over the SMR and TEWL of *A. senex* (males and females) and *A. marindia* (females) individuals, we fitted LM models with lognormal distribution, separately for each group. We included salinity treatment and body mass as explanatory variables. For *A. marindia* females we also included the variable egg sac. The data set from Chapter 1 used for *A. senex* females did not have reproductive information, so we could not include this variable in the analysis.

We also evaluated if the salinity treatments influenced weight loss (WL) and if this differed between sexes and species, by fitting LM with normal or lognormal distribution for each group. The percentage of weight loss between days 0 and 15 was used as the response variable; salinity treatment and days in the laboratory were included as explanatory variables. The variable days in the laboratory was included to control if the individuals that spent more days in the inter-experimental period had a different pattern of body mass change. Moreover, we included egg sac as an explanatory variable for *A. marindia* females. Post-hoc comparisons between salinity treatments were performed using the False Discovery Rate method in all cases (Benjamini & Hochberg, 1995).

Finally, to evaluate whether individuals changed their SMR and TEWL between the before- and after-treatment measurements, we first fitted linear mixed models with a lognormal distribution of residuals for each group. Each model included the ID of individuals

as random intercepts, and body mass and time (before/after) in interaction with salinity treatment as fixed effects. We checked each model for singularity (i.e., near-zero variance in random effects). When singularity was true, we fitted a linear model without the random intercept. To assess individual plasticity in response to salinity treatment, we compared before-after values within each salinity treatment using the False Discovery Rate method (Benjamini & Hochberg, 1995); this is what we report in the Results section. Because before-after data were not available for all individuals surviving to day 15 (see Methods), the analyses were conducted on a subset of the after-treatment data (see Results).

Survival

To assess the effect of salinity treatments on survival rate, we fitted Cox proportional-hazards models (survival package; Therneau, 2024; Therneau & Grambsch, 2000). We compared the survival rate of *A. senex* females and males, including salinity treatment, body mass and sex as explanatory variables. The model that compared the survival rate of *A. senex* and *A. marindia* females did not meet the proportionality assumptions (see below); thus, we fitted one model for each group. The model for *A. marindia* females included egg sac, besides salinity treatment and body mass. Post-hoc tests were conducted using the False Discovery Rate method (Benjamini & Hochberg, 1995) to check for differences between salinity treatments. In addition, since respirometry measurements were performed on day 15, we calculated the percentage of survival on days 15 and 30 for all groups.

Distribution pattern

To assess the effects of mean electric conductivity (EC; the mean of the three sampling days; *proxy* of salinity), vegetation cover and prey availability on the presence of *A. senex* and *A. marindia*, we used GLMs with binomial distribution, including linear, quadratic and cubic effects of the explanatory variables. For *A. senex*, we initially fitted separate models for adult males, adult females and juveniles. However, because the final models for males and females retained the same explanatory variables and the sample size was relatively low, we fitted a new model without including the variable sex. In the case of *A. marindia*, the species

was present in only 4 of 102 pitfalls (see Results), resulting in extremely low explanatory power and wide confidence intervals of the estimates of the models. Thus, we do not report these models.

All statistical analyses and graphics were performed using R software (R Core Team, 2023) with the packages ggplot2, fitdistrplus, multcomp, MuMIn, and DHARMA. For survival analysis we used the packages survival, ggsurvfit, gtsummary, survminer. In all cases where SMR was the response variable, both SMR and body mass were log-transformed given the known logarithmic relationship between metabolism and body mass (McNab, 2002). In all models continuous variables were standardized and centered, and collinearity among variables was checked (Inchausti, 2022). For LM, GLM and LMM, residual analysis was performed to ensure normality and homoscedasticity. For survival analysis, model assumptions were checked using the hazard proportionality test (cox.zph command), Schoenfeld residual plots, and DFBeta residual plots to detect influential data. Likewise, model concordance was confirmed to be ≥ 0.68 (standard goal is ≥ 0.50), and the values of the goodness-of-fit tests (LRT, Wald test, and Log-rank test) were all significant ($p < 0.05$). Model selection for before and after-treatment and for survival models followed a hypothesis-driven approach and was done using LRT, only in cases where interactions or control variables (e.g., days in the laboratory, body mass) were not statistically significant. Global effects of explanatory variables were assessed using ANOVA for variables with three or more levels. Post-hoc comparisons were performed when significant global effects were detected for these variables, or in particular cases where specific contrasts in the model showed significant differences (see below). For the distribution pattern analysis, model selection was done by AICc as there was no *a priori* hypothesis on which of the three explanatory variables had more influence over these species' distributions.

Results

The results of this study reveal intra and interspecific differences in the salinity tolerance of two sand-dwelling spider species in an ocean-influenced beach. Salinity treatments did not influence Standard Metabolic Rate (SMR) and Total Evaporative Water Loss (TEWL) of *A. senex* males and females, but did influence *A. marindia* females (Figure 2). Moreover, while weight loss (WL) was greater with increasing salinity for both *A. senex* males and *A. marindia* females, no effect was found for *A. senex* females (Figure 2). Finally, survival rate under high salinity treatments was lower for *A. senex* males than females (Figure 3), and *A. marindia* females experienced higher mortality with increasing salinity (Figure 4).

After-treatment analysis (day 15)

Salinity treatment did not affect SMR in *A. senex* females ($F_{2, 17} = 1.96$, $p = 0.17$) and males, but SMR increased with body mass in both sexes. In contrast, salinity treatment did influence SMR in *A. marindia* females ($F_{2, 46} = 5.78$, $p < 0.01$), but SMR was not affected by body mass (Figure 2, Table 1). *A. marindia* females under the 70 ‰ treatment had higher SMR values than females at 35 ‰ and 0 ‰ (Post-hoc test: 70 ‰ - 0 ‰: $t(46) = 3.03$, $p = 0.007$; 70 ‰ - 35 ‰: $t(46) = 2.96$, $p = 0.007$), with no difference between 0 ‰ and 35 ‰ ($t(46) = -0.05$, $p = 0.961$).

Regarding TEWL, salinity treatment did not affect water loss in *A. senex* males or females ($F_{2, 17} = 0.45$, $p = 0.65$; Figure 2), but in both sexes TEWL increased with body mass (Table 2). In contrast, the final model showed that TEWL increased in *A. marindia* females at 70 ‰ compared to 0 ‰ ($t(45) = 2.91$, $p = 0.017$), but TEWL did not differ among the other treatments (Post-hoc test: 35 ‰ - 0 ‰: $t(45) = 1.06$, $p = 0.295$; 70 ‰ - 35 ‰: $t(45) = 1.90$, $p = 0.095$) (Figure 2). Nonetheless, salinity did not have a significant overall effect on TEWL ($F_{2, 45} = 2.10$, $p = 0.14$). Moreover, *A. marindia* females that laid an egg sac during the experiment had greater TEWL values than those that did not (Table 2).

Weight loss was influenced by salinity treatments in *A. senex* males, as those under the 70 ‰ treatment lost more weight than those at 35 ‰ (Figure 2, Table S7). Regarding *A. marindia* females, salinity did not have a significant overall effect ($F_{2, 52} = 1.60, p = 0.21$). However, the model did detect statistical differences that held in the post-hoc test. In this sense, females under the 35 ‰ and 70 ‰ treatments lost more weight than those at 0‰ (35 ‰ - 0 ‰: $t(52) = 2.62, p = 0.017$; 70 ‰ - 0 ‰: $t(52) = 3.11, p = 0.009$), with no difference between 70 ‰ and 35 ‰ ($t(52) = 0.80, p = 0.428$). In addition, weight loss in *A. marindia* females was higher in those that laid an egg sac and increased with the number of days in the laboratory, although the effect of days in the laboratory was small compared to salinity treatment. In contrast, salinity treatments did not affect weight loss in *A. senex* females ($F_{2, 23} = 0.35, p = 0.71$). However, the model explained only 3 % of data variability. Therefore, the weight loss of *A. senex* females was explained by other factors not contemplated in the model. The table for the after-treatment model selection can be consulted in Supplementary Material (Table S8).

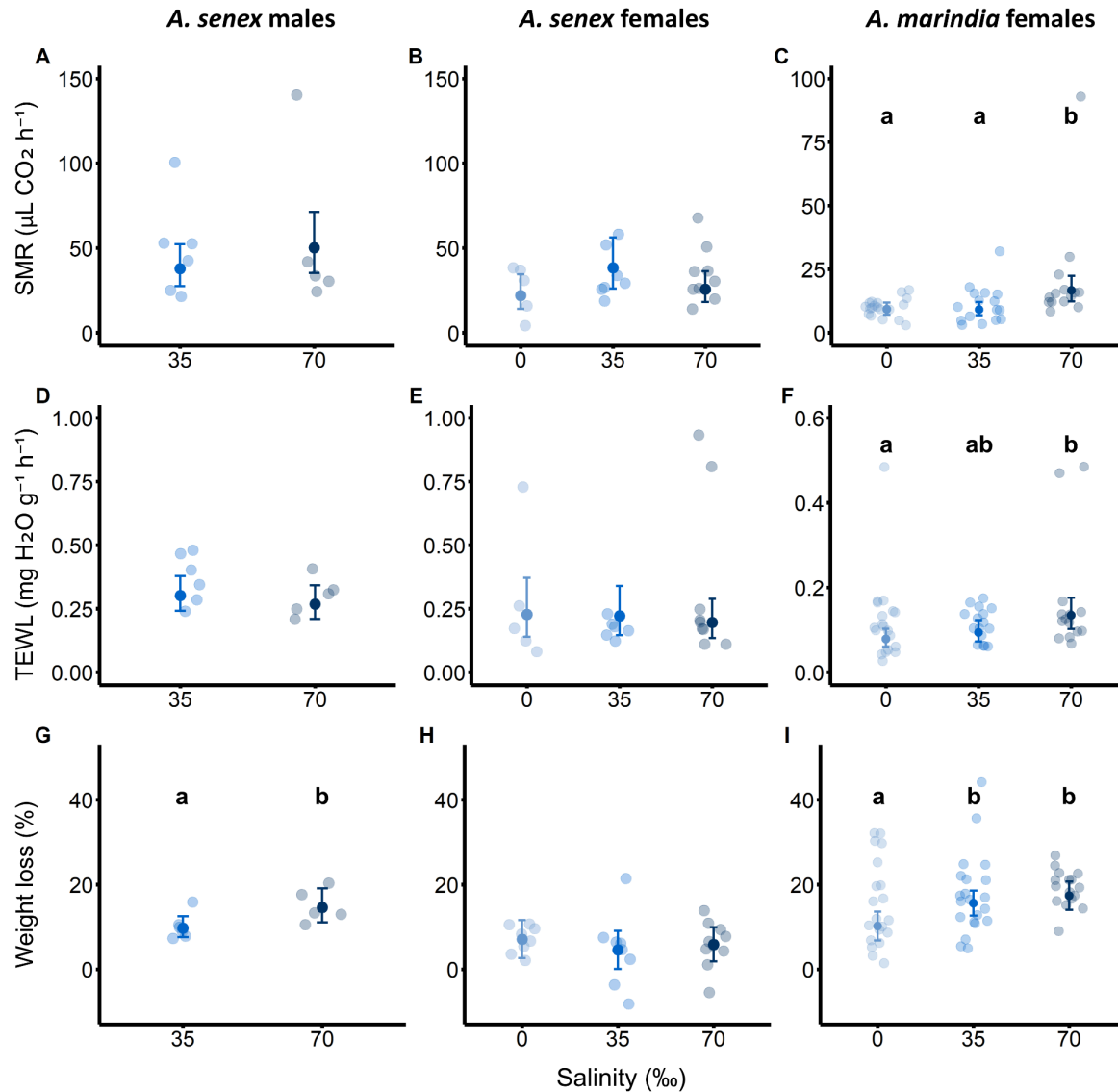


Figure 2. Standard Metabolic Rate (SMR, A-C), Total Evaporative Water Loss (TEWL, D-F) and Weight loss (%), G-I) values of adult *Allocosa senex* males (A, D, G) and females (B, E, H), and adult *A. marindia* females (C, F, I), from an oceanic-influenced beach. Individuals were exposed to salinity treatments (0 ‰, 35 ‰ and 70 ‰) for 15 days. Mean and 95 % CI are shown. Different letters are shown only in cases where statistically significant differences between treatments were found. Note that panels C and F have a different scale for clarity purposes.

Table 1. Summary of the best model for Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) for *Allocosa senex* males and females, and *Allocosa marindia* females, exposed to different salinity treatments (0 ‰, 35 ‰ and 70 ‰) for 15 days. Values are expressed in log-scale. Body mass was log-transformed. S) Salinity treatment, Mb) Body mass (g, standardized). Note that *A. senex* males were only exposed to the 35 ‰ and 70 ‰ treatments.

Standard Metabolic Rate				
<i>A. senex</i> males				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	3.63	0.14	26.26	<0.001
S (70 ‰)	0.28	0.21	1.33	0.219
Mb	0.53	0.11	4.83	0.001
Observations	11			
<i>df</i>	8			
<i>R</i> ²	0.75			
<i>A. senex</i> females				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	3.09	0.21	14.67	<0.001
S (35 ‰)	0.55	0.27	2.02	0.060
S (70 ‰)	0.15	0.27	0.55	0.588
Mb	0.39	0.12	3.36	0.004
Observations	21			
<i>df</i>	17			
<i>R</i> ²	0.47			
<i>A. marindia</i> females				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	2.22	0.13	17.48	<0.001
S (35 ‰)	-0.01	0.19	-0.05	0.961
S (70 ‰)	0.59	0.20	3.03	0.004
Observations	49			
<i>df</i>	46			
<i>R</i> ²	0.20			

Table 2. Summary of the best model for Total Evaporative Water Loss (TEWL, mg H₂O g⁻¹ h⁻¹) for *Allocosa senex* males and females, and *Allocosa marindia* females exposed to different salinity treatments (0 ‰, 35 ‰, and 70 ‰) for 15 days. Values are expressed in log-scale. S) Salinity treatment, Mb) Body mass (g, standardized), ES) Egg sac. Note that *A. senex* males were only exposed to the 35 ‰ and 70 ‰ treatments.

Total Evaporative Water Loss				
A. senex males				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-1.20	0.10	-12.31	<0.001
S (70 ‰)	-0.12	0.15	-0.82	0.436
Mb	0.22	0.08	2.84	0.022
Observations	11			
<i>df</i>	8			
<i>R</i> ²	0.58			
A. senex females				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-1.48	0.23	-6.37	<0.001
S (35 ‰)	-0.03	0.30	-0.08	0.934
S (70 ‰)	-0.15	0.30	-0.49	0.632
Mb	0.41	0.13	3.23	0.005
Observations	21			
<i>df</i>	17			
<i>R</i> ²	0.40			
A. marindia females				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-2.54	0.13	-19.37	<0.001
S (35 ‰)	0.18	0.17	1.06	0.295
S (70 ‰)	0.53	0.18	2.91	0.006
ES	0.60	0.17	3.48	0.001
Observations	49			
<i>df</i>	45			
<i>R</i> ²	0.27			

The individual plasticity to salinity exposure was assessed by comparing SMR and TEWL values before- and after-treatment within each salinity treatment. This before-after analysis revealed differences in plasticity between groups and traits (Figures S3 and S4). Linear mixed models with ID as random effect were fitted unless otherwise stated.

SMR values in *A. senex* males ($n = 11$) were analysed fitting a linear model due to singularity, suggesting low interindividual variability (Table S9). The time $\times$ salinity interaction was not significant ($F_{1,17} = 0.02$, $p = 0.89$) and within-treatment comparisons showed no evidence of plasticity. In the case of *A. senex* females ($n = 15$), the before-after analysis revealed plasticity in SMR values and interindividual variability explained 4.5 % of SMR variation (Table S9). Although the time $\times$ salinity interaction was not significant ($F_{2,12} = 0.55$, $p = 0.59$), within-treatment comparisons showed a significant decrease in SMR at 70 ‰ ($t(12.3) = -3.300$, $p = 0.006$), but no significant changes were observed at 0 ‰ or 35 ‰. For *A. marindia* females ($n = 30$), a linear model was fitted due to singularity (Table S9). The time $\times$ salinity interaction was significant ($F_{2,54} = 4.26$, $p = 0.02$) and within-treatment comparisons showed that SMR decreased after-treatment in females exposed to 35 ‰ ($t(54) = -2.384$, $p = 0.021$). No significant changes were observed at 0 ‰ or 70 ‰.

Regarding TEWL, *A. senex* males ($n = 11$) exhibited plasticity in this trait. The time $\times$ salinity interaction was significant ($F_{1,8.99} = 6.44$, $p = 0.03$) and within-treatment comparisons showed an increase in TEWL at 35 ‰ ($t(12.5) = 2.776$, $p = 0.016$), but no change at 70 ‰. Interindividual differences accounted for 38.9 % of the variance (Table S10). For *A. senex* females, interindividual differences accounted for 64.1 % of the variance (Table S10). The time $\times$ salinity interaction was not significant ($F_{2,12} = 0.20$, $p = 0.82$), and within-treatment comparisons showed no evidence of plasticity at any salinity treatment. In the case of *A. marindia* females ($n = 30$), interindividual differences explained 11.3 % of TEWL variance (Table S10). The time $\times$ salinity interaction was significant ($F_{2,27} = 4.14$, $p = 0.03$) and within-treatment comparisons showed that only females in the 0 ‰ treatment exhibited plasticity ($t(27) = -3.350$, $p = 0.002$).

