# Can differential predation of native and alien corixids explain the success of *Trichocorixa verticalis verticalis* in the Iberian Peninsula?

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## Abstract

Invasive species represent an increasing fraction of aquatic biota. However, studies on the role and consequences of facilitative interactions among aliens remain scarce. Here we investigated whether the spread of the alien water boatman *Trichocorixa verticalis verticalis* in the Iberian Peninsula is related to reduced mortality from predation compared with native Corixidae, especially since *Trichocorixa* co-occurs with the invasive fishes *Gambusia holbrooki* and *Fundulus heteroclitus.* All three invaders have a common native range in North America and are widespread in and around Doñana in SW Spain. Using laboratory experiments, we compared the predation rates by the two exotic fish and native Odonata larvae on *Trichocorixa* and the native *Sigara lateralis.* We found no evidence to suggest that *Trichocorixa* suffers lower predation rates. However, when both corixids were mixed together, predation of *Trichocorixa* by Odonata larvae was higher. Odonata larvae were size-limited predators and the proportion of corixids ingested was positively correlated with mask length. Since *Trichocorixa* is smaller than its native competitors, this may explain their higher susceptibility to predation by Odonata. This may be one of various factors explaining why *Trichocorixa* is particularly dominant in saline habitats where Odonata are rare, while it is still scarce in fresh waters.

Key words: Corixidae, *Trichocorixa*, *Sigara,* Invasive species, Predation, Doñana

## Introduction

Freshwater habitats have been subjected to massive species introductions from multiple sources for decades (Gherardi et al., 2008). As a consequence, in many of these systems, alien organisms are now a significant part of their biota (Cohen, 2002). Iberian inland waters, for example, host *ca* 73 non-indigenous freshwater species (Garcia-Berthou et al., 2007), belonging to a variety of taxa such as molluscs, crustaceans and fishes (Cobo et al., 2010).

Although negative interactions between species have been considered to be among the major drivers of biological invasions, only recently have studies highlighted the role of facilitative interactions among exotic species (Simberloff & Holle, 1999; Richardson et al., 2000). According to Courchamp et al. (2000), the co-occurrence of exotic predators and exotic prey can heavily impact native prey by hyperpredation. Adams et al. (2003) demonstrated how the invasion of the bullfrog *Lithobates catesbeianus* in North America was facilitated by the invasive bluegill sunfish *Lepomis macrochirus*, as the latter preys on macroinvertebrates that in turn prey on bullfrog tadpoles. Such positive interactions are thought to be widespread and important in exacerbating the problem of invasion (Simberloff & Holle, 1999), but studies that evaluate these interactions are still scarce (Richardson et al., 2000; Tecco et al., 2006), particularly for vertebrates (but see Adams et al., 2003).

In this study we experimentally investigated the role of predation by exotic predators (the eastern mosquitofish *Gambusia holbrooki* and the mummichog *Fundulus heteroclitus*; hereafter *Gambusia* and *Fundulus*, respectively) and native predators (dragonfly larvae) on the invasive boatman *Trichocorixa verticalis verticalis* (hereafter *Trichocorixa*) and the native, co-occurring boatman, *Sigara lateralis* (hereafter *Sigara*),in the Iberian Peninsula. *Trichocorixa* (Fieber 1851) (Heteroptera: Corixidae) is native to North American saline and freshwater habitats but now also occurs in South Africa, Iberia and Morocco (Jansson & Reavell, 1999; Kment, 2006; L’Mohdi et al., 2010). In Europe, following its initial detection in the Algarve in Portugal (Iberian Peninsula) in 1997, the species has spread along the Iberian Atlantic coast (Sala & Boix, 2005) and into the Guadalquivir delta and surrounding parts of SW Spain. Bio-climatic models predict future spread across Europe and the Mediterranean region (Guareschi et al., 2013). Established populations of *Trichocorixa* have been found at several sites in and around Doñana in the Guadalquivir delta, where it is the most abundant corixid in saline wetlands (Rodríguez-Pérez et al., 2009; Van de Meutter et al., 2010a). Despite their dominance at higher salinities, they do not show higher halotolerance as adults than native corixids (Van de Meutter et al., 2010a; Coccia et al., 2013).

