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Enhancing mosquito predation activity of Mediterranean banded killifish (*Aphanius fasciatus*) by tidal recirculation runnels in the Po River Delta area

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Abstract

Nature-protected coastal plain areas in the Po River Delta, Italy, are subjected to periodic flooding. Such phenomena create temporary water habitats conducive to the development of salt marsh mosquitoes, such as *Aedes caspius* (Pallas) and *Aedes detritus* (Haliday), which cause significant nuisance impacting the tourist industry. Mosquito control is therefore required, but conflict arises about the need to safeguard endangered bird species typically nesting in the wetlands. Despite the fact that adopted mosquito control methods are designed to cause as little disturbance as possible, authorities responsible for nature conservation require that the possible impact of larval control activities are further reduced. These concerns have served as an incentive to seek out alternative control strategies, such as the enhancement of the predatory action of fish species inhabiting the salt marsh biotopes. The most abundant of these species is the native *Aphanius fasciatus* (Valenciennes) (Cyprinodontiformes Cyprinodontidae). The access of fish to isolated pools and lowlands subjected to occasional flooding can be promoted by connecting them to major permanent water bodies via shallow ditches or runnels, which are dug by hand. The results of four years of observations and larval sampling in a salt marsh area of about 4 ha, located in the natural reserve of "Sacca del Bellocchio" where a system of runnels has been planned and constructed, suggest that runnelling can be an effective, long-lasting larval control method, favouring the predatory action of fish.

Key words: *Aphanius fasciatus*, runnelling, salt marsh, mosquito control.

Introduction

Water level variations in the coastal natural areas of the Po River Delta are caused by peak tides, heavy rainfall, rising of ground-water level, and flooding tides from the coast caused by sea storms that can even go over the present dune stretches.

The occasional flooding of the pools and the lowlands allows the development of two species of highly anthropophilic mosquitoes, *Aedes* (*Ochlerotatus*) *caspius* (Pallas) and *Aedes* (*Ochlerotatus*) *detritus* (Haliday), causing major problems in the Comacchio and Ravenna areas (Ficalbi, 1901; Bellini, 1993; Bellini and Veronesi, 1994; Veronesi *et al*.,1995). The females of these species lay eggs on the humid ground, which can remain quiescent for a long time and hatch at once following a flooding phase.

The need to safeguard tourists from these problematic infestations conflicts with the need to protect the endangered bird species nesting in these natural habitats, such as *Haematopus ostralegus* L., *Himantopus himantopus* (L.), *Charadrius dubius* Scopoli, and *Sterna albifrons* Pallas.

On the other hand, despite the adoption of the most appropriate control measures to minimise disturbance, the treatments by foot on the ground as well as of the aerial distribution of *Bacillus thuringiensis* subsp. *israelensis* de Barjac (*Bti*) based larvicidal products showed limited effectiveness because of the difficulties to achieve a homogeneous distribution of the larvicidal product over the entire larval breeding sites (Celli and Bellini, 1997). This situation, therefore, requires identifying alternative or complementary control strategies.

The presence of significant larval breeding habitats of different *Aedes* species along the valleys and salt marshes behind the dunes makes it possible to assess, as possible support for larval control, the predatory action of an autochthonous fish, *Aphanius fasciatus* (Valenciennes) (Cyprinodontiformes Cyprinodontidae), commonly called the Mediterranean banded killifish.

The purpose of the present study is to provide data about the efficacy of actions taken to promote predation by autochthonous biological control agents in accordance with the guidelines of the World Health Organisation, suggesting the use of native species in control programmes against culicids (WHO, 1982; 2003).

As the water level rises, the fish disperse from permanent reservoirs by following the movement of water making its way into marshlands as shallow as 2-3 cm deep. This way, in the areas connected to the permanent water bodies subjected to this periodic water level variation, it is possible to witness the complete natural predation of the floodwater *Aedes* larvae.

Since the land is slightly undulating, when the water recedes, it leaves flooded areas in contact with the permanent reservoirs, where *A. fasciatus* circulate freely, as well as with isolated lowlands, where the absence of predators allows the *Aedes* mosquitoes to complete their development cycle.