Survival

The survival probability of *A. senex* males was lower than *A. senex* females, regardless of salinity treatment. In addition, both sexes had lower survival at 70 ‰ compared to 35 ‰ (Figure 3, Table S11). The higher susceptibility of males to salinity treatments was reflected in their average survival at day 15 (i.e., the day when respirometry measurements were performed), as it was 56 % at 35 ‰ and 40 % at 70 ‰. In contrast, average survival for females was 100 % at 35 ‰ and 50 % at 70 ‰ (Table 3).

On the other hand, the survival analysis of *A. senex* and *A. marindia* females (separately) revealed that their susceptibility to salinity treatments differed (Figure 4, Table S12). Despite salinity treatment had a significant overall effect over *A. senex* females' survival ($\chi^2_2 = 7.05$, $p = 0.03$), this was not reflected in post-hoc comparisons (35 ‰ - 0 ‰: $z = -1.13$, $p = 0.260$, 70 ‰ - 0 ‰: $z = 1.50$, $p = 0.200$, 70 ‰ - 35 ‰: $z = 2.27$, $p = 0.069$). However, the average survival of *A. senex* females at 35 ‰ doubled survival at 70 ‰ on days 15 and 30, but there was a large overlap of the 95 % CIs (Table 4). In contrast, *A. marindia* females were more susceptible to high salinity treatments ($\chi^2_2 = 34.11$, $p < 0.001$). Survival probability at 70 ‰ was ~17 times lower than at 0 ‰ ($z = 4.520$, $p < 0.001$) and ~5 times lower than at 35 ‰ (Post-hoc test: $z = 3.841$, $p < 0.001$). No significant differences were found between 0 ‰ and 35 ‰ ($z = 1.906$, $p = 0.057$). Notably, at day 15, 95 % of *A. marindia* females had survived at 35 ‰ and 0 ‰, while 55 % had survived at 70 ‰ (Table 4). The table for model selection can be found in Table S13.

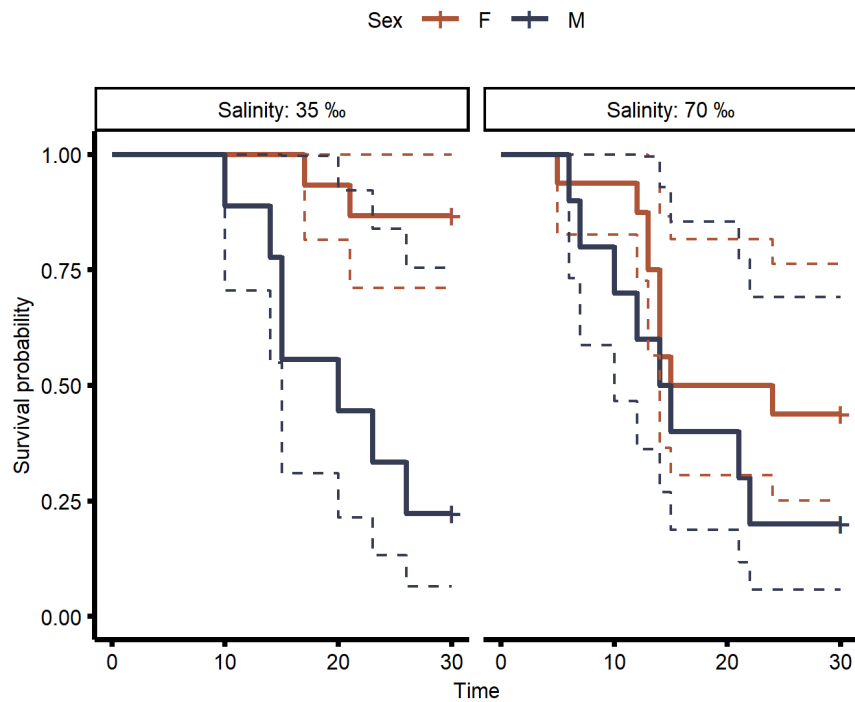


Figure 3. Survival curves of *Allocosa senex* males and females exposed to two salinity treatments (35 ‰ and 70 ‰) for 30 days at 25 °C. Solid lines indicate mean values and dashed lines correspond to 95 % CIs. Females are shown in orange and males in violet.

Table 3. Survival probability of *Allocosa senex* males and females exposed to two salinity treatments (35 ‰ and 70 ‰) for 30 days at 25 °C. Survival is shown for day 15 and day 30 of the experiment. Mean values and 95% CIs in parenthesis are shown.

Sex	Salinity	Day 15	Day 30
Females	35 ‰	100 % (100–100)	87 % (71–100)
	70 ‰	50 % (31–82)	44 % (25–76)
Males	35 ‰	56 % (31–100)	22 % (6,5–75)
	70 ‰	40 % (19–85)	20 % (5,8–69)

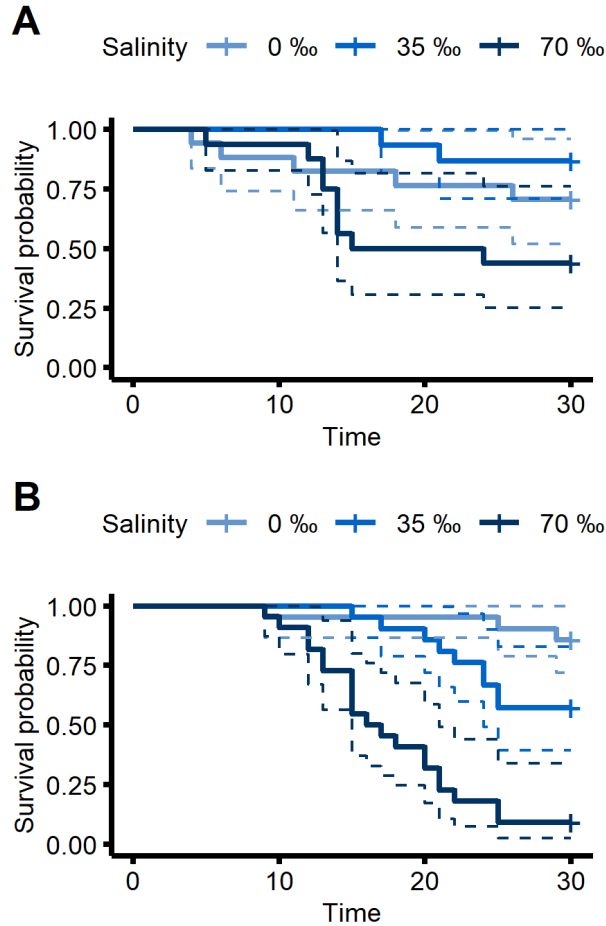


Figure 4. Survival curves of *Allocosa senex* (A) and *A. marindia* (B) females exposed to three salinity treatments (0 ‰, 35 ‰ and 70 ‰) for 30 days at 25°C. Solid lines indicate mean values and dashed lines correspond to 95 % CIs.

Table 4. Survival probability of *Allocosa senex* and *A. marindia* females exposed to three salinity treatments (0 ‰, 35 ‰ and 70 ‰) for 30 days at 25 °C. Survival is shown for day 15 and day 30 of the experiment. Mean values and 95 % CIs in parenthesis are shown.

<i>A. senex</i> females			<i>A. marindia</i> females	
Salinity	Day 15	Day 30	Day 15	Day 30
0 ‰	82 % (66–100)	71 % (52–96)	95 % (87–100)	86 % (72–100)
35 ‰	100 % (100–100)	87 % (71–100)	95 % (87–100)	57 % (39–83)
70 ‰	50 % (31–82)	44 % (25–76)	55 % (37–80)	9,1 % (2,4–34)

Distribution pattern

Regarding the distribution pattern of *A. senex* adults, six models were retained as equivalent ($\Delta AICc < 2$), and the most parsimonious one (i.e., the one with the least number of parameters) was selected as the final model (Table S14). All six models included vegetation cover as an explanatory variable, suggesting that the probability of adult presence is higher in zones where the vegetation cover is low. This effect was marginal ($z(100) = -1.919$, $p = 0.055$) (Figure S5). On average, *A. senex* adults were found in zones with a mean conductivity of $34.4 \pm 12.9 \mu S/cm$ and a mean vegetation cover of $14.5 \pm 15.5 \%$. For *A. senex* juveniles, five models were retained as equivalent, including the null model (Table S14). The model with the lowest AICc included mean conductivity (proxy of mean salinity) as explanatory variable, but it did not have a significant effect on juveniles presence ($z(100) = -1.400$, $p = 0.161$). Thus, for parsimony, the null model was selected as the final model. On average, *A. senex* juveniles were found in zones where the mean conductivity was $28.76 \pm 9.14 \mu S/cm$ and the mean vegetation cover was $25.2 \pm 25.8 \%$.

Finally, *A. marindia* individuals were found only in 4 of the 102 pitfalls. In total, 5 individuals (2 males, 2 females and 1 juvenile) were collected. Considering these 4 points, *A. marindia* was found in zones where the mean conductivity was $24.3 \pm 7.0 \mu S/cm$ and the mean vegetation cover was $16.0 \pm 9.1 \%$ (Figure S6).

Discussion

This study explored potential differences in the factors driving habitat selection between two terrestrial wolf spider species in an outer-estuarine beach. The local distribution of *Allocosa marindia* females in the landward dune zone appears to be associated with their physiological tolerance to salinity in laboratory conditions: under the highest salinity level, females exhibited increased metabolism, water loss, and weight loss, accompanied by reduced survival. In contrast, habitat selection in adult *A. senex* is not shaped by their physiological tolerance to salinity as shown by both laboratory and field results. Nonetheless, significant sex-specific differences in osmoregulatory capacity were observed: females physiology and survival were largely unaffected by salinity, whereas males exhibited decreased survival and greater weight loss at the highest salinity levels despite their larger body size. These physiological responses are generally consistent with the species' natural distribution pattern, suggesting that the dune system as a whole represents a physiologically suitable habitat for *A. senex*. Therefore, habitat selection in this species is likely shaped by ecological factors, such as species interactions, rather than physiological constraints.

Physiological tolerance to salinity

Contrary to our hypothesis, *A. senex* males have lower osmoregulatory capacity than females, as evidenced by their lower salinity tolerance. Males' SMR values did not change either between treatments in the after-treatment analysis or within treatments in the before-after analysis. This suggests they did not regulate their metabolism to cope with salinity. Regarding TEWL, no significant differences were observed between salinity treatments at the after-treatment stage. However, males exposed to 35 ‰ increased their TEWL after treatment, whereas no changes were observed in males at 70 ‰. This suggests that males did not alter their cuticle composition or their breathing rate to reduce water loss (Chown & Nicolson, 2004; Malmos et al., 2021). However, other physiological mechanisms such as an enhanced osmotic tolerance, increased hemolymph osmolarity (Foucreau et al.,

2012; Witteveen et al., 1987) and/or hormone production (e.g., octopamine, Herrmann, 2015) may have been involved .

Moreover, males experienced greater weight loss under hypersaline conditions (i.e., 70 ‰), which may reflect a reduction in body mass due to water loss and/or the mobilization of fat reserves. Additionally, they exhibited lower survival rates than females regardless of the salinity treatment, and by day 15, nearly half of the males exposed to 35 and 70 ‰ had already died. Since TEWL remained unchanged across salinity treatments, whereas body weight decreased at the highest salinity, it is possible that the males that survived until day 15 did so either because they had higher tolerance to water loss (an osmoregulatory mechanism well documented in insects, Chown & Nicolson, 2004), or because they were able to mobilize energy reserves to sustain physiological mechanisms that helped them cope with osmotic stress. Thus, individual differences in survival and weight loss may be explained by genetics, environmental conditions during development or maternal effects. This is supported by the fact that almost 40 % of TEWL variance was explained by individual variation.

In contrast, consistent with our hypothesis, *A. senex* females exhibited high osmotic stress tolerance. Neither physiological traits nor survival rates differed across salinity treatments. Interestingly, their survival probability at 35 ‰ (equivalent to ocean salinity) and 70 ‰ (the maximum salinity reached in salt marshes) did not differ from survival at 0 ‰, even though these salinity levels far exceed those typically experienced by *A. senex* females in nature. Moreover, the decrease in SMR found in females exposed to 70 ‰ (before-after analysis), suggests a potential adaptive benefit of metabolic depression for survival under extreme salinity conditions. Additionally, despite the fact that we did not find a plastic response in TEWL, previous work on females from this location (Chapter 1 of this thesis) demonstrated their ability to decrease TEWL when exposed to both high salinity and high temperature treatments. These results further support females' tolerance to extreme salinity conditions, likely due to life-history traits and the highly dynamic environments they inhabit

(Lorrain-Soligon et al., 2024). Still, as previously discussed for males, we cannot discard that *A. senex* females may have exhibited other osmoregulatory mechanisms.

Notably, the larger body size of males did not confer greater osmotic tolerance as previously reported in other wolf spider species (Pétillon et al., 2011). This may be explained by *A. senex*' reversed sex role. In this species, females are the mobile courting sex and dig shallow silk capsules instead of deep burrows (Aisenberg et al., 2007; Aisenberg & Costa, 2008), making them more frequently exposed to sea spray—at night, while moving on the surface, and during the day, due to their shallow capsules being closer to the salt-moistened sand. Thus, the higher osmotic tolerance of females could be explained by other physiological mechanisms here not studied, and may reflect an adaptive response linked to their life-history traits.

On the other hand, according to our hypothesis, females of *A. marindia* exhibited distinct physiological and survival responses than *A. senex*, indicating lower salinity tolerance. The after-treatment analysis showed that *A. marindia* females increased their SMR, TEWL and weight loss at high salinity (70 ‰), and had a lower survival rate. The decrease in SMR found comparing before-after values in females at 35 ‰, but not in those at 70 ‰, may suggest that the females at 70 ‰ that survived up to day 15 were naturally more tolerant to osmotic stress (due to genetic factors, environmental conditions during development, maternal effects), but they did not exhibit any physiological response on the traits here measured. However, the decrease in TEWL observed in the before-after analysis in females at 0 ‰ is difficult to interpret, as a reduction in osmotic stress would eventually lead to an increase in water loss. Overall, our findings align with the fact that *A. marindia* is significantly smaller than *A. senex*, and therefore, has a higher surface to volume ratio, which increases their susceptibility to dehydration and metabolic costs, reflected in the increased SMR and TEWL at hypersaline conditions (Huey, 1991; Pulz, 1987). Nonetheless, it is worth noting that responses in *A. marindia* females were only evident under hypersaline conditions (70 ‰), despite 35 ‰ also representing a challenging salinity level. In this sense, female

survival at 35 ‰ was 95 % on day 15 (i.e., respirometry measurement day), but dropped to 55 % by day 30. This likely explains the absence of significant differences between medium-high and high salinity treatments in respirometry measurements at day 15.

As expected, *A. marindia* females that produced egg sacs showed greater weight loss. Notably, they also lost more water despite no change in metabolic rate. This may suggest that lipid mobilization for egg production may alter cuticular hydrocarbon availability, reducing the efficiency of the cuticular barrier against water loss (Qiao et al., 2021). Unlike the wolf spider *Arctosa fulvolineata* (Foucreau et al., 2012), where reproduction reduced survival under hypersaline conditions, reproduction did not impair survival in *A. marindia*. As mentioned before, we did not have this information for *A. senex* females, and therefore, could not analyse the effect of laying an egg sac on the measured traits.

Physiological implications on habitat selection

As reflected in this study, sand salinity does not restrict the spatial distribution of adult *A. senex*. This species exhibited a high salinity tolerance and survival rate under highly stressful salinity conditions that exceed its naturally experienced range (i.e., 35 ‰ and 70 ‰). Despite that males exhibited lower osmoregulatory abilities than females, the cumulative effect of salinity exposure in nature may not have enough impact on their physiology to influence their habitat selection. Given that *A. senex* is the most abundant spider species reported on Uruguayan sandy beaches (Costa, 1995; Costa et al., 2006) and a generalist predator (including intraguild predation on *A. marindia*; Aisenberg et al., 2009) it raises the question of why adults primarily occupy open dunes, where environmental conditions are harsher.

The dune morphology in the beach here studied is highly variable within a few meters scale, which implies that exposure to salinity can be widely variable, while vegetation cover is a much more stable factor (see Supplementary Material Figures S7 and S8). In this sense, the marginal effect found for vegetation cover may be associated with other relevant variables. On one hand, the cryptic coloration of these spiders allows them to blend with the

sand, reducing their predation risk by the parasitoid wasp *Anoplius bicinctus*, one of the few known predators of *A. senex* (Costa et al., 2006; Stanley et al., 2013). Furthermore, although *A. senex* preys on other spiders, the area where most potential competing species are found, including *A. marindia*, is where vegetation cover is higher. Thus, their distribution on this salinity-influenced beach appears more shaped by ecological factors like competition and predation than by physiological tolerance.

