

Effects of environmental factors on *Tigriopus fulvus*, Fischer 1860, a Mediterranean harpacticoid copepod

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Abstract

Tigriopus fulvus (Fischer, 1860) is a benthic harpacticoid copepod of the Mediterranean supralittoral zone. The transitional characteristics of this environment forced this species to develop high resistance to changes of environmental parameters. Nevertheless, *T. fulvus* life-cycle is influenced from the splashpools physical-chemical parameters. In this paper, we present the results of a supralittoral monitoring performed in 2014, confirming the influence of some of these environmental parameters on population buildups. Because of recent worldwide climate change effects, a threat might have been posed on this particularly exposed organism, whose population density decreased of a sixfold value in the last 30 years. During the three pools (A, B, C) monitoring, the maximum copepod density recorded was 1456 Ind/l (September 2014, Pool C), alongside first records of extinction event for *T. fulvus*.

Introduction

The marine rocky littoral system is divided into well-defined

areas^{1,2} and represents a transitional environment between land and sea. Here, environmental conditions show sudden and critical variations to which resident organisms need to react with refined adaptation and tolerance techniques.³ These collections of temporary water represent, particularly in the supralittoral zone, an ubiquitously distributed environment. Because of low tidal activity, in Mediterranean splashpools most of water inputs come from sea storms and rainfall.⁴ Many aspects of the ecology of this habitat were studied and described during the last decades.⁵⁻¹⁵ The benthic harpacticoid copepod *Tigriopus* sp. is known to be one of the most representative and adapted species in the supralittoral environment. The genus *Tigriopus* includes many different species, with worldwide distribution ranging from North-American coasts¹⁶ to Antarctica.¹⁷ Two European species are described, but *Tigriopus fulvus* is the only one living along the Mediterranean coasts.¹⁸ *T. fulvus* is also known to be a useful test species in ecotoxicology, whose reliability was tested with different matrices and pollutants.¹⁹⁻²⁷ This species inhabits splashpools or rockpools, and therefore it is exposed to a wide variety of environmental conditions that promoted refined adaptation and resistance characteristics. Such variability is ascribed to cycles of drying out or inundation of rockpools, depending on rainfall, waves exposition and sunlight. This paper resumes a seasonal study on splashpools and a comparison with historical *T. fulvus* population data, in order to explore the health-state of copepod population.

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Materials and Methods

Study area

The studied *T. fulvus* population can be currently found in Genova Nervi splashpools (Ligurian Sea, 44°22'52.561 N; 9°2'12.570 E). Three different splashpools (A, B, C) having different structural characteristics were chosen at different height and distance from the sea (Figure 1). Studied pools are classified as splashpools, defined as *located above the high-tide line and receiving marine water inputs only by sprays during heavy seas and storms and not located in the intertidal zone*.²

Splashpools number was considered sufficient as the coastal morphology of the study sites concentrates many pools in relatively narrow space. Pools with the same sunlight exposition were chosen, this means that no structural coverage might cause differences in pool water warming. Mean pool surface and mean depth were about 2 m² and 0.5 m, respectively. Height from the sea level ranged between 4 and 6 meters (A to C). The splashpools were chosen taking into account the occurrence of *T. fulvus* at the beginning of samplings. Samplings followed a fortnightly check, from March to September 2014.

Environmental and atmospheric parameters

Splashpools temperature and salinity were acquired through a multiparametric probe (YSI 30M/50 FT; 0.1°C and 0.1 resolution), a different probe was needed for pH measures (Etekcity 009, 0.1 resolution, 0.1 accuracy at 20°C). Chlorophyll a and Total Particulate Matter (TPM) analysis were performed on water samples collected with opaque plastic bottles. Chlorophyll a (Chl_a) analysis was performed following a spectrophotometric method (Analytic Jena Spekol 1300). Water samples were filtered on glass-microfibre filters (GF/A Whatman), and subsequently exposed to an overnight acetone:distilled water (90:10 v:v) extraction prior to spectrophotometric analysis.²⁸ To evaluate TPM, water samples were filtered on 0.45 µm porosity Sartorius cellulose acetate filters. Ponderal analysis was performed on analytic scale, after 2 hours filters oven-drying (130°C) followed by 1.5 hours in silica-gel dryer. Organic percentage composition of TPM was evaluated with ash weighing after a 2h/550°C burning in muffle furnace. Rainfall data were obtained from the Liguria Region Weather Hydrological Observatory, a data collection web composed of almost 230 measuring stations spread across Liguria Region.