The predation of the fish in the lowlands and occasionally flooded, isolated pools can be promoted by making surface runnels that connect the lowlands to the main, permanently flooded areas. To avoid disturbance, the runnels can be fashioned by hand digging, following the natural water flow, thus allowing the water to circulate freely in the event of rising tides and heavier rainfall. Therefore, the main purpose of the runnels is not to drain but to connect in a network the isolated lowlands.

These interventions require precise knowledge of the distribution of the mosquitoes' productive areas and their activation dynamics. Experiences developed starting from around 1990 in Australian and American coastal areas showed highly effective permanent control of mosquito problem, as indicated in the "rotary ditching", "runnelling" and "open marsh water management" versions (Hulsman *et al.*, 1989; Easton, 1990; Dale *et al*, 1993; Kramer *et al*., 1995; Wolfe, 1996; Dale, 1997; 2005; Carlson *et al*., 1999; Knight *et al*., 2022).

The pilot trial we present here was authorised by the State Forestry Corps (*Corpo Forestale dello Stato*) and the Po Delta Regional Park of Emilia-Romagna (*Parco Regionale del Delta del Po dell'Emilia-Romagna*).

Short note on the *Aphanius fasciatus* bio-ecology

A. fasciatus belongs to the Cyprinodontiformes order and to the Cyprinodontidae family. *A. fasciatus* includes many synonyms (Froese and Pauly, 2022). Kottelat and Wheeler (2001) contributed to the debate on the nomenclature of the genus *Aphanius* Nardo.

A. fasciatus is a small fish, with the female about 6 cm long and the male 4-5 cm long, characterised by moderate sexual dimorphism. It commonly inhabits saltmarsh waters, river mouths, and lagoons in contact with the sea along the whole of the Italian peninsula and coasts of Sardinia and Sicily (Tortonese and Lanza, 1968; Gandolfi, 1973; Cottiglia, 1980; Alessio and Gandolfi, 1983; Ferrito and Tigano, 1996). Sommani (1967) attributed the observed considerable reduction in the diffusion area of *A. fasciatus* to reclamation works done during anti-*Anopheles* campaigns. In the Po Delta area, *A. fasciatus* is found in the inland saltmarsh lagoons with high saline concentration (Cavicchioli, 1962).

Its diet is quite varied, ranging from the meiobenthos to the ticoplankton. It voraciously feeds on culicids halophilous larvae, which it actively seeks in areas that are occasionally flooded (Leonardos, 2008).

It is an oviparous species that lays eggs in the water vegetation in spring. In the Mediterranean basin, two other species of the same genus exist, *Aphanius iberus* Valenciennes and *Aphanius dispar* (Ruppell), neither of which is found in Italian waters (Louis and Albert, 1988; Fletcher *et al.*, 1992).

Materials and methods

Study area

The area involved in the trial is a samphire saltmarsh behind the dunes (44°37'14"N 12°16'18"E) in the Sacca del Bellocchio Nature Reserve in the Regional Park of the Po River Delta area of Emilia-Romagna, about 2 km south of the tourist area of Lido di Spina in the municipality of Comacchio. Flooding due to intense rainfall and high peak tides creates extended breeding sites of *Ae. caspius* (Bellini *et al*., 2002). In phytosociological terms, the area is characterised by perennial halophyte vegetation, which is dominated by *Arthrocnemum fruticosum* (L.) Moq. (Piccoli *et al*., 1996).

In May 2002, over a surface area of about 4 ha in the south-eastern part close to the Gobbino-Bellocchio Canal, a number of runnels were dug by hand, that is with shovels, starting from a permanently flooded area and directed to the larval breeding grounds (figure 1). Interventions were completed in 2003. Thirty-one runnels, with a total length of 422 m and about 40 cm wide and 30 cm deep, were dug.