Regarding *A. senex* juveniles, the best distribution models included mean electrical conductivity (a *proxy* of salinity concentration) though this effect was non statistically significant and the null model was selected. The inclusion of electrical conductivity in most models could relate to the fact that *A. senex* juveniles are smaller than adults. Thus, they likely have a higher susceptibility to water loss through their surface, increasing the risk of desiccation. Overall, the whole study area constitutes a physiologically suitable area to *A. senex*, but adults prefer to occupy open dune areas.

Conversely, the physiological tolerance to salinity of *A. marindia* could play a role in shaping habitat selection. Although distribution models for *A. marindia* adults could not be run due to scarce presence data, individuals were absent from locations where mean conductivity exceeded 30 $\mu\text{S}/\text{cm}$ and vegetation cover was below 6 %. This aligns with previous studies on this species distribution in an inner-estuarine beach (Costa, 1995; Costa et al., 2006; Simó et al., 2017). The relatively limited osmoregulatory abilities of *A. marindia*, combined with its lower capacity to respond to sudden floods compared to *A. senex* (Albín et al., 2022), its susceptibility to predation by *A. senex*, and less cryptic coloration in sand (Aisenberg et al., 2009, 2023), likely restrict its habitat use. In this sense, by occupying more sheltered areas within the dune system, *A. marindia* may gain several benefits, especially in oceanic-influenced beaches where constant exposure to sea spray, salty prey and high sand salinity could otherwise severely impair its fitness.

Conclusions

Our study contributes to the understanding of habitat selection by terrestrial arthropods in coastal ecosystems. Future studies should explore additional osmoregulatory mechanisms, such as cuticular composition. Furthermore, it would be interesting to assess the effect of salted prey on osmoregulation and to evaluate if the patterns found in this study are observed in beaches with only ocean influence.

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Supplementary Material

Morphologic analysis

A. senex males exhibited higher body condition (BCI) than *A. senex* females when measured directly from the field ($t(49) = 2.93$, $p = 0.005$; $R^2 = 15\%$) (Table S1). The higher BCI of *A. senex* males may reflect sex-specific reproductive behaviors, as during the reproductive period females face high physiological demands, related to their higher mobility, the egg production, and the offspring carrying. Nonetheless, BCI has been shown to be a highly variable metric, as it can change between locations and sexes (e.g., Bollati et al., 2017; Postiglioni et al., 2025), likely influenced by abiotic conditions and prey availability. Therefore, our results likely reflect the interplay of multiple non-exclusive factors.

On the other hand, BCI did not differ between *A. senex* and *A. marindia* females ($t(93) = -1.54$, $p = 0.128$; $R^2 = 7\%$) (Table S2). However, the R^2 of the model was $<10\%$, which implies that other variables beyond species better explain BCI variability. Thus, differences in BCI between females would not influence this study's results. Model selection can be consulted in Table S3.

Table S1. Summary of the best model for the intraspecific Body Condition Index comparison of adult *Allocosa senex* females and males from an oceanic-influenced beach. Values are expressed in log-scale for *A. senex*. SX) Sex, CD) Collection Date (standardized).

Body Condition Index				
<i>A. senex</i>				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-0.08	0.04	-2.20	0.033
SX (Males)	0.20	0.07	2.93	0.005
CD	-0.08	0.03	-2.25	0.029
Observations	52			
<i>df</i>	49			
R^2	0.15			

Table S2. Summary of the best model for the interspecific comparison of Body Condition Index between *Allocosa senex* and *A. marindia* females from an oceanic-influenced beach. Values are expressed in log-scale. SP) Species, CD) Collection Date (standardized).

Body Condition Index				
Females				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	0.03	0.02	1.11	0.269
SP (<i>A. senex</i>)	-0.08	0.05	-1.54	0.128
CD	-0.07	0.03	-2.63	0.010
Observations	96			
<i>df</i>	93			
<i>R</i> ²	0.07			

Table S3. Model selection via Likelihood Ratio Test for Body Condition Index (BCI) of *Allocosa senex* females and males, and *A. marindia* and *A. senex* females from an oceanic-influenced beach. The models were run to evaluate intra and interspecific differences between both species. SP) Species, SX) Sex, CD) Collection date.

Intraspecific					
<i>A. senex</i>	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
SX × CD	48	1.37			
SX + CD	49	1.43	-1	-0.0667	0.13
Interspecific					
Females	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
SP × CD	92	2.52			
SP + CD	93	2.57	-1	-0.0505	0.17

Before-treatment analysis

A. senex males had lower SMR values than *A. senex* females, beyond their difference in body mass (Figure S1, Table S4). For both *A. senex* and *A. marindia*, SMR decreased with the number of days in the inter-experimental period, but only for *A. senex*, SMR increased with body mass (Table S4). This could be related to the decrease in activity levels under the inter-experimental conditions. The fact that *A. marindia*'s SMR did not change with body mass is probably related to the much lower body mass range in this group; the variation in body mass within this group is probably not enough to reflect in SMR changes.

Regarding TEWL, *A. senex* males exhibited higher water loss than females (Figure S1, Table S4). For both species, TEWL was positively influenced by body mass. Moreover, TEWL increased with the number of days in the inter-experimental period for *A. senex* individuals, but not for *A. marindia* females (Table S4). This probably relates to the consumption of non-salty prey and *ad libitum* water during the inter-experimental period and therefore, a reduced osmoregulatory pressure. When comparing between species, we found that females did not differ in their SMR (Figure S2, Table S5), but TEWL of *A. senex* females was higher than *A. marindia*'s, beyond their difference in body size (Figure S2). The table of model selection can be found in Table S6.

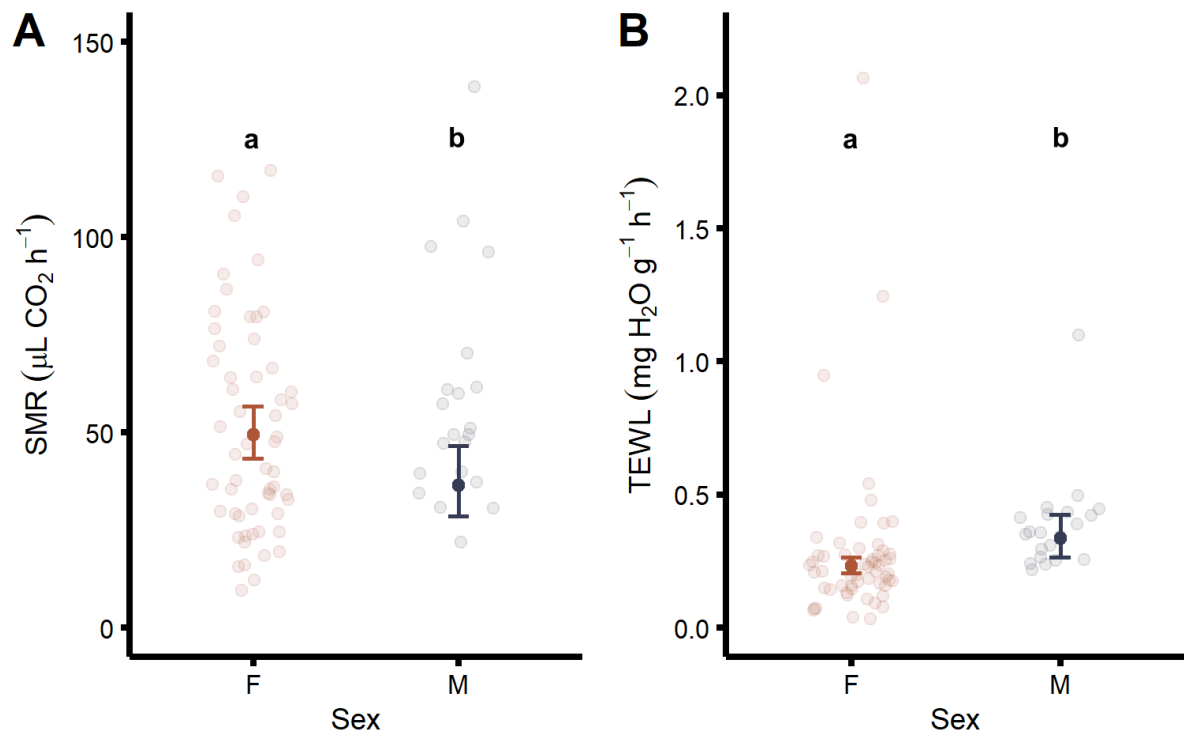


Figure S1. Standard Metabolic Rate (SMR, A) and Total Evaporative Water Loss (TEWL, B) intraspecific comparisons between adult *Allocosa senex* males and females from an oceanic-influenced beach, before-treatment (i.e., day 0). Mean and 95 % CI are shown. Different letters indicate statistically significant differences between groups.

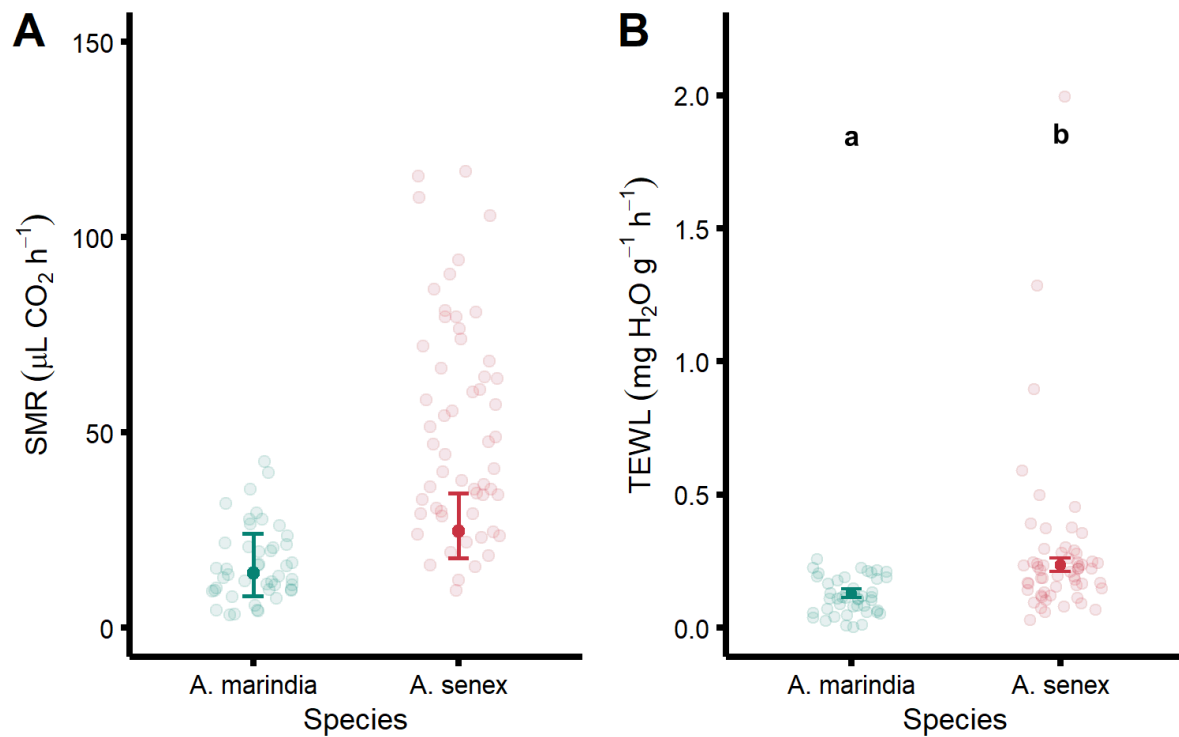


Figure S2. Standard Metabolic Rate (SMR, A) and Total Evaporative Water Loss (TEWL, B) interspecific comparisons between adult females of *Allocosa senex* and *A. marindia*, before-treatment (i.e., day 0). All individuals inhabit an oceanic-influenced beach. Mean and 95% CI are shown. Different letters are shown only when statistically significant differences between species were found.

Table S4. Summary of the best model for Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) and Total Evaporative Water Loss (TEWL, $\text{mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) for intraspecific comparisons of adult *Allocosa senex* males and females, and adult *A. marindia* females before-treatment (i.e., day 0). Values are expressed in log-scale. Body mass was log-transformed for the SMR model. SX) Sex, D) Days in the laboratory (standardized), Mb) Body mass (g, standardized).

Intraspecific - <i>A. senex</i>				
Standard Metabolic Rate				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	3.90	0.07	57.80	<0.001
SX (M)	-0.30	0.15	-2.01	0.048
Mb	0.35	0.07	5.33	<0.001
D	-0.14	0.06	-2.53	0.014
Observations	78			
<i>df</i>	74			
<i>R</i> ²	0.31			
Total Evaporative Water Loss				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-1.46	0.06	-22.58	<0.001
SX (M)	0.37	0.15	2.55	0.013
Mb	0.13	0.06	2.07	0.042
D	0.25	0.05	4.57	<0.001
Observations	78			
<i>df</i>	74			
<i>R</i> ²	0.37			
<i>A. marindia</i> females				
Standard Metabolic Rate				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	2.62	0.09	29.40	<0.001
D	-0.24	0.09	-2.65	0.011
Observations	45			
<i>df</i>	43			
<i>R</i> ²	0.14			
Total Evaporative Water Loss				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-2.06	0.03	-60.15	<0.001
Mb	0.10	0.03	2.76	0.008
Observations	45			
<i>df</i>	43			
<i>R</i> ²	0.15			

Table S5. Summary of the best model for Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) and Total Evaporative Water Loss (TEWL, $\text{mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) for interspecific comparisons of *Allocosa senex* and *A. marindia* females, before-treatment (i.e., day 0). Values are expressed in log-scale. Body mass was log-transformed for the SMR model. SP) Species, D) Days in the laboratory (standardized), Mb) Body mass (g, standardized).

Interspecific - Females				
Standard Metabolic Rate				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	2.64	0.28	9.59	<0.001
SP (<i>A. senex</i>)	0.57	0.32	1.81	0.074
Mb	-0.00	0.25	-0.01	0.992
D	-0.21	0.06	-3.66	<0.001
SP (<i>A. senex</i>) $\times$ Mb	0.66	0.32	2.10	0.039
Observations	102			
<i>df</i>	97			
<i>R</i> ²	0.58			
Total Evaporative Water Loss				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	-2.05	0.06	-34.13	<0.001
SP (<i>A. senex</i>)	0.60	0.08	7.49	<0.001
D	-0.09	0.03	-1.46	0.147
SP (<i>A. senex</i>) $\times$ D	0.38	0.08	4.75	<0.001
Observations	102			
<i>df</i>	98			
<i>R</i> ²	0.47			

Table S6. Model selection via Likelihood Ratio Test for Standard Metabolic Rate (SMR) and Total Evaporative Water Loss (TEWL) of *Allocosa senex* and *A. marindia* individuals from an oceanic-influenced beach. The models were run to evaluate intra and interspecific differences for both species. SP) Species, SX) Sex, Mb) Body mass (g), D) Days in the laboratory.

Standard Metabolic Rate					
<i>Intraspecific</i>					
<i>A. senex</i>	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
SX × Mb + SX × D	72	15.67			
SX × Mb + D	73	16.04	-1	-0.3661	0.195
SX + Mb + D	74	16.74	-1	-0.7021	0.074
<i>A. marindia</i>	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
Mb + D	42	15.3			
D	43	15.3	-1	-0.00367	0.92
<i>Interspecific</i>					
Females	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
SP × Mb + SP × D	96	29.90			
SP × Mb + D	97	29.97	-1	-0.07255	0.629
Total Evaporative Water Loss					
<i>Intraspecific</i>					
<i>A. senex</i>	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
SX × Mb + SX × D	72	14.76			
SX × Mb + D	73	15.24	-1	-0.4776	0.127
SX + Mb + D	74	15.28	-1	-0.04124	0.657
<i>A. marindia</i>	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
Mb + D	42	2.08			
Mb	43	2.27	-1	-0.188	0.052
<i>Interspecific</i>					
Females	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
SP × Mb + SP × D	96	15.11			
SP × D + Mb	97	15.28	-1	-0.1667	0.304
SP × D	98	15.71	-1	-0.4321	0.0977

After-treatment analysis

Table S7. Summary of the best model for % of Weight Loss for *Allocosa senex* males and females and *Allocosa marindia* females exposed to different salinity (0 ‰, 35 ‰ and, 70 ‰) treatments for 15 days. Males' values are expressed in log-scale the *A. senex* males' model; as males were exposed to the 35 ‰ and 70 ‰ treatments, the mean weight loss at 35 ‰ was 9.68 % and at 70 ‰ it was 14.59 %. S) Salinity treatment, ES) Egg sac, D) Days in the laboratory (standardized).