In its native area, *Trichocorixa* co-occurs in rivers, brackish pools, potholes, and salt marshes with the above two euryhaline fish species(*Gambusia* along the east coast of USA from New Jersey to Florida and *Fundulus* along the North American Atlantic coast from Canada to Florida, see Sala & Boix, 2005)*.* Various studies illustrate the coexistence of this corixid species with one of these fish species in the same waterbody within the native range (e.g. Harrington & Harrington, 1972; Cherry et al., 1979; MacKenzie, 2005; Rochlin et al., 2011; J. Simonis, pers. comm. 2014). These fishes were introduced to the Iberian Peninsula from North America around 1921 and 1970 respectively (Cobo et al., 2010). They now occur in a multitude of aquatic habitats during all the hydrological cycle in Doñana, where they are far more abundant than any native freshwater fish (Moreno-Valcárcel et al., 2013). Both species have been shown by gut content analysis to include corixids in their diet in permanent saline fish ponds in Doñana, where Odonata are absent or only recorded at low densities (Pyke, 2005; Dreamer-John, 2012; Rodríguez-Pérez & Green, 2012). In contrast, Odonata larvae represent the most important predators of corixids in temporary ponds in Doñana, where the invasive and native corixids co-exist but fishes rarely occur (Florencio et al., 2009; Rodríguez-Pérez et al., 2009).

As exotic predators can have dramatic effects on both invasive and native prey (Lodge, 1993; Knapp et al., 2001), only organisms with specific and effective anti-predatory mechanisms are likely to coexist with them (Sih et al., 2010). Although some studies have shown native prey to possess better defenses against native than alien predators (Gomez-Mestre & Díaz-Paniagua, 2011), prey species may also undergo rapid evolutionary change to counter a novel predation pressure (Griffin, 2004; Boyero, 2011). However, predator detection has a cost and performance can be lost with similar speed if the predator is removed (Strauss et al., 2006).

We hypothesized that alien predators (fish) and those native to Doñana (Odonata larvae) would have different interactions with the alien (*Trichocorixa*) and native (*Sigara*) corixids. Specifically, we tested the following hypotheses: i) predator responses to different prey depend on co-evolutionary history; thus, the exotic predators *Fundulus* and *Gambusia* are more successful at detecting and catching *Trichocorixa*, while Odonata are more successful at detecting and catching *Sigara*; and ii) differences in predation also depend on predator and prey size. We discuss the evidence that facilitative interactions can account for *Trichocorixa* success in southern Iberia and whether the scarcity of Odonata larvae in permanent, saline habitats in Doñana could be a factor promoting its dominance in this area (Rodríguez-Pérez et al., 2009; Van de Muetter et al., 2010b).

## Material and methods

### Methods

Adults of *Fundulus* and *Gambusia* were collected during spring 2012 using either minnow traps or a D-framed pond net (500 μm mesh; 16 × 16 cm), respectively. Native Odonata larvae and adults of *Trichocorixa* and *Sigara* were sampled using the same D-framed pond net. Experiments were run from 28 March to 16 May 2012. *Fundulus* and *Trichocorixa* were obtained from Veta la Palma fish ponds within Doñana Natural Park, *Gambusia* from an artificial pond called “pozo salinas” located in the stabilized sands (a system of stable dunes formed by marine drift during the Holocene period) in the west part of Doñana Biological Reserve and *Sigara* were sampled in two artificial ponds within the garden of the Cartuja Monastery (within the city of Seville). Finally, Odonata larvae were collected in artificial ponds either within Doñana National Park (FAO pond) or the Cartuja Monastery (see Serrano et al., 2006 for position of the Doñana sites).

Water conductivity varied between sites, being 32-36 mS cm-1 in Veta la Palma, and 1.8-5 mS cm-1 in the other ponds.

After collection, predators and prey were placed separately in plastic bags (a maximum of 4 fishes per bag) filled with water from the collection site and transported to the laboratory within thermally insulated polystyrene boxes to minimise thermal fluctuations. Once in the laboratory, each predator was transferred to its own 6-L aquarium (27x17x18 cm), containing water at conductivities that resembled those of sites from which they were collected [17 mS cm-1for *Fundulus*; 5 mS cm-1 for *Gambusia* (ca. 11 cm depth); and 2 mS cm-1*,* for Odonatalarvae (ca. 6 cm depth)]. Water from the collection point was used for the treatments of 5 and 2 mS cm-1, whereas for high conductivity treatments, water was prepared by mixing pond water (32-36 mS cm-1) with dechlorinated tap water until the required conductivity of 17 mS cm-1 was obtained. As we did not test for turbidity effects, all treatments were conducted in clear water. Aquaria were provided with sand and were aerated for the fish treatments. Predators were acclimated for 24 h before predation experiments; this period is considered sufficient to allow fishes to explore the aquaria (Primavera, 1997). No food was provided to predators during these 24 h.