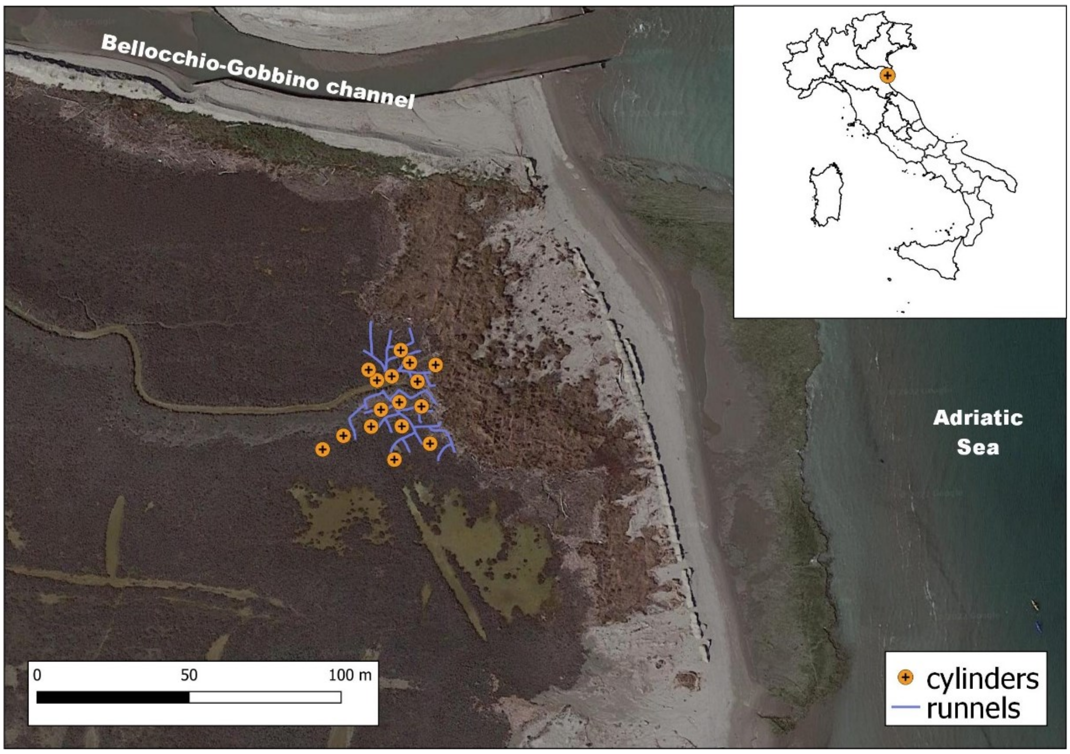

Figure 1. Sacca del Bellocchio Nature Reserve and study area.

Larval sampling

Sixteen polythene cylinders, each 63 cm in diameter and 34-45 cm in height, were randomly positioned in the study area in mid-April of each year from 2002 to 2005 and removed in October. Each cylinder was driven about 3-5 cm into the ground in order to perfectly isolate the internal area from the surroundings (figure 2). To allow the free flow of water in and out of the cylinders and at the same time to prevent access to fish, four windows, each 10 cm high and 1.5 cm wide and covered with a fine wire mesh, were made on the edge of each cylinder in contact with the ground. Each cylinder was numbered and the position kept the same by means of pegs.

The distance of the cylinders from the closest runnel ranged between 0.5 m and 5 m.

Larval sampling was carried out on day three to seven after flooding, when the L_3-L_4 larvae were present. Using standard dippers of capacity 0.5 litre, water samples were collected in four random stations inside and four random stations outside each cylinder (up to about 3 m from the cylinder). The contents of the dipper were checked and recorded immediately by reversing the sample into a white plastic tray. Sampled larvae were put back where collected. Following the larval sampling, the top of each cylinder was closed with a tulle cover with a closable opening in the centre. This way, the adult mosquitoes emerging in the cylinders were restrained and collected through an electric portable aspirator following every larval sampling, counted, and their species determined in the laboratory.

At larval samplings, the average water depth outside and inside the cylinders were recorded and, on two occasions, the salinity (‰ NaCl) was measured by means of a portable refractometer (Optech-Optical Technology, Alessandrini Strumentazione S.p.a.). The amount of rainfall was also measured with a pluviometer positioned near the study area. Field data collections were completed by September of each year.