Weight Loss				
A. senex males				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	2.27	0.11	20.64	<0.001
S (70 ‰)	0.41	0.16	2.48	0.035
Observations	11			
<i>df</i>	9			
<i>R</i> ²	0.41			
A. senex females				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	7.12	2.17	3.28	0.003
S (35 ‰)	-2.56	3.07	-0.83	0.414
S (70 ‰)	-1.23	2.92	-0.42	0.676
Observations	26			
<i>df</i>	23			
<i>R</i> ²	0.03			
A. marindia females				
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>
(Intercept)	10.21	1.68	6.09	<0.001
S (35 ‰)	5.40	2.07	2.62	0.012
S (70 ‰)	7.18	2.31	3.11	0.003
ES	13.00	2.15	6.06	<0.001
D	2.38	0.89	2.68	0.010
Observations	57			
<i>df</i>	52			
<i>R</i> ²	0.47			

Table S8. Model selection via Likelihood Ratio Test for Standard Metabolic Rate, Total Evaporative Water Loss, and percentage of Weight Loss, of *Allocosa senex* and *A. marindia* individuals from an oceanic-influenced beach, after being exposed to salinity (0 ‰, 35 ‰ and 70 ‰) treatments for 15 days. S) Salinity treatment, Mb) Body mass (g, standardized), D) Days in the laboratory (standardized), ES) Egg sac.

Standard Metabolic Rate					
<i>A. marindia</i>					
	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
S + Mb + ES	44	13.85			
S + ES	45	14.10	-1	-0.2478	0.375
S	46	14.12	-1	-0.01869	0.807
Total Evaporative Water Loss					
<i>A. marindia</i>					
	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
S + Mb + ES	44	11.15			
S + ES	45	11.26	-1	-0.1089	0.512
Weight Loss					
<i>A. senex</i> males					
	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
S + D	8	0.6551			
S	9	0.6552	-1	-0.00005	0.98
<i>A. senex</i> females					
	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
S + D	22	868.4			
S	23	869.3	-1	-0.8987	0.88

Table S9. Summary of the final model for the before-after comparison analysis on Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) of *Allocosa senex* males and females and *Allocosa marindia* females exposed to different salinity (0 ‰, 35 ‰ and, 70 ‰) treatments for 15 days. For *A. senex* males and *A. marindia* females, a linear model was adjusted; for *A. senex* females a linear mixed model was adjusted. Values are expressed in log-scale. Note that *A. senex* males were exposed to the 35 ‰ and 70 ‰ treatments. S) Salinity treatment, T) Time (before/after), Mb) Body mass (g, standardized).

Standard Metabolic Rate					
A. senex males					
Predictors	Estimates	Std. Error	t value	p	
(Intercept)	3.76	0.11	32.99	<0.001	
T (Before)	0.03	0.16	0.16	0.878	
S (70 ‰)	0.27	0.17	1.54	0.141	
Mb	0.52	0.06	8.02	<0.001	
T (Before) × S (35 ‰)	-0.04	0.24	-0.16	0.878	
Observations	22				
df	17				
R ²	0.80				
A. senex females					
Predictors	Estimates	Std. Error	t value	p	df
(Intercept)	2.95	0.25	11.62	<0.001	22.25
T (Before)	0.60	0.33	1.79	0.099	12.16
S (35 ‰)	0.53	0.33	1.60	0.124	22.55
S (70 ‰)	0.21	0.33	0.64	0.528	22.03
Mb	0.24	0.11	2.26	0.044	11.19
T (Before) × S (35 ‰)	-0.10	0.45	-0.22	0.832	12.03
T (Before) × S (70 ‰)	0.31	0.43	0.71	0.490	11.94
Observations	30				
Marginal R ² / Conditional R ²	0.52 / 0.56				
A. marindia females					
Predictors	Estimates	Std. Error	t value	p	
(Intercept)	2.24	0.18	12.44	<0.001	
T (Before)	0.48	0.25	1.89	0.064	
S (35 ‰)	0.02	0.26	0.08	0.940	
S (70 ‰)	0.82	0.30	2.77	0.008	
T (Before) × S (35 ‰)	0.15	0.37	0.41	0.680	
T (Before) × S (70 ‰)	-1.03	0.42	-2.45	0.018	
Observations	60				
df	54				
R ²	0.21				

Table S10. Summary of the final model for the before-after comparison analysis on Total Evaporative Water Loss (TEWL, mg H₂O g⁻¹ h⁻¹) of *Allocosa senex* males and females and *Allocosa marindia* females exposed to different salinity (0 ‰, 35 ‰ and, 70 ‰) treatments for 15 days. Values are expressed in log-scale. Note that *A. senex* males were exposed to the 35 ‰ and 70 ‰ treatments. S) Salinity treatment, T) Time (before/after), Mb) Body mass (g, standardized).

Total Evaporative Water Loss					
A. senex males					
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>	<i>df</i>
(Intercept)	-1.14	0.09	-12.94	<0.001	9.59
T (Before)	-0.16	0.06	-2.81	0.015	12.61
S (70 ‰)	-0.13	0.13	-0.97	0.354	9.67
Mb	0.23	0.06	3.58	0.004	10.92
T (Before) × S (35 ‰)	0.19	0.07	2.54	0.032	8.99
Observations	22				
Marginal <i>R</i> ² / Conditional <i>R</i> ²	0.54 / 0.93				
A. senex females					
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>	<i>df</i>
(Intercept)	-1.89	0.25	-7.43	<0.001	16.00
T (Before)	-0.06	0.19	-0.29	0.775	12.00
S (35 ‰)	0.20	0.34	0.59	0.559	16.00
S (70 ‰)	0.31	0.33	0.93	0.360	16.00
T (Before) × S (35 ‰)	0.10	0.26	0.40	0.696	12.00
T (Before) × S (70 ‰)	0.16	0.25	0.64	0.540	12.00
Observations	30				
Marginal <i>R</i> ² / Conditional <i>R</i> ²	0.09 / 0.73				
A. marindia females					
Predictors	Estimates	Std. Error	<i>t</i> value	<i>p</i>	<i>df</i>
(Intercept)	-2.56	0.12	-22.21	<0.001	52.85
T (Before)	0.50	0.15	3.35	0.002	27.00
S (35 ‰)	0.40	0.17	2.38	0.021	52.88
S (70 ‰)	0.69	0.19	3.62	0.001	52.88
T (Before) × S (35 ‰)	-0.37	0.22	-1.68	0.104	27.00
T (Before) × S (70 ‰)	-0.70	0.25	-2.83	0.009	27.00
Observations	60				
Marginal <i>R</i> ² / Conditional <i>R</i> ²	0.23 / 0.35				

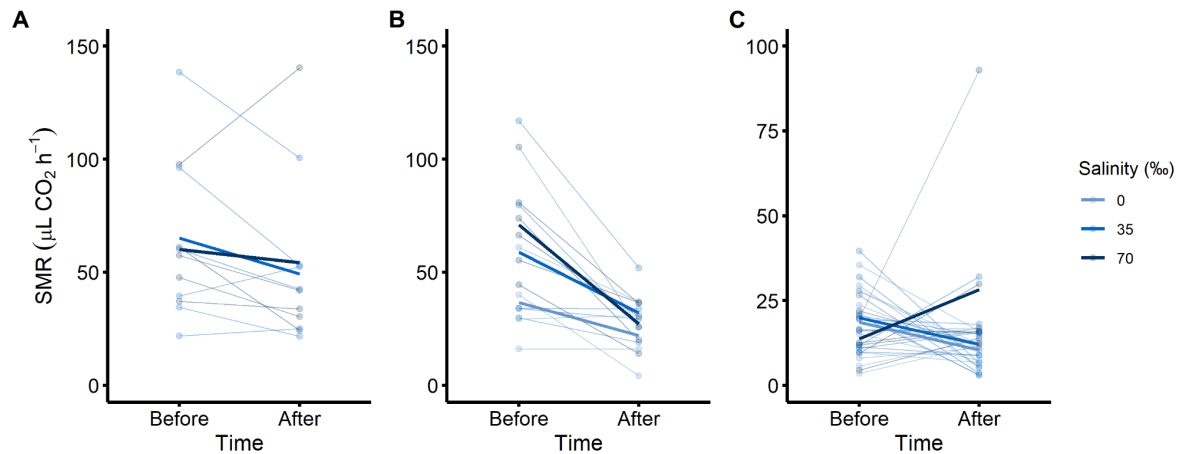


Figure S3. Visual comparison of before- and after-treatment changes in Standard Metabolic Rate (SMR, $\mu\text{L CO}_2 \text{ h}^{-1}$) values of *Allocosa senex* males (A), *A. senex* females (B), and *A. marindia* females (C), by salinity treatment. Each thin line connects the individual's before- and after-treatment SMR values, illustrating within-individual change. The bold lines represent the average change for each experimental group, based on raw data. Notice C has a different scale for clarity purposes.

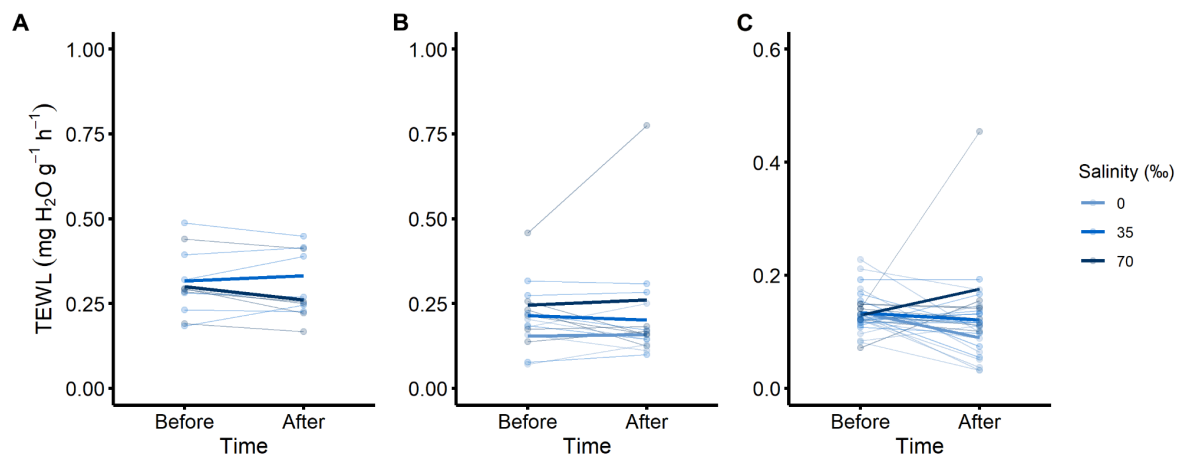


Figure S4. Visual comparison of before- and after-treatment changes in Total Evaporative Water Loss (TEWL, $\text{mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) values of *Allocosa senex* males (A), *A. senex* females (B), and *A. marindia* females (C), by salinity treatment. Each thin line connects the individual before- and after-treatment TEWL values, illustrating within-individual change. The bold line represents the average change for each experimental group, based on raw data. Notice C has a different scale for clarity purposes.

Survival

Table S11. Summary of the best Cox-hazard model for the survival comparison of *Allocosa senex* females and males exposed to different salinity (35 ‰ and 70 ‰) treatments for 30 days. The Estimate value corresponds to the log (Hazard Ratio) value. SX) Sex, S) Salinity treatment.

Predictors	Estimate	Hazard Ratio	Std. Error	z value	p
SX (M)	1.10	3.01	0.40	2.75	0.006
S (70 ‰)	0.92	2.50	0.41	2.21	0.027
Observations	50				
Events	26				
Likelihood ratio test $\chi^2(2) = 13.1$, $p = 0.001$					
R^2 Nagelkerke	0.24				

Table S12. Summary of the best Cox-hazard model for *Allocosa senex* and *A. marindia* females exposed to different salinity treatments (0 ‰, 35 ‰, and 70 ‰) for 30 days. The Estimate value corresponds to the log (Hazard Ratio) value. S) Salinity treatment.

<i>A. senex</i>					
Predictors	Estimate	Hazard Ratio	Std. Error	z value	p
S (35 ‰)	-0.94	0.39	0.84	-1.13	0.26
S (70 ‰)	0.84	2.32	0.56	1.50	0.13
Observations	48				
Events	16				
Likelihood ratio test $\chi^2(2) = 7.05$, $p = 0.03$					
R^2 Nagelkerke	0.15				
<i>A. marindia</i>					
Predictors	Estimate	Hazard Ratio	Std. Error	z value	p
S (35 ‰)	1.27	3.60	0.67	1.91	0.057
S (70 ‰)	2.86	17.40	0.63	4.52	<0.001
Observations	64				
Events	32				
Likelihood ratio test $\chi^2(2) = 34.1$, $p < 0.001$					
R^2 Nagelkerke	0.42				

Table S13. Model selection via Likelihood Ratio Test for Cox-hazard models for *Allocosa senex* females and males and *A. marindia* females exposed to different salinity treatments for 30 days. Females were exposed to 0 ‰, 35 ‰ and 70 ‰ salinity treatments, whereas males were exposed to 35 ‰ and 70 ‰. SX) Sex, S) Salinity treatment, Mb) Body mass, ES) Egg sac.

A. senex (males vs females)				
	loglik	Chisq	Df	Pr(>Chi)
SX × S + Mb	-85.64			
SX + S + Mb	-87.01	2.74	1	0.098
SX + S	-87.13	0.25	1	0.615
A. senex (females)				
	loglik	Chisq	Df	Pr(>Chi)
S + Mb	-35.90			
S	-35.92	0.04	1	0.844
A. marinida (females)				
	loglik	Chisq	Df	Pr(>Chi)
S + ES + Mb	-105.14			
S + ES	-105.16	0.03	1	0.866
S	-106.55	2.79	1	0.095

Distribution pattern

Table S14. Model selection via AICc for *Allocosa senex* presence probability. Only models with $\Delta\text{AICc} < 2$ are shown. V) Vegetation cover (%), C) Mean conductivity ($\mu\text{S}/\text{cm}$), P) Prey abundance.

Adults					
	Df	logLik	AICc	delta	weight
V	2	-46.52	97.2	0.00	0.11
V + V ²	3	-45.83	97.9	0.74	0.08
V + C	3	-45.98	98.2	1.05	0.07
V + P	3	-46.14	98.5	1.37	0.06
V + V ² + V ³	4	-45.28	99.0	1.80	0.05
V + P + P ²	4	-45.34	99.1	1.92	0.04
Juveniles					
	Df	logLik	AICc	delta	weight
C	2	-35.64	75.4	0.00	0.13
-	1	-36.95	75.9	0.52	0.10
C + C ²	3	-35.33	76.9	1.50	0.06
C + V	3	-35.33	76.9	1.50	0.06
C + P	3	-35.37	77.0	1.58	0.06

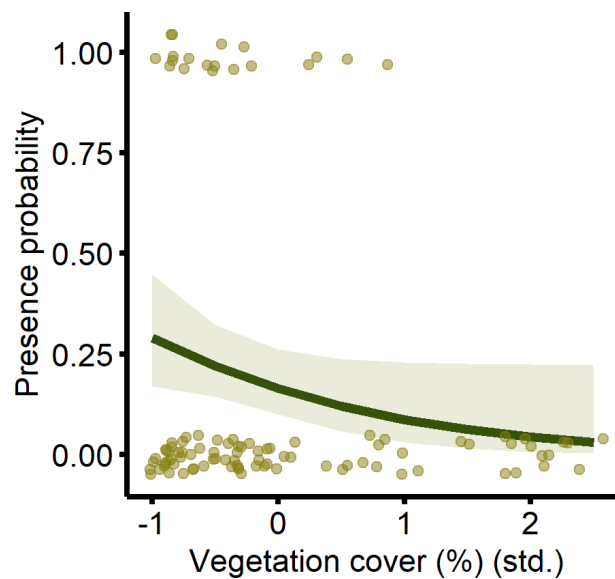


Figure S5. Effect of vegetation cover percentage on the probability of *A. senex* adults presence. *A. senex* adults show a lower presence probability where vegetation cover is higher ($p = 0.055$). Mean and 95 % CI are shown. Vegetation cover percentage is standardized (mean = 0, SD = 1).