Experiments were conducted in a climatic chamber set at 15ºC under a 12 h: 12 h D:L regime. Ten corixids (balanced for sex when possible) were added to each aquarium containing a predator individual. When fishes were used as predators, six treatments were created by crossing corixid species (*Trichocorixa* alone, *Sigara* alone and both species together) and refuges (with and without an artificial plant, Online Resource 1a,b). In the case of Odonata larvae, only three treatments were employed (the three corixid combinations) as aquaria were always provided with artificial plants to provide a perch for the larvae. Five replicates of each treatment were used for each fish species and 10 replicates for dragonflies. The first check for surviving corixids was made after 6 h. Thereafter, aquaria were checked every 24 h. At each check, live corixids were retrieved using a hand net, counted, identified to species and visually sexed. After that, the corixids were returned to their aquarium. Consumed corixids were not replaced. After a maximum of 72 h, predators were retrieved from each aquarium. Each predator was used only once and sacrificed under licence after the experiment, using anaesthetic. Fishes were weighed using a ALC\_2100.1 balance (Sartorious Ltd, U.K.) and the length of each individual was measured using a plastic millimeter sheet. Mean predator sizes (± SE) were as follows: 6.9±0.13 cm for *Fundulus* and 3.8±0.04 cm for *Gambusia.* The mean lengths (± SE) of masks of Odonata larvae (4.03±0.14 mm) were measured on images taken with a digital camera (AxioCam Icc1) connected to a Zeiss microscope (Discovery V8) (Online Resources 2-3). Odonata larvae were mainly (70%) final instars, and the remaining 30% were mid instars (according to Askew 1998). Prey size was not measured, as it would have involved a strong handling stress that may have altered corixid behaviour. The typical lengths were 5-6 mm for *Sigara* (Nieser et al., 1994) and 3.5-5.4 mm for *Trichocorixa* (authors’unpublished data).

### Data analysis

We quantified the proportion of corixids (either *Sigara* or *Trichocorixa*) that were eaten by predators (Proportion of Corixids Eaten, PCE = 1 – final/initial number of corixid individuals), for each predator (*Fundulus*, *Gambusia*, and Odonata) and for each corixid treatment (single species vs. both species together). Although we used larvae of several dragonfly genera of the families Libellulidae and Aeshnidae [*Orthetrum* spp. (*n*=10); *Aeshna* spp. (*n*= 19); *Sympetrum* spp. (*n*= 10); and *Crocothemis* spp. (*n*=1)], we pooled the data for final analyses presented because results were the same as when using only the most frequent genus (*Aeshna* spp., other taxa could not be analysed separately because there were not enough individuals, see Online Resource 4). Initial models showed that there were no significant differences in PCE for fish between treatments with and without an artificial plant (results not shown). Thus, these treatments were pooled for further analyses comparing PCE between predator and prey species.

We used general linear models (GLMs) followed by Tukey tests to compare, for each corixid species separately, the effect of predator identity, corixid treatment and their interaction on PCE, which was arcsin square root transformed to improve normality and homogeneity of variances (tested with Shapiro-Wilk’s and Levene’s tests, respectively). Given the differential consumption rates between *Fundulus* (the most voracious), *Gambusia* and dragonflies, we initially calculated PCE for several experimental time intervals (6 and 24h for *Fundulus*; 6, 24, 48 and 72h for *Gambusia* and dragonflies), but we present results only for the final time (24h for *Fundulus* and 72h for *Gambusia* and dragonflies) because (1) *Gambusia* and dragonflies started eating after 24h and (2) results of analyses were the same between 6 and 24h for *Fundulus* and between 48 and 72h for *Gambusia* and dragonflies.

As the interaction between predator identity and corixid treatment was significant for *Trichocorixa*, we then used separate GLMs for each predator species to further explore effects of corixid treatment on PCE, for each corixid species separately. In these analyses we included the log (x+1) transformed predator size (for *Fundulus* and *Gambusia*) or dragonfly mask length as a covariate. Finally, we compared PCE between corixid species for the ‘both species together’ treatment with one-way ANOVA for each predator separately.

## Results

Predator identity had a significant effect on PCE (Table 1), although results differed slightly for each corixid species. For *Sigara*, *Fundulus* showed the highest predation rate followed by Odonata larvae and *Gambusia*. For *Trichocorixa*, *Fundulus* and Odonata were equally efficient, and *Gambusia* was again the least efficient predator (Fig. 1). Corixid treatment had no effect on PCE, but the interaction between predator identity and corixid treatment was significant for *Trichocorixa* (Table 1); differences between corixid treatments were significant for Odonata (*P* < 0.05), which consumed significantly more *Trichocorixa* when *Sigara* was also present, but not for the other predators (Fig. 1).

When the effect of different predators was analyzed separately, there was no significant effect of either corixid treatment or predator body length for *Fundulus* and *Gambusia* (results not shown). For Odonata larvae, corixid treatment had no effect for *Sigara*; however, corixid treatment did have a significant effect for *Trichocorixa*. Mask length had a positive effect on PCE for both species (Table 2): dragonflies with larger masks ate more corixids (Fig. 2). When both corixid species were together, there were no significant differences between the number of *Sigara* and *Trichocorixa* eaten by any predator (*Fundulus*: *F*1,42 = 0.06, *P* = 0.78; *Gambusia*: *F*1,38 = 1.28, *P* = 0.27; dragonflies: *F*1,38 = 3.50, *P* = 0.069); however, the marginal result for dragonflies shows they tended to eat more *Trichocorixa* individuals (Fig. 3). Hence, the predation efficiency of Odonata larvae increased with their size, and was marginally influenced by prey size.

