**RESEARCH ARTICLE**

# Productivity in natural and artificial habitats in brackish water systems: an example from *Aphanius fasciatus* populations

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## Abstract

1 - Venice Lagoon is characterized by many different shallow-water habitats, where salt marshes represent important habitats for many nektonic species. On the other side, small lagoon islands show a typical landscape dominated by agricultural gardens and orchards, that still host small ditches once used in traditional fishery activities but now abandoned.

2 - Aims of this study is to determine the productivity of these habitats for the nektonic community, referring to the small teleost *Aphanius fasciatus* (Valenciennes, 1821), listed in Annex II of 'Habitat' Directive 92/43/CEE.

3 - One year monthly samplings were performed in four sites: two natural salt marshes and two systems of artificial ditches. All collected fish were photographed and then released. Only a sub-sample was preserved for laboratory analysis.

4 - Data show that artificial habitats host higher biomass of *A. fasciatus*, yielding higher secondary production respect to natural salt marshes, while P:B ratios vary in accordance to different environmental features and the related life history strategies adopted by the four populations.

5 - Results of this study highlight the relevance of these artificial habitats in the conservation of an important resident fish species.

Keywords: *Aphanius fasciatus*, secondary production, Venice lagoon, conservation.

## Introduction

The Habitats Directive (92/43/EEC) aims to ensure "biodiversity through the conservation of natural habitats and of wild fauna and flora in the European territory". Coastal areas, wetlands and estuaries in particular suffer of major threats, due to loss and alteration of natural habitats (Ibanez *et al*., 2000; Kennish, 2002; Airoldi and Beck, 2007). Within the Venice lagoon, a severe reduction of salt marsh areas was registered during the last century, from about 120 km² at the beginning of the XIX century to about 40 km² today (Molinaroli *et al*., 2009; Franco et al., 2010).

Salt marshes represent important habitats, in particular for many species of birds, fishes and invertebrates. With the current knowledge (Malavasi *et al*., 2004; Franco *et al*., 2006; Franzoi *et al*., 2010), among the

nekton community of the Venice lagoon, four species are listed in Annex II of habitat directive. They are the killifish *Aphanius fasciatus* (Valenciennes, 1821), two species of small gobies, *Pomatoschistus canestrinii* (Ninni, 1883) and *Knipowitschia panizzae* (Verga, 1841), and the shad *Alosa fallax* (Lacepede, 1803).

The two species of small gobies and the killifish belong to the eco-functional guild of estuarine residents, in agreement with Malavasi *et al*. (2004) and Franco *et al*. (2006). The whole life cycle of these species is spent within the lagoon, and their bio-ecology is strictly associated to the typical habitats of transitional aquatic environments. The availability of these essential habitats, providing both protection from predators and abundant trophic resources, are considered of great importance for fish populations in estuarine environments (Beck *et al*., 2001; Cabral *et al*., 2007; Martinho *et al*., 2007). This is particularly true for those resident species that play a key role within estuarine food webs, due to their intermediate position (Leitao *et al*., 2006; Dolbeth *et al*., 2007), linking the benthic compartment to higher trophic levels.

Since one of the main goal of the Habitat Directive is "to ensure the restoration or maintenance of natural habitats and species of Community interest at a favourable conservation status", the analysis of the bio-ecology of protected species becomes a fundamental tool in this context, in order to evaluate the viability of the populations considered. Both anthropogenic pressures and natural variability control the status of resident fish populations within estuarine habitats, and their conservation depends mainly on environmental quality (Nicolas *et al*., 2007). In this perspective, secondary production estimates are quite relevant, since they integrate many different driving forces, influencing both individual growth and population mortality (Cusson and Bourget, 2005; Dolbeth *et al*., 2005). Thus, information about habitat productivity constitutes a useful tool in decision-making processes regarding management and protection measures (Dolbeth *et al*., 2008).

According to Gandolfi *et al*. (1991), *A. fasciatus* is the only Italian species of the family Cyprinodontidae (Cyprinodontiformes), the so-called killifishes. They are characterized by small size and short life cycle. The word kill, literally ditch in old Dutch, designates small water bodies, such as fresh- and brackish-water creeks and rivulets, which are habitats of election for these fish. Among the three species mentioned as estuarine resident, *A. fasciatus* appears to be the most adapted to brackish environments characterised by extremely shallow depth, high instability and marked spatio-temporal fluctuations of the main environmental parameters (Tryantafillidis *et al*., 2007). In the lagoon context, previous surveys of the fish community have highlighted the relationship of the species with salt marsh habitats (Malavasi *et al*., 2004; Franco *et al*., 2006). Furthermore, this species could play a key role within the trophic web of transitional waters, as a link from the benthic food web to higher trophic levels (i.e. birds and piscivorous fish).