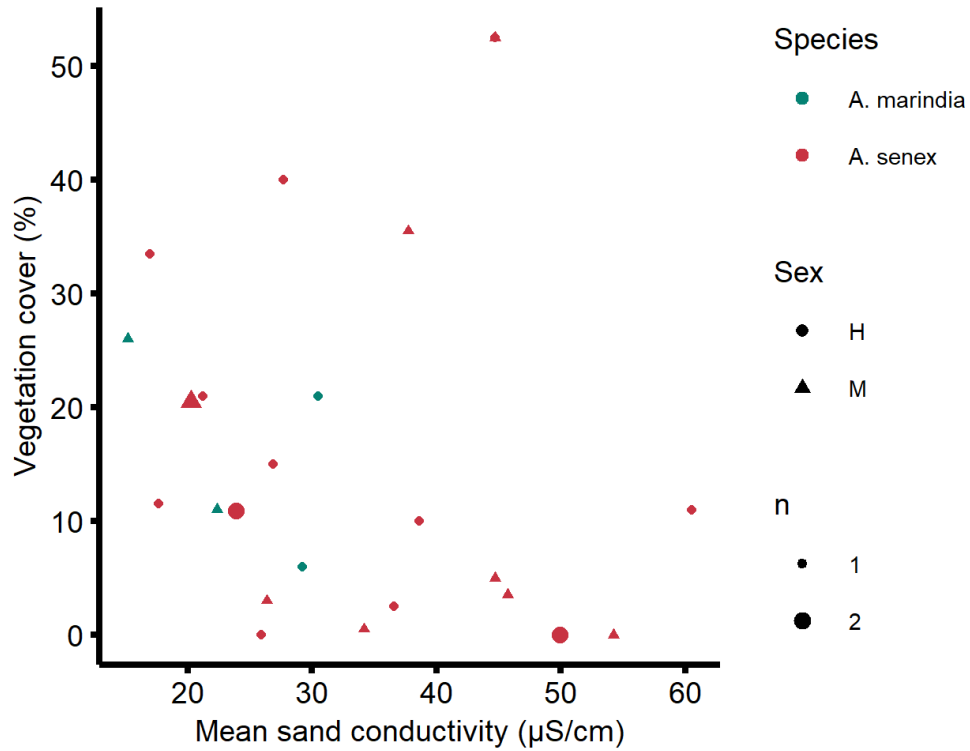


Figure S6. Presence of adult *Allocosa marindia* and *A. senex* regarding the percentage of vegetation cover and the mean sand electric conductivity.

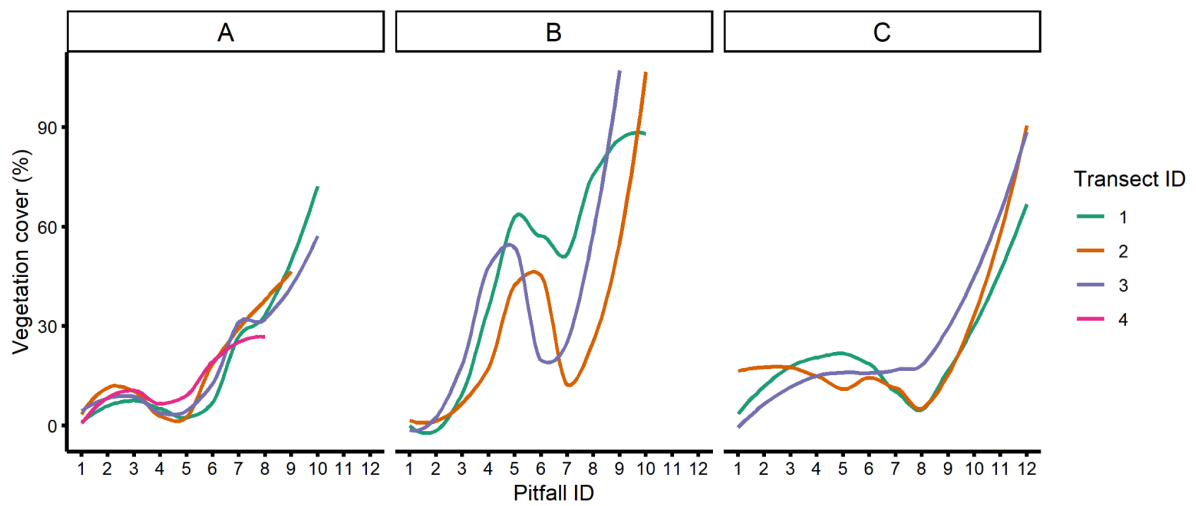


Figure S7. Variability in mean vegetation cover (%) between zones (A to C) and transects (1 to 4) within an outer-estuarine beach, Las Grutas, Maldonado, Uruguay. Each transect comprised at least 8 pitfall points, where vegetation cover was estimated.

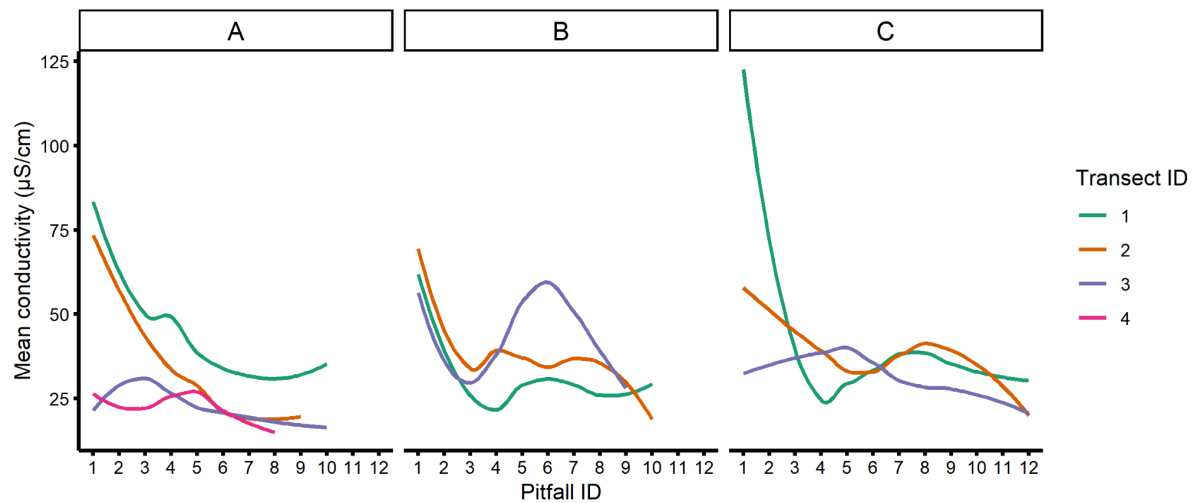


Figure S8. Variability in mean electric conductivity ($\mu\text{S}/\text{cm}$) between zones (A to C) and transects (1 to 4) within an outer-estuarine beach, Las Grutas, Maldonado, Uruguay. Each transect comprised at least 8 pitfall points, where sand samples were taken to measure sand conductivity in the laboratory.

Conclusiones generales y perspectivas

En esta tesis se profundizó en los mecanismos fisiológicos que median la respuesta a variables ambientales como salinidad y temperatura, así como en los procesos de selección de hábitat en artrópodos terrestres de ecosistemas costeros. Las especies de arañas lobo del género *Allocosa* constituyeron un interesante modelo de estudio. Por un lado, los resultados obtenidos al analizar el efecto de la exposición simultánea a alta salinidad y temperatura en hembras de *A. senex* de distintas localidades, destacan la complejidad de las respuestas fisiológicas cuando los organismos enfrentan múltiples estresores. Predecir la magnitud y la dirección de la interacción entre factores es difícil, ya que su efecto varía a nivel intraespecífico y también depende del rasgo fisiológico considerado. Por otro lado, al evaluar la capacidad osmorreguladora entre sexos de *A. senex* provenientes de una localidad con influencia salina, encontramos diferencias en su fisiología y sobrevivencia, probablemente asociadas a sus rasgos de historia de vida. Finalmente, mostramos que en *A. senex* y *A. marindia*, dos especies costeras con rasgos de historia de vida similares, rasgos como el tamaño corporal pueden influir en su tolerancia a la salinidad y afectar diferencialmente su selección de hábitat.

Un aporte significativo de esta investigación al conocimiento general es el análisis de la interacción de dos procesos fisiológicos clave como lo son la osmorregulación -agua y solutos- y la termorregulación -temperatura- en organismos terrestres. Aunque existen numerosos estudios que abordan estos factores de forma separada (ej., González-Tokman et al., 2020; Renault et al., 2014, 2016; Segura-Hernández et al., 2024), son escasos aquellos que evalúan su interacción y el modo en que esta afecta el desempeño de los organismos (Lahondère, 2023; Rozen-Rechels et al., 2019). Asimismo, estos estudios han sido realizados en organismos acuáticos (ej., artrópodos crustáceos: Kondo et al., 2021; Torres et al., 2020, 2021; peces: Vargas-Chacoff et al., 2018, 2020). Esta tesis a través de un enfoque mecanicista, aporta evidencia sobre las potencialidades y limitaciones de la

respuesta fisiológica en artrópodos terrestres de ambientes costeros (Lorrain-Soligon et al., 2024).

Debido a que los ecosistemas costeros, como las playas arenosas, son altamente dinámicos (McLachlan & Defeo, 2017), la alta plasticidad fisiológica y las adaptaciones constituyen rasgos clave para la subsistencia de las especies que allí habitan (ej. Scapini et al., 2019). En este sentido, en esta tesis se encontraron diferencias entre sexos en una dirección no esperada según la teoría y evidencia previa (Huey, 1991; Pétilion et al., 2011; Pulz, 1987), destacando la importancia de contemplar los rasgos de historia de vida al evaluar la respuesta fisiológica de los organismos. Asimismo, este trabajo se inserta en dos líneas de investigación consolidadas en Uruguay, sobre las playas arenosas (Celentano et al., 2022; Lercari & Defeo, 2015; Orlando et al., 2020; Scapini et al., 2019) y las arañas del género *Allocosa*. A pesar de que estas arañas son un modelo relevante para estudios de comportamiento y ecología en nuestro país desde hace varias décadas (ej. Aisenberg & Costa, 2008; Albín et al., 2022; Bollatti et al., 2023; Cavassa et al., 2022), aún persisten interrogantes clave sobre sus estrategias fisiológicas. Los datos aquí presentados aportan hacia una comprensión más integrada de los patrones observados en estos ambientes.

Dado que parte de los objetivos de esta tesis buscaron describir el potencial fisiológico de estas especies, la presente investigación se desarrolló principalmente en condiciones de laboratorio. Sin embargo, este enfoque no está exento de limitaciones. Aunque permitió un control riguroso de las variables y la reducción de factores de confusión, también implicó la exposición de los individuos a escenarios experimentales que no necesariamente reflejan las condiciones ambientales naturales. Esto último limita la posibilidad de extrapolar directamente los resultados al contexto natural, por lo que futuros estudios podrían realizar mediciones de respirometría en individuos de distintas localidades directamente colectados del campo. Esto permitiría evaluar si existen efectivamente diferencias entre sexos/especies en su metabolismo y pérdida de agua.

A pesar de las limitaciones identificadas, el aporte de esta tesis al conocimiento en ecofisiología es relevante y abre la puerta a diversas líneas de investigación futuras. Por ejemplo, utilizando el presente diseño experimental se podría evaluar el efecto del consumo de presas salinas sobre la osmorregulación. Asimismo, considerando la variabilidad intraespecífica detectada y la aparente ausencia de adaptación local o plasticidad del desarrollo, trabajos futuros podrían buscar criar estas especies en laboratorio bajo distintas condiciones salinas y de temperatura. Este enfoque además permitiría evaluar la influencia de los efectos maternos sobre la fisiología de los individuos, un aspecto relevante en la osmorregulación de crustáceos acuáticos (Torres et al., 2020). Por último, las diferencias entre sexos observadas en *A. senex* sugieren la necesidad de ampliar este análisis a *A. marindia* y de incluir el estudio de machos sometidos a la exposición simultánea de factores ambientales, lo que podría revelar dinámicas aún no exploradas.

En el contexto actual de cambio climático, resulta crucial comprender cómo la exposición simultánea a múltiples variables ambientales afecta la fisiología y supervivencia de las especies, para realizar predicciones pertinentes. La variabilidad fisiológica intra- e interespecífica observada en este trabajo resalta la necesidad de ampliar estos estudios incorporando otros factores ambientales relevantes (ej. humedad, disponibilidad de oxígeno), así como un componente clave del cambio climático: la mayor variabilidad y frecuencia de eventos extremos. Además, analizar aspectos como el éxito reproductivo y el comportamiento permitirá evaluar de forma más integral las respuestas de estas y otras especies terrestres. Finalmente, el hecho de que *A. senex*, una especie de araña lobo neotropical, presenta tolerancia y supervivencia comparables a las del género *Pardosa* de marismas europeas (Pétillon et al., 2011), sugiere la relevancia de desarrollar investigaciones con un enfoque filogenético sobre la evolución de la tolerancia a la salinidad en Lycosidae.

En conclusión, el presente trabajo resalta la importancia de evaluar los mecanismos fisiológicos que subyacen la presencia de los organismos con hábitos terrestres en ambientes con alto grado de dinamismo, como los ecosistemas costeros. Asimismo, contribuye al creciente conocimiento sobre la interacción de procesos fisiológicos frente a cambios ambientales.

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Anexos

Aquí se presentan dos anexos que incluyen resultados complementarios obtenidos de esta tesis. Si bien dichos resultados no son partes fundamentales de las preguntas de investigación de cada capítulo, son relevantes para su presentación por lo novedoso de los mismos o por su complementariedad para entender los resultados prioritarios de la tesis. El Anexo 1 incluye el análisis de la variación en tamaño corporal de *Allocosa senex* entre temporadas reproductivas y localidades, así como una comparación del Índice de Dimorfismo Sexual (SDI) de *A. senex* y *A. marindia* de diferentes localidades. El Anexo 2 se centra en describir los tres patrones de respiración encontrados durante las mediciones de respirometría de estas dos especies, los cuales no han sido aún descritos para Araneomorphae. Se prevé publicar el contenido de ambos anexos como comunicaciones cortas en revistas arbitradas.

Anexo 1: Variabilidad temporal en medidas morfométricas y masa corporal

Para evaluar si existe variación en el tamaño corporal en función de la estación reproductiva, se ajustaron modelos lineales (LM) con distribución normal o lognormal. En total se realizaron 4 comparaciones: (1) entre hembras de *Allocosa senex* provenientes de dos localidades (playa ribereña, $n = 178$, y playa oceánica, $n = 237$) y en dos temporadas reproductivas (2021 y 2022); (2) entre machos de *A. senex* provenientes de dos localidades (playa ribereña y playa oceánica) en la temporada reproductiva de 2022; (3) entre machos de *A. senex* de la playa oceánica entre dos temporadas reproductivas (2022 y 2023) y (4) entre hembras y machos de *A. senex* de dos localidades en la temporada reproductiva de 2022. Los datos morfológicos de hembras de 2022 y de machos de 2023 corresponden a esta tesis, mientras que los de hembras de 2021 y machos de 2022 son parte de la base de datos del grupo de investigación.

En cada caso se ajustó un modelo para la masa corporal, uno para el ancho de prosoma (indicador de tamaño corporal; Moya-Laraño et al., 2003) y uno para el Índice de

Condición Corporal (BCI por sus siglas en inglés, *Body Condition Index*). El BCI se calculó mediante el método de residuales de Jakob et al., (1996). Previo al cálculo de los residuales de BCI se evaluó si existen diferencias en la relación $\log(\text{masa}) \sim \log(\text{prosoma})$ para cada comparación (explicado en detalle en la metodología del Capítulo 1). Los modelos lineales incluyeron como variables explicativas la playa de origen, el sexo y/o el período reproductivo (es decir, el año de colecta), según correspondiera. Además, todos los modelos incluyeron el día de colecta (*proxy* de avance del período reproductivo) centrado y estandarizado como variable de control. El día de colecta se calculó considerando el 1° de octubre como día 0, es decir, como el día de inicio de la temporada reproductiva. En los casos en los que había más de un día de colecta para todos los grupos analizados, se evaluó la interacción entre día de colecta y la/s otra/s variables de respuesta. De lo contrario, se evaluó solo el efecto simple. Se realizó selección de modelos por Likelihood Ratio Test, removiendo solamente interacciones o la variable día de colecta cuando su efecto no era significativo. Cuando fue necesario, se calcularon las diferencias pareadas entre grupos utilizando el paquete *predictmeans* o *emmeans* de R según correspondiera. En todos los casos se realizó un análisis de residuales para asegurar el correcto ajuste del modelo final. Todos los análisis se realizaron en R Studio (R Core Team, 2023).

Por otra parte, se calculó el Índice de Dimorfismo Sexual (SDI por sus siglas en inglés, *Sexual Dimorphism Index*). Este es un índice cualitativo, que permite evaluar la magnitud de la diferencia de tamaño entre hembras y machos de una especie. Se calculó el SDI para *A. senex* de la playa oceánica y ribereña en el año 2022 y para *A. marindia* de la playa oceánica para el año 2023. Se tomaron estos años dado que fueron aquellos en los que había datos tanto de hembras como de machos. Siguiendo la metodología de Aisenberg et al., (2023), el SDI se calculó como el dimorfismo sexual de tamaño (ancho de prosoma de los machos / ancho de prosoma de las hembras) menos uno. Asimismo, se siguió el siguiente criterio comparativo: 0, no existe dimorfismo de tamaño entre sexos, 0–0,5, ligero dimorfismo con sesgo masculino; 0,5–1, fuerte dimorfismo con sesgo masculino; -0,5–0,

ligero dimorfismo con sesgo femenino; y -1– -0,5, fuerte dimorfismo con sesgo femenino. Se utilizaron los datos abiertos publicados por Aisenberg et al., (2023) para calcular el SDI de ambas especies en localidades no evaluadas en esta tesis: *A. marindia* en Playa de Marindia, Canelones (estuario interno, baja influencia salina) y *A. senex* en Arroyo Santana, Paysandú (fluvial, sin influencia salina).