## Discussion

In a multiple invasion context, where invaders at different trophic levels co-exist, several scenarios are possible. For example, alien predators may prefer native prey, native predators may prefer alien prey, or predators may easily detect/recognize prey because they share a co-evolutionary history (Sih et al., 2010).

Our results indicate that the invasive *Fundulus* and the native Odonata larvae are considerably more efficient than the invasive *Gambusia* in the rate of consumption of corixids. However, the only evidence to suggest that predators distinguish between alien and native corixids was on the basis of body size. *Trichocorixa* body mass is 35% lower than that of *Sigara* (Coccia et al., 2013), and our results suggest that for that reason they suffer higher predation by Odonata, and that this may help explain *Trichocorixa* dominance in saline waters in SW-Spain.

We found no evidence to suggest that corixids show different predator avoidance behaviour depending on whether they encountered predators sharing a common native range or predators of a different origin. However, our study did not allow us to distinguish between the existence of anti-predator responses in corixids vs. prey detection/capture mechanisms in predators, and we did not collect behavioural data. The absence of an efficient anti-predator response can lead to species decline (Courchamp et al., 2000), and the apparent inefficiency of anti-predator responses contradict field data confirming the co-occurrence of both exotic fishes and corixids in Doñana (Van de Meutter et al., 2010b; authors' unpublished data). Under confined laboratory conditions and high water clarity, it is likely that predator efficiency was greater than that in the field, where habitats are more diverse with reduced spatial or temporal overlap in habitat use between predator and prey, and turbidity can be high. For example, Kelts (1979) showed an increased rate of consumption of *T.verticalis* var *sellaris* by *Fundulus* when algae were removed from aquaria. The plastic plant we used as a refuge had no similar effect and was probably insufficient to hinder prey detection (Online Resource 1a,b).

In Doñana, *Fundulus* and *Gambusia* occur in different types of habitat (see Online Resource 5), however *Fundulus* prefers saline waters, and is generally absent in fresh or brackish ponds, which are inhabited mainly by *Gambusia* (Moreno-Valcárcel et al., 2013). *Trichocorixa* and *Sigara* often co-occur in sites of 3-15 mS cm-1 (Van de Meutter at al., 2010a), whereas *Trichocorixa* is the dominant corixid at higher salinities and *Sigara* at lower salinities. Thus, we might expect a strong effect of *Fundulus* for both corixids at salinities between 9-23 mS cm-1 where *Fundulus* dominates (Moreno-Valcárcel et al., 2013). However, in most of the sites were *Trichocorixa* was found with native corixids within the Doñana area, *Gambusia* was the only fish present (Van de Meutter et al., 2010b). At low conductivities (0.3-8.5 mS cm-1), Moreno-Valcárcel et al. (2013) also found *Gambusia* to be the dominant species. The low observed predation rate by *Gambusia* in our experiments suggests that the predator-prey interaction between *Gambusia* and both the corixids is less important than that of *Fundulus*, a finding in agreement with field data showing that *Gambusia* ate mainly copepods and relatively few corixids (Dreamer-John 2012). In contrast, our results suggest that *Fundulus* and Odonata larvae may have a major impact on the invasive corixids in permanent saline waters and low salinity ponds respectively.

Permanent salinewaters in SW Spain are often inhabited by both *Fundulus* and *Trichocorixa* (Sala & Boix, 2005). It has been suggested that the unpalatability of *Trichocorixa* late instars and adults may reduce predation by *Fundulus* in North America (Campbell & Denno, 1978; Kelts, 1979), but our observations do not support this*.* The extent of predation on *Trichocorixa* by *Fundulus* is likely to partly depend on the availability of alternative prey such as copepoda, isopoda and decapod shrimps (Dreamer-John, 2012). Furthermore, the distribution and abundance of *Fundulus* is likely to be limited by its own predators, such as larger fish and piscivorous birds, both of which are present at high density in saline parts of Doñana where *Trichocorixa* is dominant (Rendón et al., 2008, Rodríguez-Pérez & Green 2012). Additionally, co-occurrence with *Fundulus* may also be related to *Trichocorixa* preferences forshallow, vegetated waters (authors personal observation) avoided by fish (Kelts, 1979; Lewin et al., 2004).At the same time,the osmoregulatory abilityof the invasive corixid at the egg and nymph stagetogether withitshigh fecundity and the absence of parasitic infections might be some important determinants of its success at higher salinities (authors’ unpublished data).