Statistical analysis

The data collected with the sample inside and outside the cylinders underwent ANOVA analyses to test the significance of the difference between the averages, with T-testing for dependent samplings.

Figure 2. Detail of the area during flooding with two cylinders.

Results

Hydrometric regime

Not all the rainfalls were sufficient to allow larval developments, depending on the pluviometric regime of the period. The number of flooding events causing larval development and when larval sampling were conducted was three in 2002 (April 12; May 27; August 18), three in 2003 (June 28; August 31; September 2), eight in 2004 (April 10; April 19; April 25; June 5; June 20; July 6; July 27; September 17), and three in 2005 (August 1; August 5; August 9) (figures 3 and 4). The depth of the water recorded in the area inside and outside the cylinders at each flooding ranged from 0 (in these cases water concentrated in the sparse and scattered puddles around the cylinders, where the level reached a maximum of 2 cm) to 10 cm. The water salinity measured on April 19th 2002 was 29‰ and on July 3rd 2003 was 35‰.

During the periods when the area was dry, the runnels were frequently flooded by the normal daily tide.

Dynamics of larval densities

The larval sampling conducted before the excavation of the runnels showed that the larval density was similar inside and outside the cylinders (table 1).

The invertebrate macrofauna were not very present in the sampled plots, with *Chironomus* spp. (Diptera Chironomidae) adults sampled in almost all the cylinders in the first sampling in 2002 (April 19th) and only two specimens of *Berosus spinosus* (Steven) (Coleoptera Hydrophilidae) sampled also during the sampling of April 19th, 2002.

Following runnel excavation, larval sampling outside the cylinders fell by close to 100% up to the last two samplings of 2004, in which few larvae were found both inside and outside the cylinders (figure 4). This might be due to the high frequency of flooding allowing the development of more larvae. In the first six samplings, no larvae were found inside and outside the cylinders; in the sampling of July 30th, some larvae were sampled in three out of 16 cylinders, while in the external area, larvae were sampled only around three cylinders (70% of the larvae were found outside). In the last sampling of 2004 (September 22nd), larvae were sampled in three cylinders out of 16 (five cylinders were dry), and the larval density inside the cylinders was four times higher compared to the external area. Unfortunately, a sea storm (September 24th) brought intense flooding to the entire area, submerging the cylinders and preventing the adults from being found.

In 2005, no larvae were ever sampled either inside or outside the cylinders (figure 4).

With reference to 2002, both before the excavation of the runnels (sampling of 19th April) and in the following two checks, on average fewer larvae were sampled inside the cylinders than outside, although the difference was statistically significant only in the first sampling (30th May) after the excavation of the runnels (table 1; figure 4).

All the mosquito adults that emerged from the cylinders belonged to the *Ae. caspius* species.