Resultados del análisis

- (1) Las hembras de la playa ribereña tuvieron mayor masa corporal que las de la playa oceánica, sin importar la temporada reproductiva. Sin embargo, las hembras de ambas localidades exhibieron menor masa corporal en la temporada reproductiva de 2022 respecto a la de 2021 (Tabla A1). Respecto al tamaño corporal, en 2021 las hembras de la playa ribereña tuvieron mayor ancho de prosoma que las de la playa oceánica, pero no hubo diferencias entre localidades en 2022. Asimismo, las hembras de ambas playas tuvieron menor tamaño corporal en 2022 respecto a 2021 (Tabla A1, Figura A1). Finalmente, el BCI no difirió ni entre localidades ni entre temporadas reproductivas (Tabla A1); el R^2 del modelo explicó 0 % de la variabilidad de los datos. Sin embargo, es de destacar que la relación entre la masa y el tamaño corporal mostró diferencias significativas entre localidades en el período 2022 (Post-hoc: $t = 11,25$, $p < 0,001$) y entre períodos reproductivos en la playa ribereña (Post-hoc: $t = 7,67$, $p < 0,001$) (Tabla A1, Figura A2). Se muestra la selección de modelos en la Tabla A2.

Tabla A1. Resumen del mejor modelo para la masa corporal, ancho de prosoma, Índice de Condición Corporal (BCI) y relación logarítmica masa corporal-tamaño corporal, de hembras de *Allocosa senex* de dos playas con exposición a salinidad naturalmente contrastante (Oceánica y Ribereña) y dos temporadas reproductivas (2021 y 2022). B) Playa, RS) Estación Reproductiva, CD) Fecha de colecta (estandarizada). Los valores del modelo de BCI están en escala logarítmica.

Masa corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	0,36	0,01	37,77	<0,001
B (Ribereña)	0,13	0,01	12,36	<0,001
RS (2022)	-0,08	0,02	-4,82	<0,001
CD	-0,04	0,01	-3,66	<0,001
B (Ribereña) × CD	0,03	0,01	2,36	0,019
Observaciones	412			
R ²	0,31			
Ancho de prosoma				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	5,29	0,06	91,66	<0,001
B (Ribereña)	0,77	0,08	9,90	<0,001
RS (2022)	-0,39	0,11	-3,72	<0,001
CD	-0,16	0,04	-3,78	<0,001
B (Ribereña) × RS (2022)	-0,63	0,12	-5,08	<0,001
Observaciones	415			
R ²	0,29			
Índice de Condición Corporal (BCI)				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-0,00	0,02	-0,00	1
B (Ribereña)	-0,00	0,02	-0,00	1
RS (2022)	-0,00	0,02	-0,00	1
Observaciones	412			
R ²	0			
Relación masa corporal-tamaño corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-4,77	0,26	-18,46	<0,001
log (Pro.)	2,22	0,16	14,16	<0,001
B (Ribereña)	0,48	0,47	1,02	0,309
RS (2022)	-0,78	0,41	-1,91	0,057
log (Pro.) × B (Ribereña)	-0,24	0,27	-0,88	0,379
log (Pro.) × RS (2022)	0,45	0,25	1,79	0,074
B (Ribereña) × RS (2022)	4,09	0,60	6,83	<0,001
log (Pro.) × B (Ribereña) × RS (2022)	-2,37	0,36	-6,68	<0,001
Observaciones	412			
R ²	0,67			

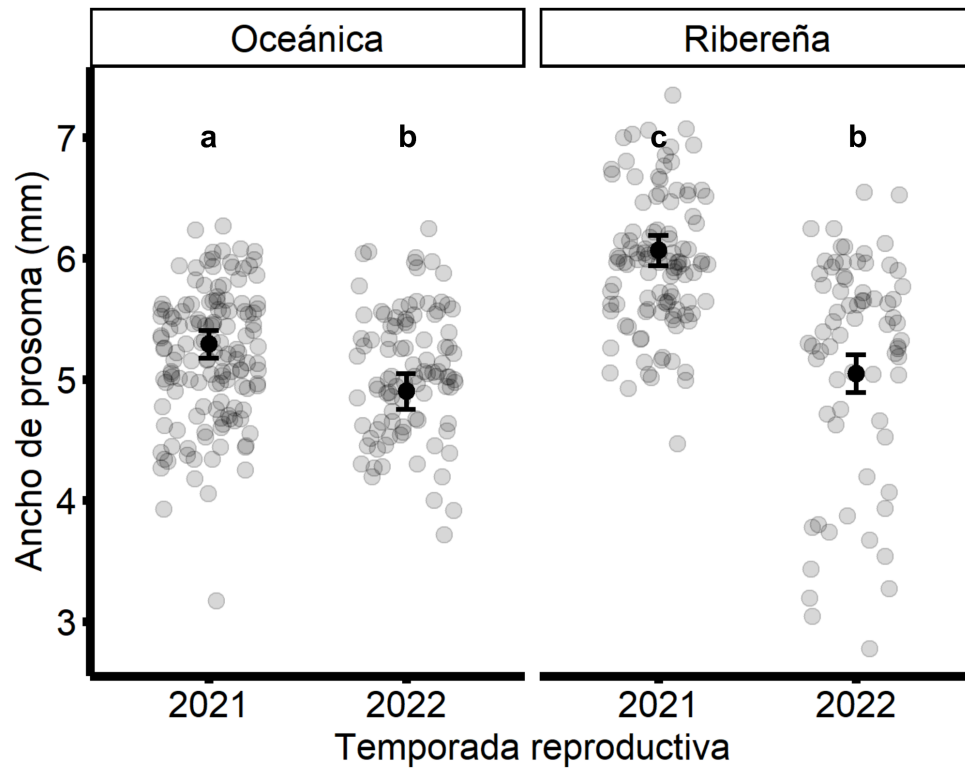


Figura A1. Ancho de prosoma (mm) de hembras adultas de *Allocosa senex* de dos playas con salinidad naturalmente contrastante (Oceánica y Ribereña), en dos temporadas reproductivas. Diferentes letras indican diferencias estadísticamente significativas entre los grupos. Se muestra la media y el IC 95 %.

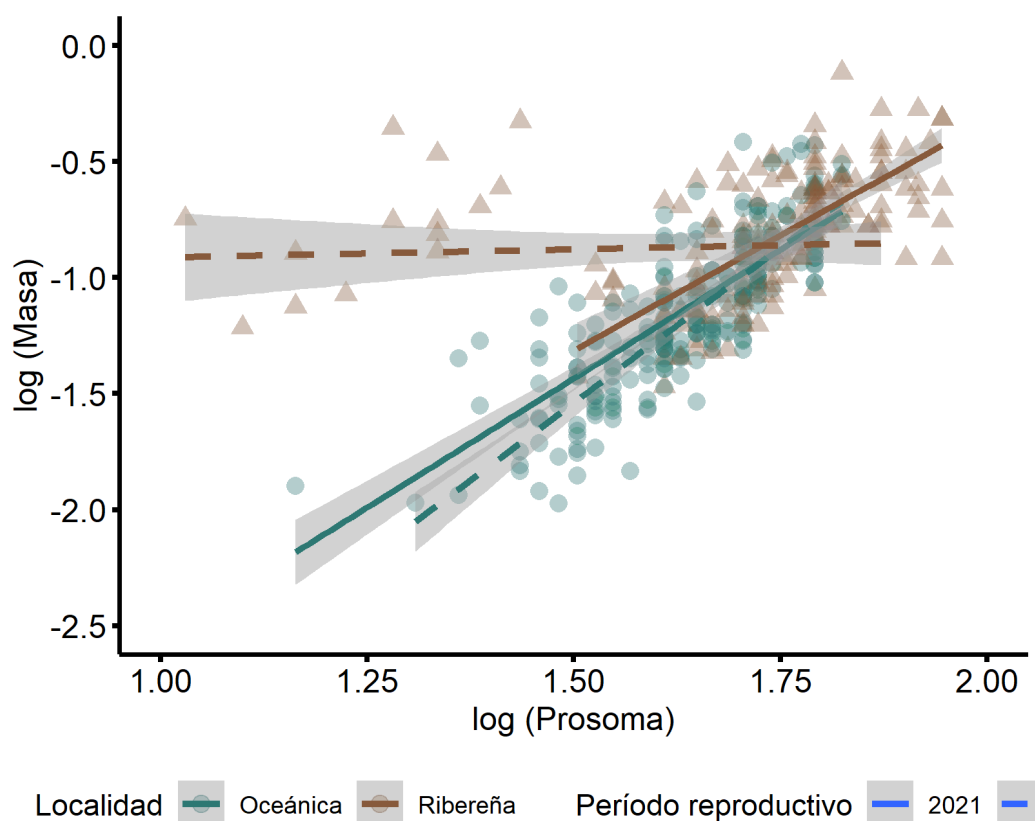


Figura A2. Relación entre el logaritmo de la masa (g) y el ancho de prosoma (mm) entre hembras de *Allocosa senex* provenientes de dos localidades diferentes (playa oceánica y playa ribereña) en dos temporadas reproductivas (2021 y 2022). Con círculos y líneas turquesa se representan los valores de la playa oceánica y con triángulos y líneas marrones los valores de la playa ribereña. Los periodos reproductivos se distinguen según el tipo de línea.

Tabla A2. Selección de modelos vía Likelihood Ratio Test para medidas morfológicas de hembras adultas de *Allocosa senex* provenientes de dos localidades con salinidad contrastante y en dos temporadas reproductivas. B) Playa, RS) Temporada reproductiva, CD) Día de colecta.

Masa corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B × RS + B × CD	405	4,94			
RS + B × CD	406	4,96	-1	-0,0199	0,2
Ancho de prosoma	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B × RS + B × CD	408	150			
CD + B × RS	409	151	-1	-1,28	0,062
Índice de Condición Corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B × RS + CD	407	16,40			
B + RS + CD	408	16,40	-1	-0,00015	0,95
B + RS	409	16,40	-1	-0,01433	0,55

(2) Los machos de *A. senex* de la playa ribereña tuvieron mayor masa corporal y ancho de prosoma que los de la playa oceánica en el período reproductivo de 2022 (Tabla A3). Sin embargo, su BCI no difirió entre localidades ($R^2 = 0 \%$, Tabla A3), pero se detectaron diferencias significativas en la relación masa-tamaño corporal de cada localidad (Tabla A3, Figura A3). La tabla de selección de modelos se puede consultar en la Tabla A4.

Tabla A3. Resumen del mejor modelo para la masa corporal, ancho de prosoma, Índice de Condición Corporal (BCI) y y relación logarítmica masa corporal-tamaño corporal, de machos adultos de *Allocosa senex* de dos playas con exposición a salinidad naturalmente contrastante (Oceánica y Ribereña). B) Playa, CD) Fecha de colecta (estandarizada).

Masa corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	0,45	0,05	9,86	<0,001
B (Ribereña)	0,25	0,05	4,57	<0,001
Observaciones	53			
R^2	0,29			
Ancho de prosoma				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	5,28	0,16	33,31	<0,001
B (Ribereña)	1,45	0,19	7,73	<0,001
CD	-0,26	0,08	-3,11	0,003
Observaciones	53			
R^2	0,55			
Índice de Condición Corporal (BCI)				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-0,00	0,04	-0,00	1
B (Ribereña)	-0,00	0,05	-0,00	1
Observaciones	53			
R^2	0			
Relación masa corporal-tamaño corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-6,14	0,63	-9,80	<0,001
log (Pro.)	3,13	0,37	8,44	<0,001
B (Ribereña)	2,02	0,83	2,43	0,019
log (Pro.) × B (Ribereña)	-1,17	0,47	-2,48	0,017
Observaciones	53			
R^2	0,81			

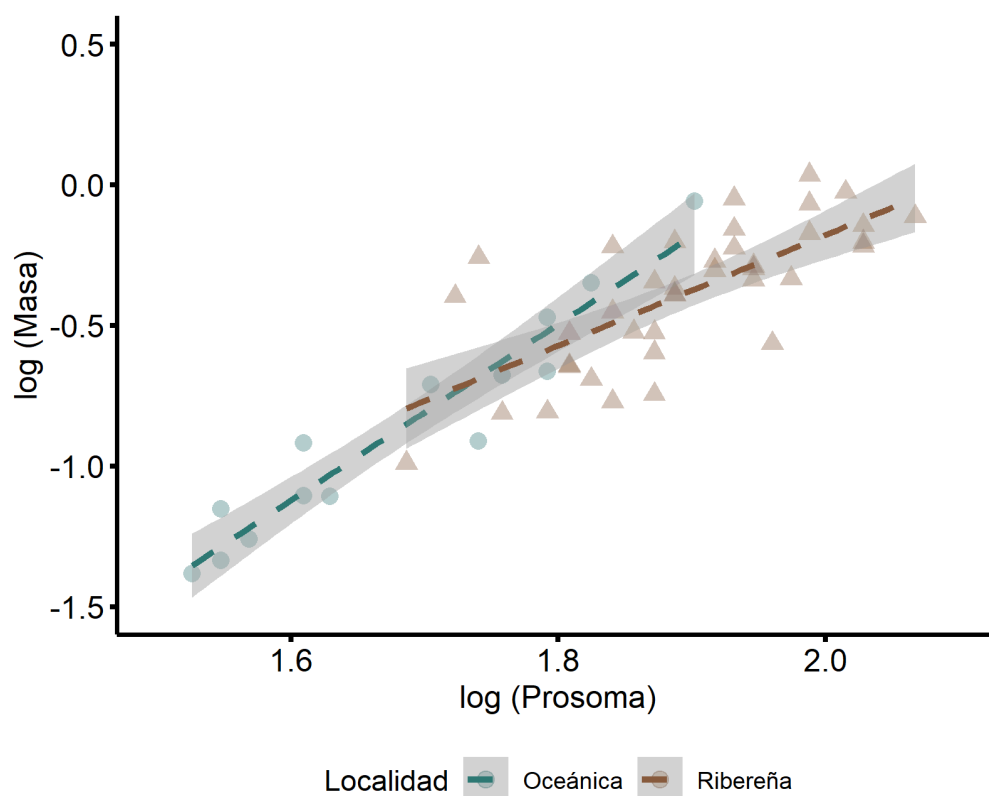


Figura A3. Relación entre el logaritmo de la masa (g) y el ancho de prosoma (mm) de machos de *Allocosa senex* provenientes de dos localidades diferentes, playa oceánica y playa ribereña. Con círculos y líneas turquesas se representan los valores de la playa oceánica y con triángulos y líneas anaranjadas los valores de la playa ribereña.

Tabla A4. Selección de modelos vía Likelihood Ratio Test para la masa corporal y el Índice de Condición Corporal de machos adultos de *Allocosa senex* provenientes de dos localidades con salinidad contrastante. B) Playa, CD) Día de colecta.

Masa corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B + CD	50	1,42			
B	51	1,52	-1	-0,095	0,068
Índice de Condición Corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B + CD	50	1,28			
B	51	1,28	-1	-0,0029	0,735

(3) Los machos de la playa oceánica tuvieron mayor masa corporal y ancho de prosoma (Figura A4) en la temporada reproductiva de 2023 respecto a la de 2022 (Tabla A5). El BCI no varió entre temporadas reproductivas ($R^2 = 3,0 \%$, Tabla A5) ni se encontraron diferencias en la relación masa-tamaño corporal entre temporadas reproductivas. La selección de modelos se puede consultar en la Tabla A6.

Tabla A5. Resumen del mejor modelo para la masa corporal, ancho de prosoma, Índice de Condición Corporal (BCI) y relación logarítmica masa corporal-tamaño corporal, de machos de *Allocosa senex* de una playa estuarina externa con influencia oceánica (Oceánica) en dos temporadas reproductivas (2022 y 2023). RS) Estación Reproductiva, CD) Fecha de colecta (estandarizada). Los valores del modelo para masa corporal están en escala logarítmica.