In temporary ponds, corixids arrive *en masse* in spring to reproduce, and the absence of fish maximises nymphal survival (Brown, 1951). Odonata larvae and both corixid species are often found together in Doñana ponds, especially during late spring (Florencio et al., 2009; Rodríguez-Pérez et al., 2009). Odonata larvae are top predators in temporary aquatic habitats, able to feed on different prey ranging from invertebrates (Hopper, 2001) to small fishes (Walker, 1953). Our results illustrate how Odonata larvae are size-limited predators, and suggest that predation of *Trichocorixa* by these major pond predators is increased by their smaller size compared to native *Sigara* spp. (including *S. scripta*, *S. stagnalis* and *S. selecta*, Rodríguez-Pérez et al. 2009, Van de Meutter et al., 2010b). The predator size-prey size relationship is one of many factors that can lead to greater use of exotic species as prey by native predators (Anholt & Werner, 1998). There are many previous example of native predators consuming exotic prey (King et al., 2006; Bulté & Blouin-Demers, 2008), sometimes as the dominant food items (Carlsson et al., 2009). In temporary ponds, predation by Odonata larvae may provide biotic resistance to invasion (Levine et al., 2004), limiting the abundance of *Trichocorixa* which frequently reproduces in temporary ponds in the native range (Brown, 1951). Selective predation can often mediate similar co-existence of similar prey (Ciros-Pérez et al., 2004).

## Conclusion

*Trichocorixa* is a successful invader in SW Spain and is particularly dominant over native corixid species in saline wetlands (Rodríguez et al., 2009, Van de Meutter et al., 2010b). Our findings provide no support for the hypothesis that the *Trichocorixa* invasion has been promoted by lower predation rates compared to native corixids. On the other hand, *Trichocorixa* may suffer particularly higher predation rates from native Odonata larvae, which may partly explain why this species is particularly successful in saline habitats where Odonata are rare. Further studies are required to compare the life histories of the alien and native corixids. Priorities for future research include direct interactions (aggression, predation) between *Trichocorixa* and native heteropterans, as well the trophic ecology of *Trichocorixa* and its role in invaded food webs.

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**Table 1** Results (degrees of freedom, sum of squares, F-statistic and p-value) of GLMs showing the effects of predator identity (*Fundulus heteroclitus*, *Gambusia hoolbroki* or dragonflies larvae), corixid treatment (single species or both species together), and their interaction, on the proportion (arcsin sqrt transformed) of corixids eaten by predators.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source of variation** | **df** | **SS** | **F** | **P** |
| ***Sigara lateralis*** |  |  |  |  |
| **Predator** | 2 | 14.51 | 36.93 | <0.0001 |
| **Corixid** | 1 | 0.01 | 0.03 | 0.87 |
| **Predator × Corixid** | 2 | 0.54 | 1.38 | 0.26 |
| **Error** | 85 | 16.70 |  |  |
| ***Trichocorixa v. verticalis*** |  |  |  |  |
| **Predator** | 2 | 17.14 | 41.17 | <0.0001 |
| **Corixid** | 1 | 0.12 | 0.60 | 0.44 |
| **Predator × Corixid** | 2 | 1.49 | 3.58 | 0.032 |
| **Error** | 85 | 17.69 |  |  |

**Table 2** Results of GLMs showing the effects of corixid treatment (single species or both species together) and log (x+1) transformed mask length of the arcsin sqrt transformed proportion of corixids eaten by Odonata larvae.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source of variation** | **df** | **SS** | **F** | **P** |
| ***Sigara lateralis*** |  |  |  |  |
| **Corixid** | 1 | 0.48 | 2.82 | 0.10 |
| **Dragonfly mask length** | 1 | 0.93 | 5.47 | 0.027 |
| **Error** | 27 | 4.57 |  |  |
| ***Trichocorixa v.verticalis*** |  |  |  |  |
| **Corixid** | 1 | 1.50 | 10.95 | 0.003 |
| **Dragonfly mask length** | 1 | 1.41 | 10.36 | 0.003 |
| **Error** | 27 | 3.69 |  |  |

## Figure



**Fig. 1** Arcsin sqrt-transformed mean ± SE proportion of corixids eaten (PCE = 1 - final/initial number of corixid individuals) for each corixid species (SL, *Sigara lateralis*; TV, *Trichocorixa v. verticalis*), by different predators (*Fundulus heteroclitus*, *Gambusia holbrooki*, and Dragonflies), and in two different corixid treatments (single species vs. both species together). Asterisks indicate significant differences in the PCE of TV by Dragonflies compared to the other predators when SL was also present.