Sampling of specimens

A plastic 250 mL bottle was used to collect samples that were subsequently fixed in 70% ethanol (v:v); sampling bottle was dragged along the top of the south facing pool wall, every sampling day at noon, as made in previous fieldwork.¹² *T. fulvus* specimens were collected to follow the population variability in terms of density and composition. Copepods counting was carried out under light microscope (Nikon SM7-U); Five categories were assigned to determine population structure, males, females, ovigerous females, copepodites (C1-C5) and nauplii (N1-N6). Ovigerous females frequency was considered as an indicator for reproductive events constancy, and to evaluate a possible *source and sink* dynamic between the studied splashpools as well. Copepodite stages were recognized by size and morphology, while nauplii were identified from body shape and a maximum of four thoracic segments.

Statistical analysis

Spearman rank correlation analysis were performed by the mean of StatSoft STATISTICA 10 software. Boxplot presence/absence analysis was performed in the R environment.

Results

Environmental parameters

Spatial and temporal variability of the supralittoral environment was highlighted during the samplings; temperature and salinity data are displayed in a T-S graph (Figure 2). Pool C showed wide mean variability of environmental parameters, with peaks in salinity (55.8±24.2‰), density (1035±15.6) and pH (8.4±0.5). Pool A, showed wider oscillations during the sampling period (24.8±8.8°C). The highest salinity value was reached in pool C with 90‰ (July 2014). Pool C also showed the lowest pH value (pH=7.3, July 2014).

TPM and chlorophyll resulted higher in pool C, where trophic conditions seem to be more favorable for *T. fulvus* population, in terms of quantity (mean 193 mg/L TPM) and quality (mean organic particulate composition of 58.6%) than in Pool A (49.15 mg/L and 39.6%). Pool A population (Figure 3A) reached a top value of 424 individuals per liter (Ind/l) in April 2014, but it was nevertheless recognized as the most scarcely populated pool, with a mean

population density of 155.6 Ind/l. Pool B and Pool C showed higher mean population densities (285 ind/l and 448,9 ind/l, respectively), but different population behaviors. Pool B population (Figure 3B) reached a maximum of 1428 Ind/l (September 2014) and did not show extinction events. Pool C (Figure 3C) reached the population peak in September 2014 as well (1456 Ind/l), and the minimum in August 2014 (136 Ind/l). Pool C went into extinction in the first two weeks of July 2014.

Pool B showed significant reproductive events, where ovigerous females almost reached the 50% of the total population (Figure 4); pool A showed the highest (24.2%) mean value for this parameter. Regarding Pool A population, the occurrence of ovigerous females (Figure 4) was reported during all the examined period, nevertheless the scarce mean density does not allow to highlight reproduction events. Mean Spearman Rank analysis between population and ovigerous females density highlights a high correlation (mean $r_s=0.77$, $P<0.05$).



Figure 1. Study area map and splashpools distribution.

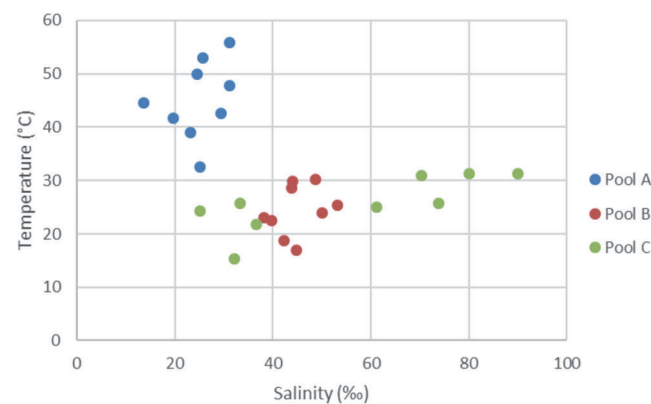


Figure 2. Temperature and salinity distribution in Pool A, B and C (March-October 2014).

Discussion

Meteorological parameters influence the supralittoral environment, determining a wide variability of salinity, temperature and general pools condition. Splashpools populations are influenced by such parameters and by phytoplankton biomass availability. Studies on *Tigriopus brevicornis* showed that this organism has a wide tolerance toward environmental parameters variability, that nevertheless affect copepod survival and development.^{29,30} It was suggested that a considerable energetic cost is paid by the supralittoral inhabitants to survive and fight environmental changes.³¹ *Tigriopus japonicus* can survive at 1.8‰ water salinity, but the most suitable range for reproduction is between 27.1 and 34.3‰.^{32,33} The wide variability of environmental parameters may overcome *Tigriopus* sp. survival mechanisms, such as osmotic, temperature and desiccation tolerance, as it was observed in *Tigriopus californicus*.³⁴⁻³⁶ On this latter species, a limit of adaptation to raising temperatures was found, putting this well-adapted organism under the climate change threat.¹⁶