Masa corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-0,86	0,09	-10,10	<0,001
RS (2023)	0,26	0,11	2,35	0,024
Observaciones	37			
R^2	0,14			
Ancho de prosoma				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	5,41	0,16	34,63	<0,001
RS (2023)	0,58	0,20	2,91	0,006
Observaciones	37			
R^2	0,20			
Índice de Condición Corporal (BCI)				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	0,03	0,03	0,86	0,398
RS (2023)	-0,04	0,04	-1,09	0,285
Observaciones	37			
R^2	0,03			
Relación masa corporal-tamaño corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-6,14	0,44	-13,81	<0,001
log (Pro.)	3,13	0,26	11,90	<0,001
RS (2023)	0,60	0,67	0,91	0,371
log (Pro.) $\times$ RS (2023)	-0,38	0,38	-0,99	0,330
Observaciones	37			
R^2	0,90			

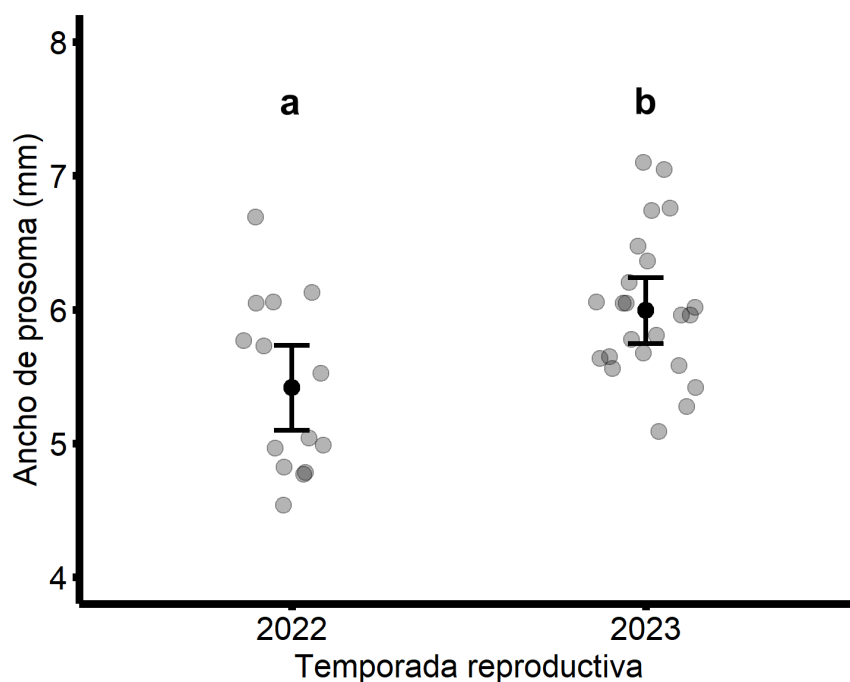


Figura A4. Ancho de prosoma (mm) de machos adultos de *Allocosa senex* de una playa estuarina externa con influencia salina en dos temporadas reproductivas. Diferentes letras indican diferencias estadísticamente significativas. Se muestra la media y el IC 95 %.

Tabla A6. Selección de modelos vía Likelihood Ratio Test para medidas morfológicas de machos adultos de *Allocosa senex* provenientes de una playa estuarina externa en dos temporadas reproductivas. RS) Temporada reproductiva, CD) Día de colecta.

Masa corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
RS + CD	34	3,58			
RS	35	3,59	-1	-0,007	0,8
Ancho de prosoma	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
RS + CD	34	11,8			
RS	35	12,0	-1	-0,217	0,43
Índice de Condición Corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
RS + CD	34	0,434			
RS	35	0,450	-1	-0,0166	0,25

(4) Los machos presentaron mayor masa corporal que las hembras y a su vez los individuos de la playa ribereña exhibieron mayor masa que los de la playa oceánica (Tabla A7). Este patrón se cumplió también para el ancho de prosoma, pero además se encontró una interacción entre sexo y localidad, siendo los machos de la playa ribereña significativamente de mayor tamaño que el resto de los grupos (Tabla A7, Figura A5). No se encontraron diferencias en el BCI ni entre localidades ni entre sexos ($R^2 = 0 \%$, Tabla A7). Sin embargo, la relación masa-tamaño corporal difirió entre hembras de ambas localidades ($t = -10,72$, $p < 0,001$) y sexos de la playa ribereña (Post-hoc: $t = -4,76$, $p < 0,001$) (Figura A6). La selección de modelos puede consultarse en la Tabla A8.

Tabla A7. Resumen del mejor modelo para la masa corporal, ancho de prosoma, Índice de Condición Corporal (BCI) y relación logarítmica masa-tamaño corporal, de machos y hembras adultos de *Allocosa senex* provenientes de dos playas naturalmente expuestas a salinidad contrastante (Océánica y Ribereña) en una temporada reproductiva (2022). B) Playa, S) Sexo (M = machos), CD) Fecha de colecta (estandarizada). Los valores del modelo para masa corporal y BCI están en escala logarítmica.

Masa corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-1,24	0,03	-41,85	<0,001
B (Ribereña)	0,38	0,04	8,99	<0,001
S (M)	0,45	0,05	8,91	<0,001
Observaciones	220			
R ²	0,50			
Ancho de prosoma				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	4,82	0,09	53,42	<0,001
B (Ribereña)	0,24	0,11	2,22	0,027
S (M)	1,06	0,26	4,12	<0,001
CD	-0,39	0,10	-4,06	<0,001
B (Ribereña) × S (M)	1,21	0,23	5,20	<0,001
Observaciones	221			
R ²	0,47			
Índice de Condición Corporal (BCI)				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	0,00	0,02	0,00	1
B (Ribereña)	-0,00	0,03	-0,00	1
S (M)	0,00	0,03	0,00	1
Observaciones	220			
R ²	0			
Relación masa corporal-tamaño corporal				
Predictores	Estimados	Error Std.	t valor	p
(Intercepto)	-5,54	0,33	-16,60	<0,001
log (Pro.)	2,67	0,21	12,94	<0,001
B (Ribereña)	4,56	0,39	11,57	<0,001
S (M)	-0,59	0,89	-0,67	0,504
log (Pro.) × B (Ribereña)	-2,60	0,24	-10,72	<0,001
log (Pro.) × S (M)	0,46	0,53	0,88	0,381
B (Ribereña) × S (M)	-2,54	1,16	-2,19	0,030
log (Pro.) × B (Ribereña) × S (M)	1,43	0,66	2,17	0,031
Observaciones	220			
R ²	0,77			

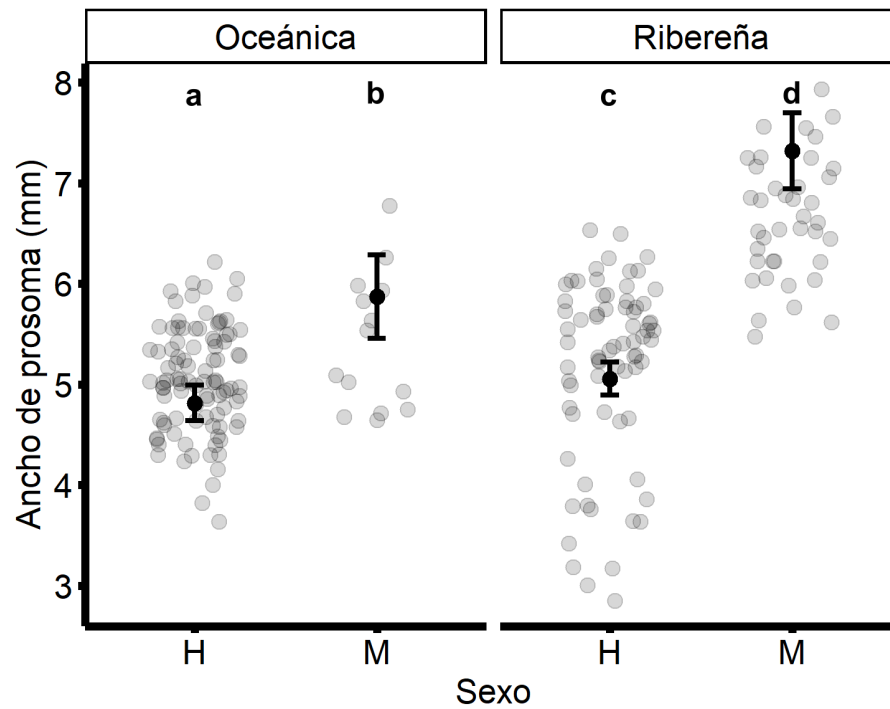


Figura A5. Ancho de prosoma (mm) de machos y hembras adultas de *Allocosa senex* de dos playas con salinidad contrastante (Oceánica y Ribereña). Diferentes letras indican diferencias estadísticamente significativas. Se muestra la media y el IC 95 %.

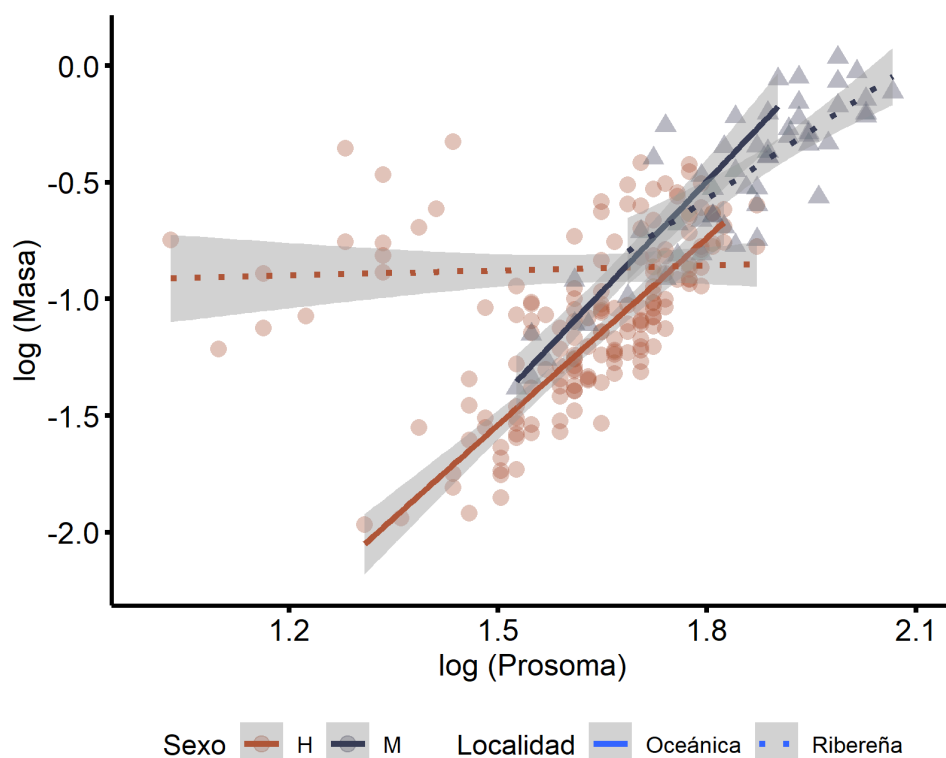


Figura A6. Relación entre el logaritmo de la masa (g) y el ancho de prosoma (mm) entre hembras y machos de *Allocosa senex* provenientes de dos localidades diferentes, playa oceánica y playa ribereña. Con círculos y líneas anaranjadas se representan los valores de las hembras y con triángulos y líneas violetas los valores de los machos. Las localidades se distinguen según el tipo de línea.

Tabla A8. Selección de modelos vía Likelihood Ratio Test para medidas morfológicas de machos y hembras adultas de *Allocosa senex* provenientes de dos playas con salinidad contrastante. B) Playa, S) Sexo, CD) Día de colecta.

Masa corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B × S + CD	215	19,8			
B + S + CD	216	19,9	-1	-0,142	0,21
B + S	217	20,2	-1	-0,276	0,08
Índice de Condición Corporal	Res.Df	RSS	Df	Sum of Sq	Pr(>Chi)
B × S + CD	215	9,35			
B + S + CD	216	9,35	-1	-0,0005	0,91
B + S	217	9,48	-1	-0,127	0,09

Índice de Dimorfismo Sexual (SDI)

El cálculo de SDI reveló que tanto *A. senex* como *A. marindia* presentan un ligero dimorfismo sexual de tamaño sesgado hacia los machos (Tabla A9, Figura A7). Asimismo, en la localidad oceánica, ambas especies exhibieron menor dimorfismo sexual de tamaño respecto a las localidades no salinas. Esta diferencia fue mayor en *A. senex*, ya que el SDI de la playa ribereña fue casi 2,5 veces mayor que en la playa oceánica. Asimismo, la localidad fluvial tuvo el mayor SDI, demostrando un claro gradiente en la diferencia sexual de tamaños en función del hábitat (Tabla A9, Figura A7).

Tabla A9. Índice de Dimorfismo Sexual (SDI) de *Allocosa senex* y *A. marindia* en función de su localidad de origen y nivel de salinidad. Para los datos de Aisenberg et al., (2023), no se tiene conocimiento de si corresponden a un mismo año o incluyen datos de años distintos. M = machos; H = hembras.

Especie	Localidad	Hábitat	Salinidad	Año	SDI	n	Referencia
<i>A. marindia</i>	Playa Marindia, Canelones	Estuario interno	Baja	Indet.	0,13	M = 46; H = 84	Aisenberg et al., 2023
	Playa Las Grutas, Maldonado	Estuario externo	Alta	2023	0,09	M = 6; H = 63	Datos propios
<i>A. senex</i>	Arroyo Santana, Paysandú	Fluvial	No salino	Indet.	0,33	M = 2; H = 11	Aisenberg et al., 2023
	Playa Zagarzazú, Colonia	Estuario interno	Baja	2022	0,29	M = 39; H = 71	Datos propios
	Playa Las Grutas, Maldonado	Estuario externo	Alta	2022	0,07	M = 14; H = 96	Datos propios

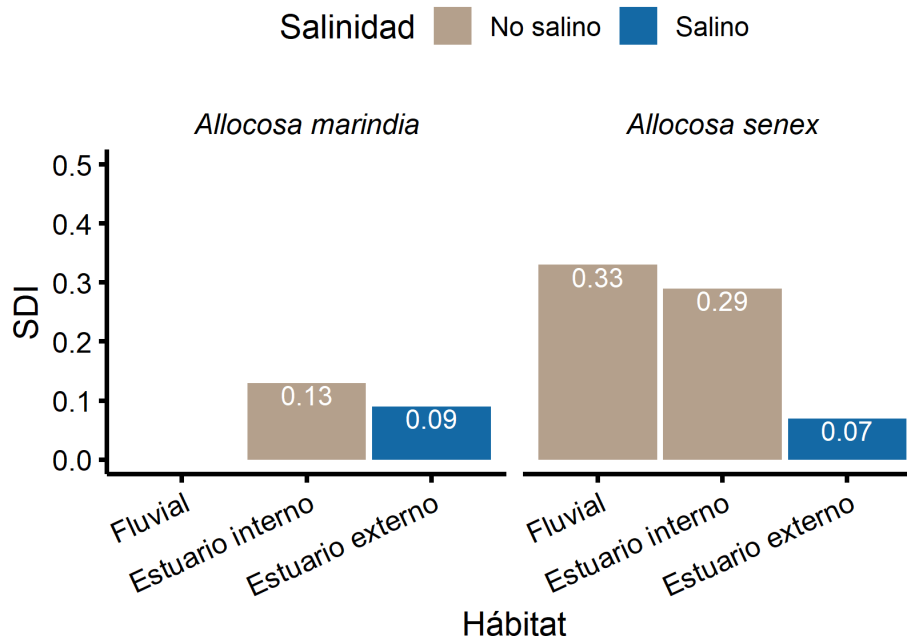


Figura A7. Índice de Dimorfismo Sexual (SDI) de *Allocosa senex* y *A. marindia* en función de su hábitat de origen y el nivel de salinidad del mismo.

Breve discusión

El análisis de los rasgos morfológicos de *A. senex* reveló que tanto la masa corporal como el ancho de prosoma (tamaño corporal) varían entre estaciones reproductivas tanto en machos como en hembras (Figuras A1 y A4). Particularmente, los resultados reflejan que en el año 2022 disminuyó el tamaño corporal en ambos sexos. En Uruguay, los efectos de la sequía que comenzó en 2019 se vieron agravados en 2022 por el impacto de La Niña (Naumann et al., 2023). Esto implicó un aumento de la salinidad en las zonas con influencia oceánica y un desplazamiento de la pluma de salinidad hacia las zonas estuarinas internas del estuario del Río de la Plata (Ministerio de Ambiente, 2023). Aunque no hay datos concretos de un aumento de la salinidad media en la playa ribereña (Playa Zagarzazú) por falta de medición, es probable que se produjeran picos de salinidad durante el año 2022. Por ende, es posible que los individuos que se desarrollaron durante ese año hayan tenido un crecimiento menor debido a un costo osmorregulador inusualmente más alto.

De forma interesante, el análisis de BCI reveló que no existen diferencias en la condición corporal entre años, localidades o sexos, una vez controlada la relación

masa-tamaño corporal. Sin embargo, sí se detectaron diferencias en dicha relación. Los individuos de la playa ribereña mostraron una menor pendiente masa-tamaño respecto a los de la playa oceánica. Esto indica que en la playa oceánica la masa aumenta más rápidamente con el tamaño corporal. Sumado a que hembras y machos de la playa oceánica son más pequeños que los de la playa ribereña, estos resultados indican que los individuos adquieren reservas energéticas con mayor rapidez, pero alcanzan un tamaño corporal menor. Posiblemente ello se deba a mayores costos osmorreguladores y por ende, a diferencias en la asignación energética a diferentes procesos (crecimiento y mantenimiento). Finalmente, la diferencia más notoria se observó en 2022, donde la pendiente de las hembras de la playa ribereña fue prácticamente nula, reflejando que no hubo casi aumento de masa con el tamaño corporal. Por ende, en esa temporada reproductiva, las hembras no difirieron en sus reservas energéticas, grasas e hídricas, en función de su tamaño. Sin embargo, el bajo tamaño corporal alcanzado por algunas hembras de esta localidad es sorprendente y posiblemente se deba a las razones previamente expuestas.