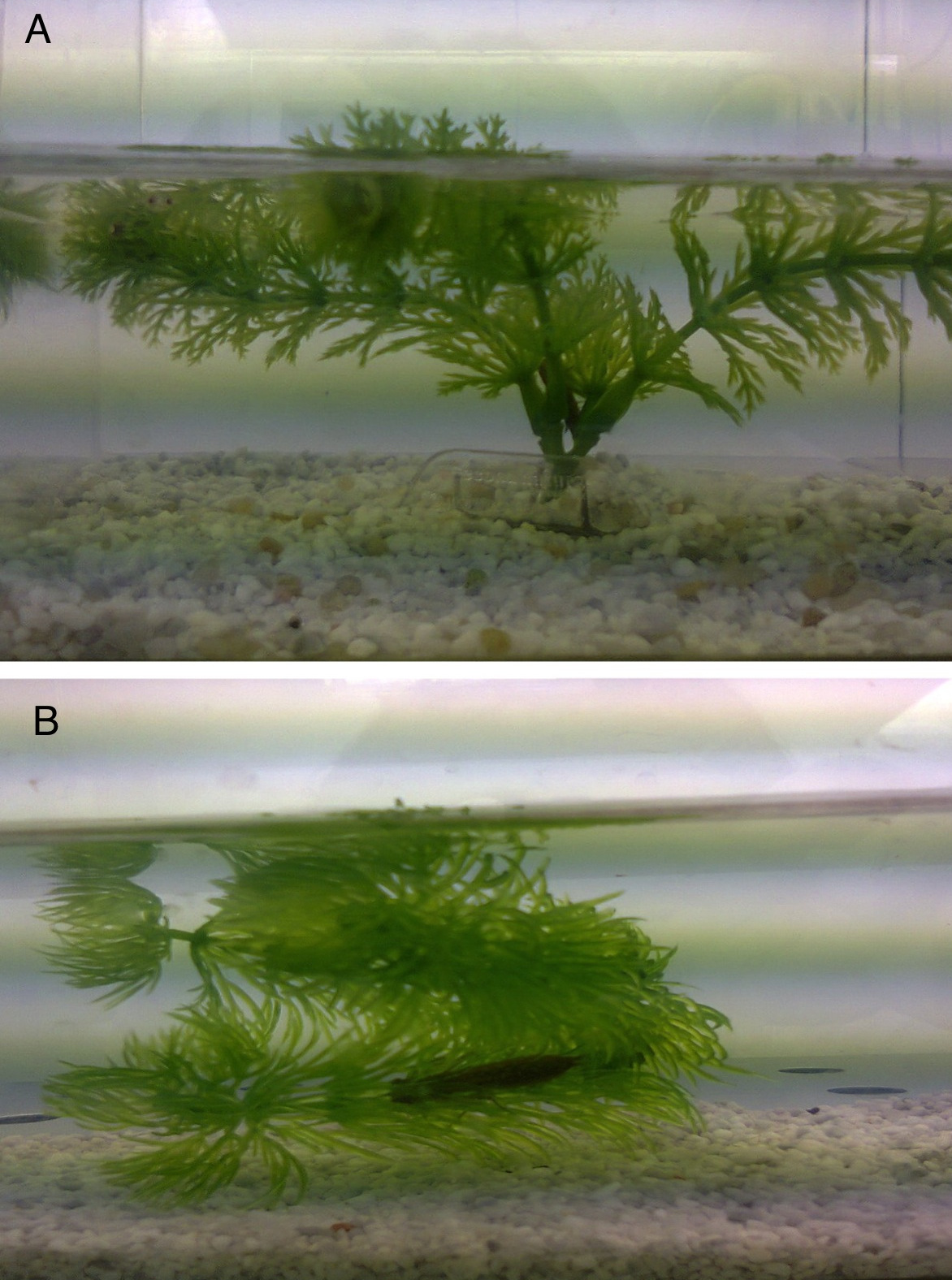
****

**Fig. 2** Linear regression (+ 95% confidence interval) between log-transformed dragonfly larva mask length and arcsin sqrt-transformed proportion of corixids eaten (PCE); SL, *Sigara lateralis (P =*0.11*, R2=*0.13*)*; TV, *Trichocorixa v. verticalis (P* = 0.001*; R2 =*0.46*).*

****

**Fig. 3** Arcsin sqrt-transformed mean ± SE proportion of corixids eaten (PCE) of each species (SL, *Sigara lateralis*; TV, *Trichocorixa v. verticalis*) in the ‘both species together’ treatment.