Aim of this study is to estimate the secondary production of four *A. fasciatus* populations from both natural and artificial habitats within the Venice lagoon, in order to evaluate the conservation importance of artificial habitats.

**Materials and methods**

Samplings were conducted in four shallow water creeks of the Venice lagoon (Figure1). Two sites (CA and CO) were situated within natural salt marsh systems, while the remaining two (AL and VI) were man-made ditches within lagoon islands.

One year nearly monthly samplings were performed in the four sites with a small beach
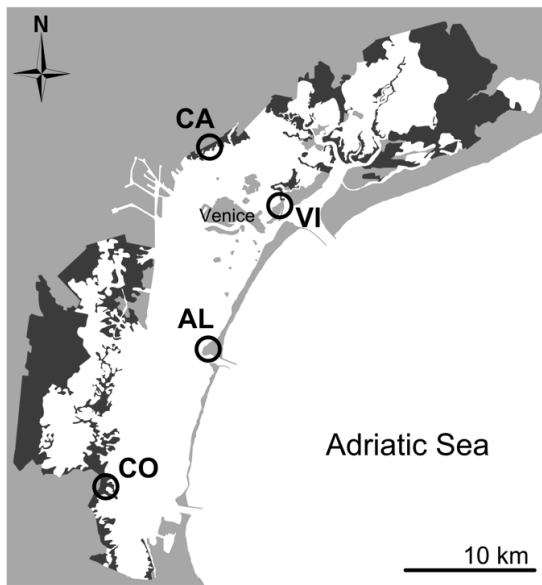
Figure 1. Location of the four sampling sites within the Venice lagoon.

seine net (8 metres length, 2 mm distance knot to knot). In each sampling occasion, three replicates were performed during the peak of spring high tide. Water temperature (digital thermometer, ±0.1 °C), salinity (optical refractometer, ±1 psu), dissolved oxygen (Winkler method, ±0.1 mg L$^{-1}$ subsequently converted in saturation percentage) and turbidity (portable nephelometer, ±0.1 ftu) were recorded during each sampling. In June, three cores of sediment (Ø 3 cm) were collected to determine the content of organic matter through the loss on ignition method, according to Heiri et al. (2001). All fish collected were photographed and then released. A subsample of 60 *A. fasciatus* (sex ratio 1:1) were sacrificed with excess of 2-phenoxyethanol and then preserved in buffered formaldehyde for laboratory analysis.

Preserved specimens were sexed, measured to nearest 0,1 mm (standard length), eviscerated and weighed (precision 0.01 grams). For each site, at least 30 specimens of both sexes were used to age determination. In each site,

length-weight regressions were calculated for both sexes. Differences between sex and across sites were tested by means of ANCOVA. Scales removed from the left side of the body, between the end of the thoracic fin and the beginning of the dorsal fin, were mounted on glass slides and age was determined according to the methodology proposed by Leonardos *et al.* (1996). Von Bertalanffy growth equations for the four population, separately for males and females, were calculated by fitting length-age data by means of non linear least square regression, as proposed by Kuparinen *et al.* (2011), using PAST software (Hammer *et al.*, 2001).

This allowed the estimate of individual weight and age for all fish sampled, limiting the number of sacrificed fish.

Secondary production was calculated according to the instantaneous growth method proposed by Ricker (1946), following recommendations of Waters and Crawford (1973) and Chapman (1978). Production P (g m$^{-2}$ year$^{-1}$) was calculated as

$$P = \sum_{c=1}^{n} \left( \sum_{t=1}^{n} G_c^t \cdot \sum_{t=1}^{n} \overline{B_c^t} \right)$$

where $G_c^t$ is the instantaneous growth rate for cohort c at time t and $\overline{B_c^t}$ is the corresponding standing stock at time t.

Instantaneous growth rate was calculated as

$$G_c^t = ln \frac{\overline{W_c^t}}{\overline{W_c^{t-1}}}$$

where $\overline{W_c^t}$ and $\overline{W_c^{t-1}}$ are mean weight for cohort c at time t and t-1. Standing stock was calculated as

$$B_c^t = \frac{D_c^{t-1} \cdot \overline{W_c^{t-1}} + D_c^t \cdot \overline{W_c^t}}{2}$$

where $D_c^{t-1}$ and $D_c^t$ represent densities of cohort c at time t-1 and t.