Respecto al SDI, nuestros resultados se alinean con los resultados de Aisenberg et al., (2023), ya que en las localidades de esta tesis (Playa Zagarzazú y Playa Las Grutas) el SDI mostró un leve dimorfismo sexual de tamaño hacia los machos. Asimismo, el SDI y por ende, la diferencia entre el tamaño de machos y hembras disminuyó con el aumento de la salinidad. Esto se cumplió tanto para *A. senex* como para *A. marindia* y en el caso de *A. senex* fue respaldado con poder estadístico en los análisis de ancho de prosoma. Sin embargo, considerando los resultados previamente expuestos, sería interesante realizar una evaluación de cómo varía el SDI en el tiempo, puesto que el ancho de prosoma es una variable altamente dinámica. El hecho de que *A. marindia* siendo la especie pequeña tenga menor dimorfismo sexual puede reflejar una limitación en el aumento de tamaño en esta especie, que implica que a pesar de exhibir dimorfismo sexual, este no sea tan pronunciado, independientemente del hábitat.

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Anexo 2: Patrones de respiración en *Allocosa senex* y *A. marindia*

Dentro de los Artrópodos existen grupos con diversos sistemas respiratorios (Hsia et al., 2013). Entre ellos, los insectos (Clase Insecta) presentan un sistema de intercambio de gases compuesto por un sistema de tráqueas que desembocan en espiráculos, y permite sostener de forma eficiente actividades demandantes de oxígeno, como el vuelo (Chown & Nicolson, 2004). Por otra parte, dentro de las arañas (Clase Arachnida, Orden Araneae), existen dos tipos de sistema respiratorio: las migalomorfas (Suborden Mygalomorphae) poseen un sistema de dos pares de pulmones en libro, que en comparación a los insectos, es menos eficiente en el intercambio de gases, pero suple los requerimientos de especies con hábitos sedentarios y/o fosoriales. Por otra parte, la mayoría de arañas araneomorfas (Suborden Araneomorphae), incluyendo a las especies de la Familia Lycosidae, tienen un sistema mixto que incluye un par de pulmones en libro y un par de túbulos traqueales que desembocan en espiráculos. En comparación con los otros dos grupos, su sistema tiene una eficiencia media, y se refleja en que las especies de este suborden son más activas (Hsia et al., 2013).

El sistema respiratorio de los insectos ha sido ampliamente estudiado, tanto por su eficiencia, como por sus particularidades. Una de ellas es que en reposo, los insectos presentan más de un tipo de respiración. Estas se separan en respiración continua, cíclica y discontinua (DGC por sus siglas en inglés, *Discontinuous Gas Exchange*) (Chown, 2011; White et al., 2007) (Figura A8). Su principal diferencia radica en el patrón de intercambio gaseoso, cuya ritmicidad está modulada por la apertura y cierre de los espiráculos, que regulan la expulsión de CO₂ y la entrada de O₂ al organismo. La respiración cíclica se caracteriza por aumentos y disminuciones regulares en la salida de CO₂, sin periodos en los que los espiráculos están completamente cerrados. En cambio, la DGC incluye fases de cierre completo de los espiráculos, seguidas por una fase de fluctuación de las válvulas espiraculares (*flutter*) y una breve fase abierta con liberación abrupta de CO₂ (Chown, 2011). Además de estos tres patrones, Chown & Nicolson (2004) han descrito el *interbust-burst*, el

cual consiste en ráfagas cortas e irregulares de liberación de gases ("*burst*"), pudiéndose considerar un intermedio entre la respiración cíclica y el DGC (Figura A8).

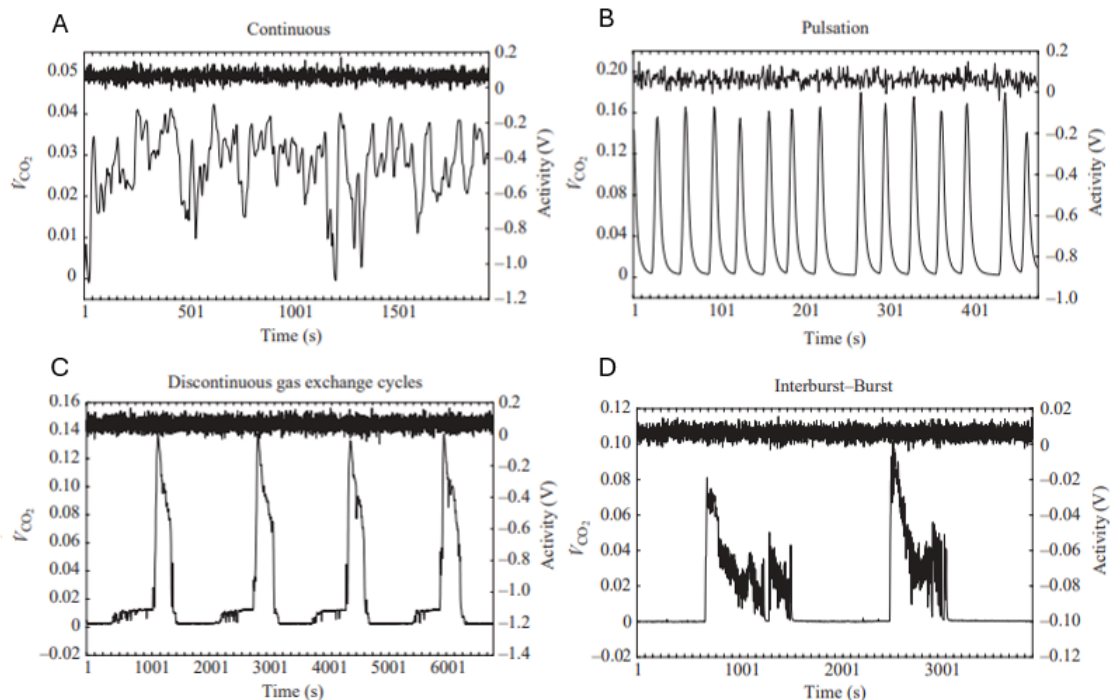


Figura A8. Patrones de respiración en insectos. A) Patrón de respiración continua; B) Patrón de respiración cíclico/de pulso; C) Patrón de Intercambio Discontinuo de Gases (DGC); D) *Interburst-burst*. Modificada de Chown & Nicolson (2004).

Aunque la diversidad de patrones respiratorios ha sido extensamente estudiada en insectos, en arañas el conocimiento sobre este tema es muy limitado. Conforme a nuestro conocimiento, solo el estudio de Mason et al., (2013) ha reportado encontrar distintos patrones respiratorios en dos especies de Mygalomorphae. En Araneomorphae, incluyendo a Lycosidae, no existen registros previos. En este trabajo reportamos el hallazgo de tres tipos de patrones respiratorios en dos especies de Lycosidae, *Allocosa senex* y *A. marindia*, las cuales habitan ecosistemas costeros y fluviales en Uruguay (Simó et al., 2017). Para clasificarlos, nos basamos en el conocimiento previo de insectos, aunque cabe destacar que, al ser especies con sistema respiratorio mixto, los registros no son tan claros como los de especies completamente traqueadas.

Breve descripción metodológica y resultados

Al analizar los registros de respirometría en *A. senex* y *A. marinida* correspondientes a los Capítulos 1 y 2 de esta tesis, tanto en el registro inicial (día 0; previo al tratamiento de salinidad/temperatura) como en el final (día 15; luego del tratamiento), se encontraron distintos patrones de respiración. Estos corresponden a respiración continua, la cual fue utilizada para los análisis de los Capítulos 1 y 2 (Figura A9, A); un segundo tipo que se asemeja al *interburst-burst* en insectos (Figura A9, B) y por último, un patrón similar al DGC en insectos (Figura A9, C). Dado que estos últimos dos no habían sido previamente reportados para especies de Araneomorphae, les llamamos genéricamente “respiración no-continua”. A continuación se detalla el análisis que se realizó para evaluar bajo qué condiciones se expresaron los distintos tipos de respiración no-continua.

En la Tabla A10 se detalla el número de registros de respiración no-continua para cada especie, sexo y localidad. De forma interesante, la respiración no-continua representó alrededor de un 11 % para ambas especies de *Allocosa* de la playa con influencia oceánica, mientras que fue solo un 3 % de los registros en las hembras de *A. senex* de la playa ribereña. Asimismo, no se registró respiración no-continua en machos de *A. senex* pero sí de *A. marindia*, la especie con menor tamaño corporal.

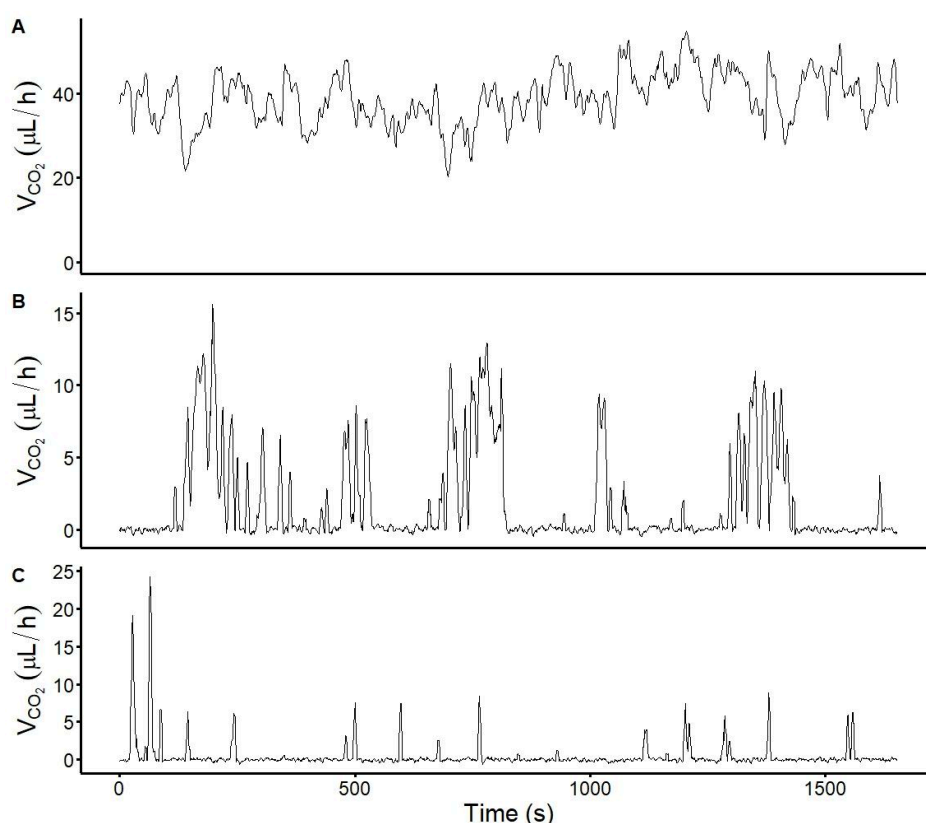


Figura A9. Tipos de respiración encontrados en hembras de *Allocosa senex* de Uruguay. A, tipo de respiración continua; B y C, corresponden a tipos de respiración no-continua, siendo B más similar al patrón *inter-burst burst* en insectos y C, al Intercambio Discontinuo de Gases (DGC) en insectos. Notar la diferente escala entre los paneles.

Tabla A10. Casos de respiración no-continua (incluyendo “*inter-burst burst*” e “*Intercambio Discontinuo de Gases, DGC*”) en hembras y machos de *A. senex* y *A. marindia*, según su localidad de procedencia (O = Oceánica, R = Ribereña), sexo (H = Hembras, M = Machos), momento del registro (Inicial = día 0, Final = día 15) y porcentaje sobre el total de registros.

Especie	Localidad	Sexo	Inicial	Final	Total Resp. Disc.	Reg. Totales	% Resp. No-cont.
<i>A. senex</i>	O	H	10	6	16	125	12,8 %
	R	H	1	2	3	98	3 %
	O	M	0	0	0	29	0 %
<i>A. marindia</i>	O	H	10	2	12	106	11,3 %
	O	M	0	1	1	10	10 %

Para evaluar si la ocurrencia de los tipos de respiración dependía de la localidad y de la exposición a salinidad o temperatura, se realizaron tablas de frecuencia y se analizaron mediante Prueba Exacta de Fisher usando el software R Studio (R Core Team, 2023). Estos test se realizaron solamente para las hembras de *A. senex*. En el caso de *A. marindia*, fueron solamente 2 y 1 los registros de respiración discontinua al final del tratamiento para hembras y machos, respectivamente. Por ende, no fue posible analizar el rol de la salinidad mediante un test estadístico.

Dado que el número de muestra de respiración no-continua para *A. senex* fue bajo, se evaluó el efecto de una sola variable a la vez, de la siguiente forma: (1) se analizó si la expresión de respiración no-continua difiere entre localidades (playa ribereña y playa oceánica), sin importar si la medición fue inicial o final. (2) Si la expresión depende de la salinidad de exposición (0, 35 y 70 ‰), utilizando solo los registros finales y no separando por localidad ni temperatura. Primero se realizaron test pareados por salinidad, pero al no haber diferencias entre salinidad 35 ‰ y 70 ‰ (Prueba Exacta de Fisher, $p = 1$), se comparó entre salino y no-salino. (3) Se evaluó si la expresión depende de la temperatura de exposición (25 y 32 °C), usando los registros finales y no separando ni por localidad ni salinidad.

Los análisis revelaron que las hembras de la playa oceánica exhibieron una mayor proporción de respiración no-continua con respecto a las ribereñas (Prueba Exacta de Fisher, $p = 0,0138$). El odds ratio (OR) fue 0,22 (IC 95 % = 0,04–0,79), lo cual indica que las hembras de la playa oceánica tienen una probabilidad ~4,5 veces mayor de expresar respiración no-continua que las de la playa ribereña. Considerando solamente los registros finales (es decir, luego del tratamiento), se encontró que la frecuencia de respiración no-continua es mayor bajo condiciones de alta salinidad (35 ‰ y 70 ‰) que no salinas (0 ‰) (Prueba Exacta de Fisher, $p = 0,047$). Dado que no se registraron eventos de respiración no-continua a 0 ‰, el OR estimado es infinito y su IC 95 % fue 1,02–Inf. Finalmente, no se encontraron diferencias significativas en la expresión de respiración no-continua en función

de la temperatura de exposición (25 y 32 °C) (Prueba Exacta de Fisher, $p = 1$; OR = 1,25, IC 95 % = 0,22–7,19).

Breve discusión

En este trabajo se describen tres patrones respiratorios encontrados para dos especies de arañas Lycosidae, *Allocosa senex* y *A. marindia*. Estos incluyen respiración continua y dos patrones de respiración no-continua: uno similar al *interburst-burst* en insectos y otro al DGC.

La expresión de respiración no-continua dependió del sexo en *A. senex* (solo lo expresaron hembras), de la localidad (más frecuente en hembras de playa con influencia oceánica en *A. senex*) y la salinidad (a alta salinidad, mayor expresión), pero no de la temperatura. En el caso de las arañas migalomorfas, ni la temperatura ni la humedad relativa explicaron la expresión de respiración no-continua (Mason et al., 2013). Estos resultados permiten discutir su posible valor adaptativo basándose en las hipótesis evolutivas previamente formuladas para insectos.

Se han propuesto numerosas hipótesis para explicar la existencia de patrones de respiración no-continuos (Chown, 2011; Terblanche & Woods, 2018; White et al., 2007). Entre ellas, destacan la hipótesis hídrica, que plantea que la DGC surge como una adaptación para reducir la pérdida de agua en ambientes xéricos; la hipótesis de estrés oxidativo, que plantea que el DGC contribuye a reducir el daño por exposición prolongada a altos niveles de oxígeno cuando la actividad y el metabolismo son bajos; y la hipótesis ctónica, que sugiere que la DGC es una adaptación para la respiración en ambientes hipóxicos o hipercápnicos, como cuevas o madrigueras. En nuestro caso, las especies estudiadas construyen cuevas en la arena, en las que se refugian durante los meses fríos y durante el día en los meses cálidos (Simó et al., 2017). Por ende, la presencia de patrones discontinuos de respiración, podría ser una adaptación para tolerar las condiciones subterráneas en reposo. Sin embargo, es llamativo que no se haya expresado en machos

de *A. senex* quienes construyen las cuevas más profundas (Aisenberg et al., 2007). Asimismo, el hecho de que la respiración discontinua se haya correlacionado positivamente con la salinidad y haya sido más prevalente en la localidad oceánica, podría relacionarse a la hipótesis hídrica, constituyendo un mecanismo que mitigue el estrés osmótico.

De todas formas, es necesario realizar estudios especialmente diseñados para evaluar bajo qué condiciones se expresan los distintos tipos de patrones respiratorios y cuáles su potencial ventaja adaptativa.

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