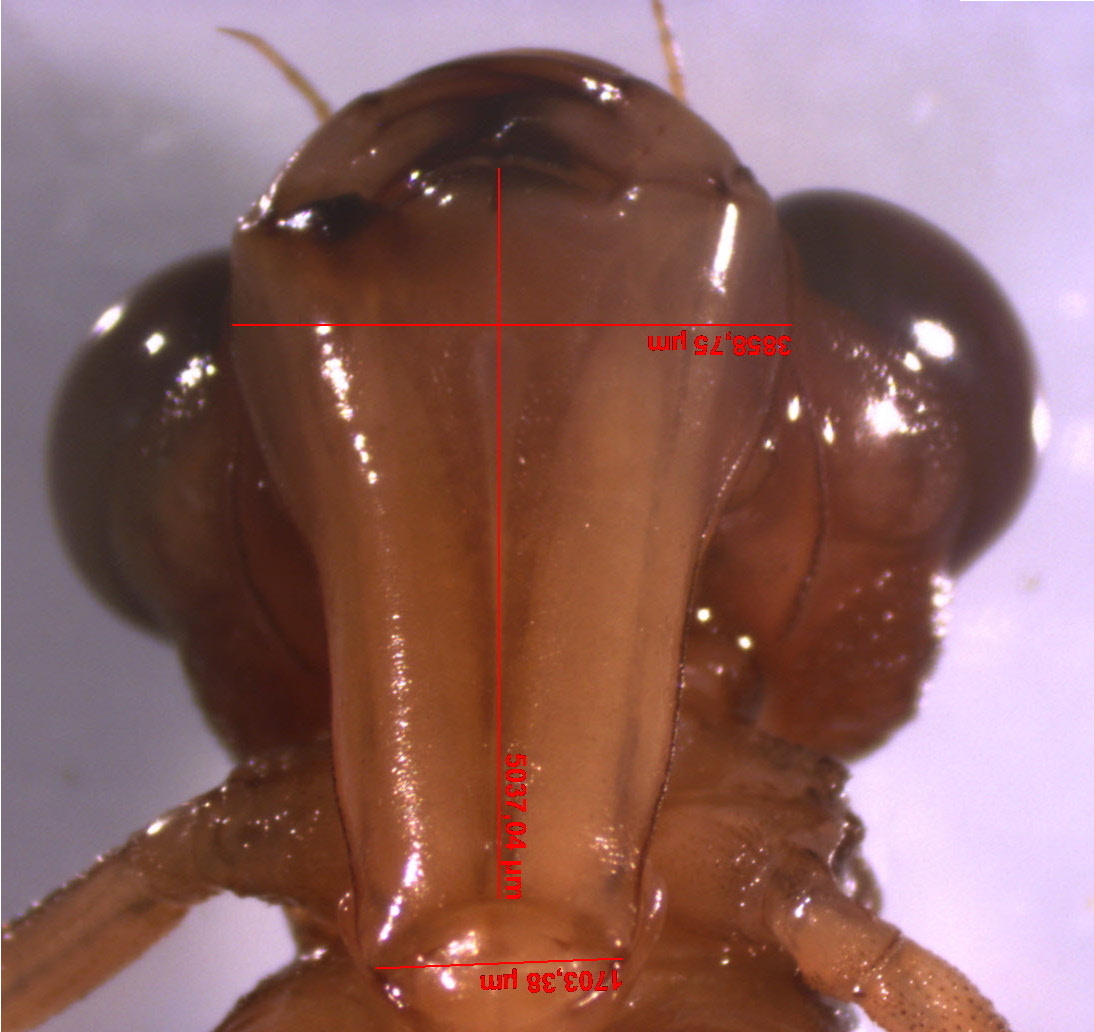
**Online Resource 1** Artificial plant used in the experiment