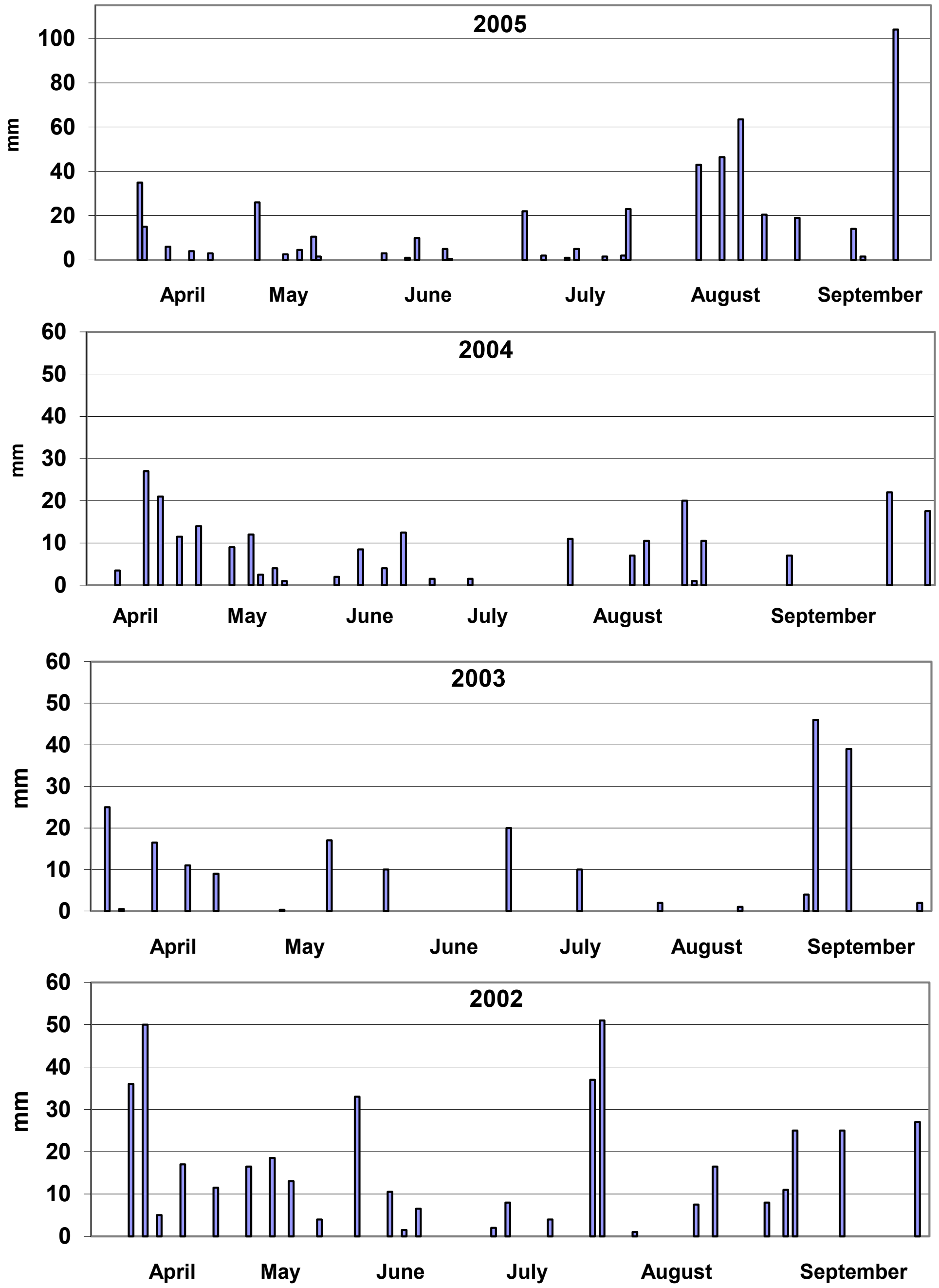

Figure 3. Rainfall trends in the test area in the 4-year period from 2002 to 2005.

Discussion and conclusion

The data collected with the first sampling (2002) showed a high larval density, slightly lower inside the cylinders than outside (table 1; figure 4). During the 2002 season, the *A. fasciatus* activity was observed to be very low, probably because of the low temperatures, as its presence in the area was not observed before June. Therefore, the difference between larval densities inside and outside the cylinders seems to be attributable to micro-environmental factors more than to predation. In contrast, in the following three years, fishes were frequently observed along the runnels as early as April.

In the second sampling 2002 (end of May), while an increase in larval density was observed outside the cylinders in comparison with the first larval sampling, a significant reduction was observed inside the cylinders (figure 4). As this reduction in larval density inside the cylinders could not have been caused by predation because of the exclusion of fish by the cylinders itself, we hypothesized that this may be attributable to the barrier effect of the cylinders on the egg laying females.

In the third and last sampling of 2002, when *A. fasciatus* was observed frequently and in significant numbers, a larval density 90-95% lower than in the previous samplings was observed outside, while inside the cylinders the larval density remained extremely low.

Data collected and observations made in subsequent years made it possible for the general results to be extended.