The *T. fulvus* population in Genova Nervi (Ligurian Sea) showed genus-typical adaptability to environmental variability. Concerning the splashpools trophic conditions, Chl_a and TPM peaks during April 2014 (Chl_a=98.32 mg/L; TPM=68.52 mg/L) and September 2014 (Chl_a=98.32 mg/L; TPM=607.1 mg/L) matched with *T. fulvus* population buildups; on the other hand, Pool C went through a drying event which caused extinction of *T. fulvus* population in July 2014. In comparison with data from 1987,¹² the results of this study show a decrease in mean *T. fulvus* population. While older samplings highlighted a mean copepod density of

2699±1058 Ind/L from March to September 1987, the data from 2014 showed a density of 580±630 Ind/L; therefore, a fivefold mean population density decrease occurred. The comparison between the data of environmental parameters highlights a remarkable temperature rise (mean=25.9°C, range=21.8–31.36°C) with respect to the previous data from the same rockpool (mean=22.1°C, range=16.5–27.0°C). Similar considerations can be made regarding water temperature and density.¹²

Conclusions

Even though two points do not constitute a trend, especially in a highly variable species, the first signs of a reached tolerance limit must be considered, because of the assessed growth rate decrease.³⁷ As already assessed,¹² thermal tolerance is a crucial factor in *Tigriopus* spp. survival in the natural environment. Despite genetic adaptation and phenotypic plasticity that might buffer the adverse effects of the environmental temperature increase, the response that *T. fulvus* shows to climate change effects might not be sufficient to overcome the subsequent stress.

All in all, *T. fulvus* presence in the three studied rockpools is determined by a combination of the mentioned environmental factors, as shown in the subsequent presence/absence analysis. Primarily, as expected, splashpools water salinity affects *T. fulvus* fitness and therefore its presence in the studied pools (Figure 5); absence data of water temperature and salinity refer to small water remainings that can be found under the bottom salt crust, no copepods were present. A further environmental temperature increase might affect the fitness of supralittoral organisms up to their tolerance limit. Moreover, environmental temperature is correlated with pool water evaporation and subsequent salinity increase, that might lead to further distress for the resident populations (Figure 5).

Metapopulation structure has been already highlighted for *T. fulvus*.¹⁵ If for *T. brevicornis*³⁸ and *T. californicus*³⁹ the individual distribution and the subsequent splashpools colonization seem to be affected by tidal activity, for *T. fulvus* this phenomenon is more connected to sea storms and rainfall. The correlation between populations B ($r=0.81$, $P<0.05$) and C ($r=0.75$, $P<0.05$) and cumulate rainfall between samplings (Figure 6) highlighted between March and September 2014 (Figure 3), could confirm this

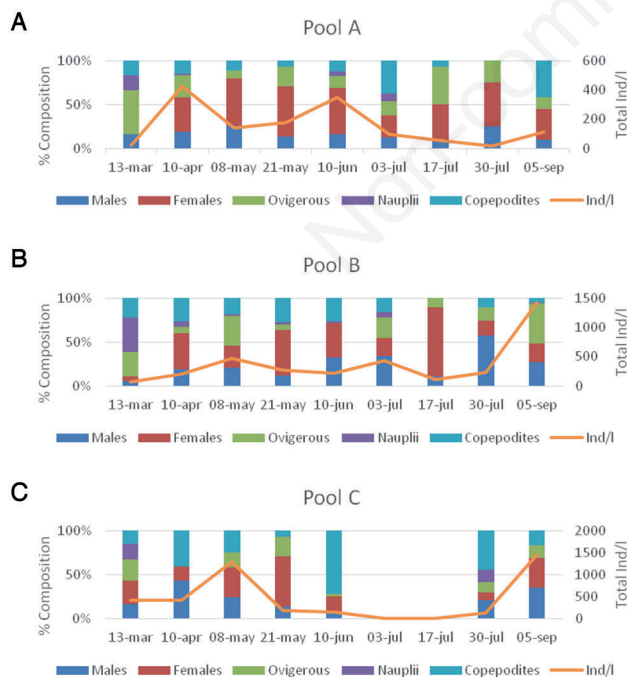


Figure 3. Percent composition of *Tigriopus fulvus* population categories in A) pool A, B) pool B and C) pool C coupled with total individual density (Ind/l) in the sample (March-October 2014).