**Online Resource 2** Dragonflies mask length.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| N | Origen | Genus | Mask Length (mm) | Treatment |
| 1 | Cartuja M. | *Orthetrum* spp | 3.5 | SL+TV |
| 2 | Cartuja M. | *Orthetrum* spp | 3.6 | SL+TV |
| 3 | Cartuja M. | *Orthetrum* spp | 3.0 | SL+TV |
| 4 | Cartuja M. | *Orthetrum* spp | 3.3 | SL+TV |
| 5 | Cartuja M. | *Orthetrum* spp | 3.1 | SL+TV |
| 6 | Cartuja M. | *Orthetrum* spp | 3.3 | SL+TV |
| 7 | Cartuja M. | *Orthetrum* spp | 2.8 | SL+TV |
| 8 | Cartuja M. | *Orthetrum* spp | 3.2 | SL+TV |
| 9 | Cartuja M. | *Orthetrum* spp | 2.6 | SL+TV |
| 10 | Cartuja M. | *Orthetrum* spp | 1.9 | SL+TV |
| 1 | FAO | *Aesha* spp | 5.6 | SL+TV |
| 2 | FAO | *Aesha* spp | 4.7 | SL+TV |
| 3 | FAO | *Aesha* spp | 5.5 | SL+TV |
| 4 | FAO | *Aesha* spp | 5.6 | SL+TV |
| 5 | FAO | *Aesha* spp | 4.3 | SL+TV |
| 6 | FAO | *Aesha* spp | 4.8 | SL+TV |
| 7 | FAO | *Aesha* spp | 4.3 | SL+TV |
| 8 | FAO | *Aesha* spp | 4.5 | SL+TV |
| 9 | FAO | *Aesha* spp | 4.6 | SL+TV |
| 10 | FAO | *Aesha* spp | 4.8 | SL+TV |
| 1 | FAO | *Aesha* spp | 4.9 | SL |
| 2 | FAO | *Aesha* spp | 5.6 | SL |
| 3 | FAO | *Aesha* spp | 4.7 | SL |
| 4 | FAO | *Aesha* spp | 3.5 | SL |
| 5 | FAO | *Sympetrum* spp | 3.5 | SL |
| 6 | FAO | *Sympetrum* spp | 3.5 | SL |
| 7 | FAO | *Sympetrum* spp | 3.4 | SL |
| 8 | FAO | *Sympetrum* spp | 3.4 | SL |
| 9 | FAO | *Sympetrum* spp | 3.7 | SL |
| 10 | FAO | *Sympetrum* spp | 3.6 | SL |
| 1 | FAO | *Aesha* spp | 4.7 | TV |
| 2 | FAO | *Aesha* spp | 4.1 | TV |
| 3 | FAO | *Aesha* spp | 4.7 | TV |
| 4 | FAO | *Aesha* spp | 4.6 | TV |
| 5 | FAO | *Aesha* spp | 5.6 | TV |
| 6 | FAO | *Crocothemis* spp | 3.4 | TV |
| 7 | FAO | *Sympetrum* spp | 3.6 | TV |
| 8 | FAO | *Sympetrum* spp | 3.6 | TV |
| 9 | FAO | *Sympetrum* spp | 3.7 | TV |
| 10 | FAO | *Sympetrum* spp | 3.9 | TV |

**Online Resource 3** Example of stereomicroscope photograph of Odonata larvae mask



|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source of variation** | **df** | **SS** | **F** | **P** |
| ***Aeshna* spp.** | | | | |
| ***Sigara lateralis*** |  |  |  |  |
| **Predator** | 2 | 16.132 | 43.737 | <0.001 |
| **Corixid** | 1 | 0.041 | 0.221 | 0.639 |
| **Predator x Corixid** | 2 | 0.044 | 0.120 | 0.886 |
| **Error** | 69 | 12.72 |  |  |
| ***Trichocorixa v. verticalis*** | | | | |
| **Predator** | 2 | 18.294 | 44.132 | <0.001 |
| **Corixid** | 1 | 0.208 | 1.003 | 0.319 |
| **Predator x Corixid** | 2 | 1.416 | 3.416 | 0.038 |
| **Error** | 70 | 14.51 |  |  |
| **All Odonata** | | | | |
| ***Sigara lateralis*** |  |  |  |  |
| **Predator** | 2 | 14.510 | 36.929 | <0.001 |
| **Corixid** | 1 | 0.005 | 0.027 | 0. 868 |
| **Predator x Corixid** | 2 | 0.543 | 1.382 | 0.256 |
| **Error** | 85 | 16.70 |  |  |
| ***Trichocorixa v. verticalis*** | | | | |
| **Predator** | 2 | 17.138 | 41.167 | <0.001 |
| **Corixid** | 1 | 0.124 | 0.597 | 0.442 |
| **Predator x Corixid** | 2 | 1.491 | 3.581 | 0.032 |
| **Error** | 85 | 17.69 |  |  |

**Online Resource 4** Comparison of the results (degrees of freedom, sum of squares, F-statistic and p-value) of GLMs performed with all Odonata genera with those including only the most frequent genus *Aeshna* spp. on the proportion (arcsin sqrt transformed) of corixids eaten by them.

**Online Resource 5** Types of habitat where *Fundulus heterolictus*, *Gambusia holbrooki*, Odonata larvae and Corixids (*Sigara lateralis* and *Trichocorixa v.verticalis*) occur in Doñana.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Habitat occurrence** | ***Fundulus heteroclitus*** | ***Gambusia holbrooki*** | **Odonata**  **larvae** | ***Sigara lateralis*** | ***Trichocorixa v.***  ***verticalis*** |
| Temporary ponds |  | x\* | x | x | x |
| Permanent fresh ponds |  | x | x | x | x |
| Small streams | x | x | x | x | x |
| Seasonal lakes | x | x | x | x | x |
| Saline fish ponds | x | x | x |  | x |
| Solar saltworks | x |  |  |  | x |
| Waterholes |  | x | x | x | x |
| Ditches | x | x | x | x | x |

\* indicates that temporary presence of the species depends on hydrological connections defined by the flooding regime.