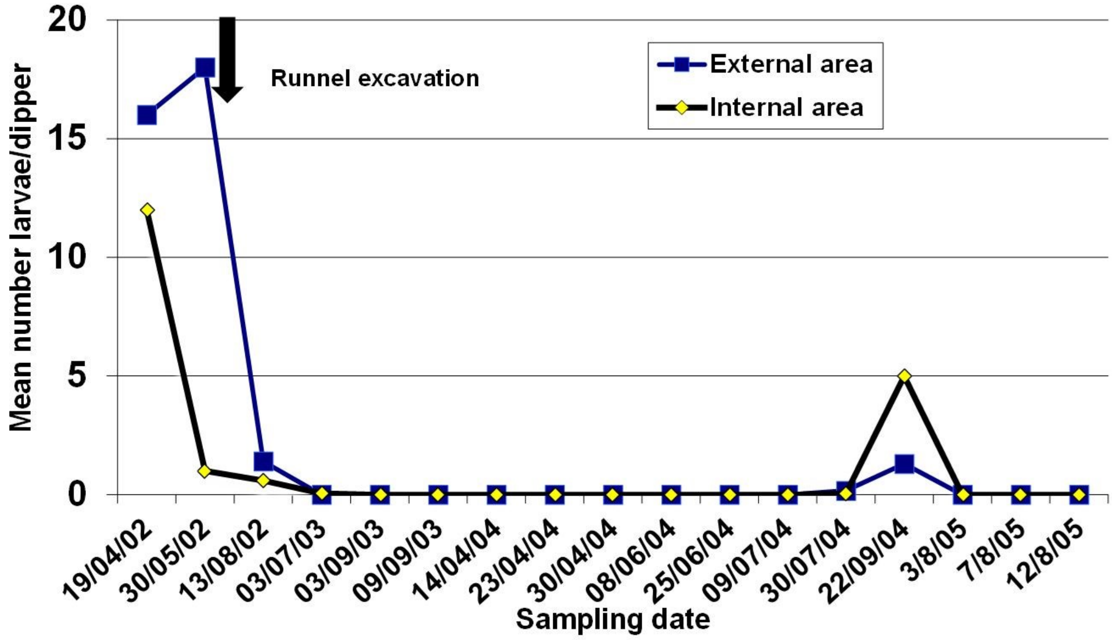

Figure 4. Dynamics of the *Ae. caspius* larvae as sampled in the four-year period 2002-2005.

Table 1. Larvae of *Ae. caspius* sampled outside and inside the cylinders in 2002.

Date	Position	Mean larvae/dipper[1]	SD	
4.19.02 (pre-runnelling)	Inside	11.82	27.59	NS
	Outside	15.48	27.75	
5.30.02	Inside	1.03	1.43	a
	Outside	18.29	29.83	b
8.13.02	Inside	0.52	1.75	NS
	Outside	2.08	3.43	

[1] 64 collections outside and the same number inside the 16 cylinders on each sampling date.
NS: not significant. Letters a and b indicate a significant difference at $p \leq 0.001$.

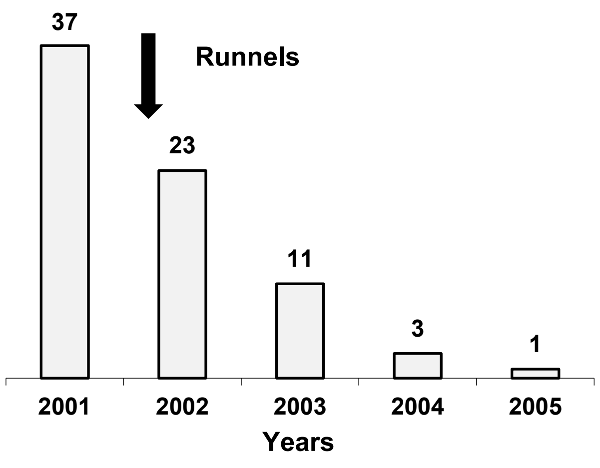

Figure 5. *Bacillus thuringiensis israelensis*-based larvicidal formulation (1200 UTI/mg) consumption, in litres, in the test area in the period 2001-2005.