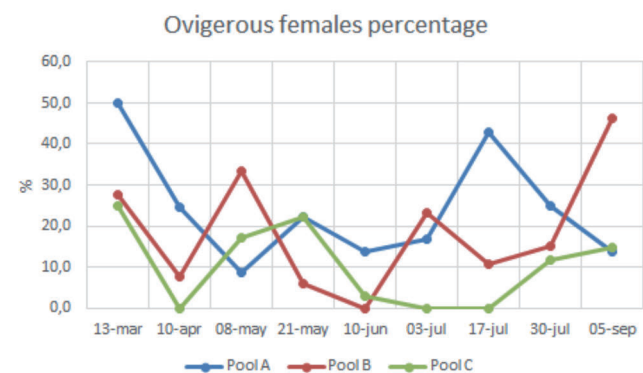


Figure 4. Ovigerous females percentage in *Tigriopus fulvus* populations of pools A, B and C.

hypothesis. Pool A lower population densities did not allow a significant statistical data treatment.

The opportunistic behavior as r-specialist showed in this study by the harpacticoid copepod *T. fulvus* seems to be triggered and sustained by rainfall. *T. fulvus* survival during warmer months seems to rely on several factors, as for this species lethal temperature and salinity seem to be, moreover, directly correlated.⁴⁰ In a context of climatic changes, wild populations of extreme environments such as splashpool copepods might be carefully monitored to locate possible local climate change effects.

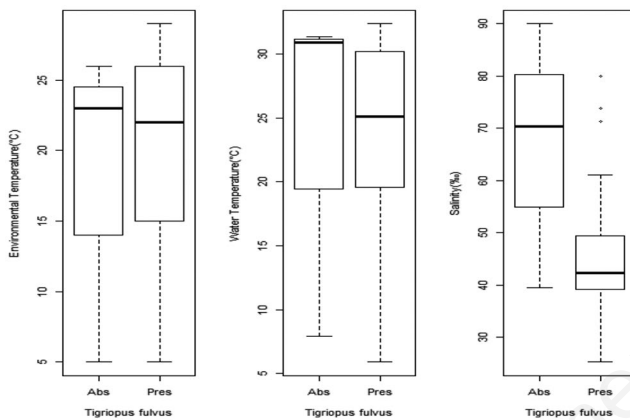


Figure 5. Presence/Absence of *Tigriopus fulvus* in the study area and environmental factors of pools A, B and C.

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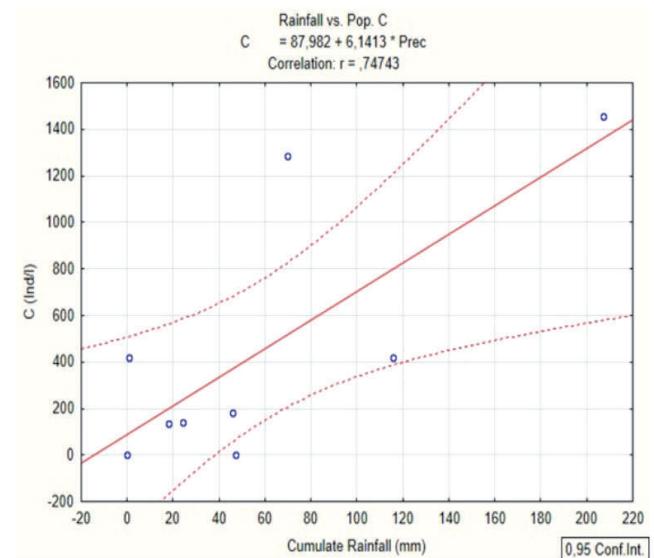
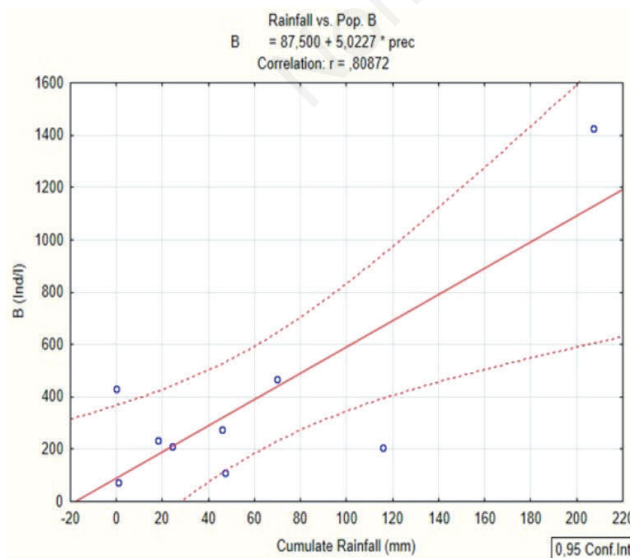


Figure 6. Spearman rank correlation between cumulate rainfall and *Tigriopus fulvus* populations (March-October 2014).

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