The action of the fish was complete during the first flooding in 2003 (July 3rd), and it was confirmed with subsequent flooding at the end of August and September. Low density or absence of larvae inside the cylinders was also observed in 2003, 2004 and 2005, confirming what has been said above regarding the possible interaction of the cylinders with the choice of the egg-laying *Ae. caspius* females. Another hypothesis is that the entire test area was indiscriminately conditioned by the presence of the fish, since the female mosquitoes seemed to be able to detect the presence of fish, as Ritchie and Laidlaw-Bell (1994) demonstrated.

The decrease in larval infestation frequency and in the total surface of colonized area brought about a progressive decrease in the number of larval control interventions and relative consumption of *Bti*-based microbiological formulations in the years from 2001 to 2005 (figure 5). The possibility of reducing/eliminating the use of larval treatments also reduces the need for operators to access the area, thus preventing any disturbance, especially during the nesting period. Moreover, the cost savings gained offset the cost of the minor annual management of the runnels to remove obstructing materials or the accumulation of sediments at the bottom, which may affect the efficiency of the system.

Another aspect to consider is whether the excavation of runnels has an impact on the local vegetation.

Generally, runnelling increases the frequency and the extent of flooding, compared to the pre-excavation situation. This implies variation in the saline concentration and aeration of the ground, as well as the production of sediments, which together with other micro-environmental factors, such as micro-topography, propagation of flooding, variability of the aquifer level, and space variability of drainage, may influence floral associations.

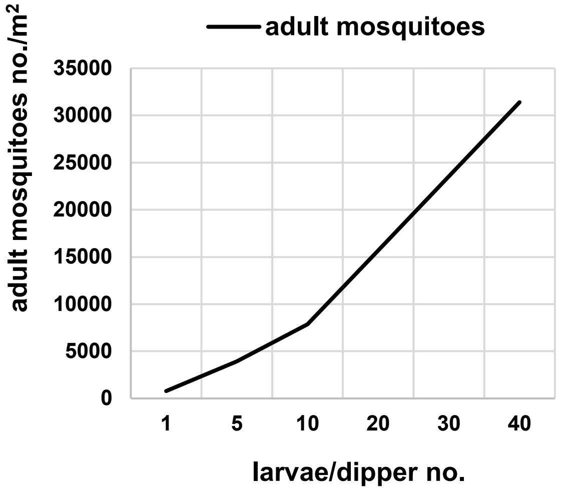

Figure 6. Productivity by m^2 of *Ae. caspius* adults in the saltmarsh test area before runnel excavation.

The topic has been widely discussed and the scientific literature reports a large number of observations and results connected with the effects produced (Hulsman *et al*., 1989; Dale *et al*., 1993; Kramer *et al*., 1995; Wolfe, 1996). Recent data obtained from an Australian coastal area (Queensland S-E) subjected to runnelling for almost 19 years have been reported by Dale (2005; 2008). These data indicate that there were no significant differences in the density and height of the two dominant halophyte species, *Sporobolus virginicus* (L.) Kunth and *Sarcocornia quinqueflora* (Bunge ex Ung.-Sternb.), compared to a “non-runnelled” area.

To guarantee the stability of the vegetation composition, it is important to create a shallow system of runnels following the original lie of the land, in order to limit, as much as possible, the depth of flooding, the water flow surface, and the accumulation of sediments, which, over the years, raises the level of the area. The general objective is, therefore, to keep the original characteristics of the saltwater marshland area intact as much as possible, as the method in this proposed study seems to guarantee.

Finally, we would like to report a datum which was indirectly obtained from the study, and which underscores the importance of controlling larval breeding grounds in natural areas.

By checking the ratio between larval density and adults collected inside the cylinders, it was 1/248 in the first samplings 2002; 1/5 in the second sampling 2002; 1/241 in the third sampling 2002; 1/316 in the sampling of 3 July 2003; 1/19 in the sampling of 30 July 2004.

From the aforementioned collected data relative to the surface bounded by a cylinder, it is possible to deduce a productivity of *Ae. caspius* adults per unit marsh surface area, in terms of the average larvae/dipper number ratio. For example, in the first sampling of 2002 where the average sample outside the cylinders was 16 larvae/dipper, each hectare of flooded marsh samphire area produced a theoretical 125,600,000 adults (figure 6).

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