Table 1 - Annual mean values of the four water chemico-physical parameters and the content of organic matter in the sediment (measured in June). Numbers in italics show the minimum and the maximum values registered.

| Parameter | AL | CA | CO | VI |
|-----------|-----|-----|-----|-----|
| Temperature | 16.4±1.43 | 16.3±1.32 | 17.7±1.58 | 17.6±1.51 |
| (° C) | *6.1-27.8* | *0.9-29.5* | *0.9-33.8* | *4.1-33.0* |
| Salinity | 35±1 | 22±1 | 21±1 | 26±1 |
| (psu) | *30-40* | *12-32* | *12-32* | *14-39* |
| Dissolved oxygen | 127.7±14.5 | 82.2±5.1 | 77.6±6.5 | 107.6±11.4 |
| (% of saturation) | *64.4-339.4* | *41.8-160.7* | *14.1-150.4* | *11.1-255.4* |
| Turbidity | 3.1±0.7 | 9.6±1.6 | 19.4±5.4 | 7.6±1.1 |
| (ftu) | *0.0-10.7* | *1.0-50.0* | *2.3-137.0* | *1.0-23.4* |
| Organic matter | 7.2±1.4 | 14.1±0.9 | 7.2±0.6 | 15.4±0.5 |
| (%) | *2.6-11.0* | *11.6-16.5* | *5.2-8.4* | *13.0-16.8* |

**Results**

The measurement of the main chemico-physical parameters allowed to highlight some differences among sites (Table 1). In particular, a lower salinity was recorded, together with lower dissolved oxygen and higher turbidity in the natural salt marsh systems respect to the artificial ditches. On the contrary, the content of organic matter (Table 1) in the natural salt marsh of CA and in the artificial site of VI resulted to be nearly double than that measured in the other two sites.

Length-weight relationships were calculated for the four populations, separately for males and females (Table 2). ANCOVA revealed significant differences for both factors (Table 3). Comparing regression slopes, the only non-significant difference was found between females from AL and CO (ANCOVA followed by post-hoc test after Bonferroni correction, p > 0.05). Again, in AL non-significant differences were found between the two sexes.

The four populations were composed by five to seven age classes, with the fish from artificial habitats showing a higher longevity. Recruitment did not occur in one single occasion, but it was extended from May to September. Differences among sites were found also considering the parameters obtained from the Von Bertalanffy growth curves (Table 4), separately for the two sexes. For females, CO exhibited the highest growth rate (0.73 years$^{-1}$) while CA the lowest (0.35 years$^{-1}$). The same value of 0.49 years$^{-1}$ was found in AL and VI. Theoretical maximum length showed a different pattern, with a maximum

Table 2 - Coefficients of the regressions between standard length and eviscerated weigth for the four populations, calculated separately for males and females.

| Site | Sex | a | b | r² |
|------|-----|-----|-----|-----|
| AL | female | -4.51 | 2.91 | 0.97 |
|    | male | -5.11 | 3.29 | 0.99 |
| CA | female | -4.61 | 3.00 | 0.93 |
|    | male | -5.12 | 3.31 | 0.98 |
| CO | female | -4.34 | 2.80 | 0.93 |
|    | male | -5.35 | 3.47 | 0.98 |
| VI | female | -4.55 | 2.96 | 0.96 |
|    | male | -5.10 | 3.28 | 0.98 |

Table 3 - Results of ANCOVA performed on length-weight relationships testing differences in sex and site

|            | SS       | d.f. | MS       | F        | p        |
|------------|----------|------|----------|----------|----------|
| Intercept  | 123.9632 | 1    | 123.9632 | 31040.59 | $p < 0.05$ |
| logSL      | 111.1127 | 1    | 111.1127 | 27822.81 | $p < 0.05$ |
| Site       | 0.1217   | 3    | 0.0406   | 10.16    | $p < 0.05$ |
| Sex        | 0.7579   | 1    | 0.7579   | 189.79   | $p < 0.05$ |
| Site x sex | 0.0718   | 3    | 0.0239   | 5.99     | $p < 0.05$ |
| Error      | 4.5088   | 1129 | 0.0040   |          |          |

of 60.1 mm in VI and a minimum of 38.3 mm in CO, while AL and CA presented intermediate values (respectively 52.1 and 53.9 mm).

As regards males, CO showed the highest growth rate ($0.60$ year$^{-1}$), while the minimum value was recorded in VI ($0.29$ year$^{-1}$). Values slightly different were calculated for AL ($0.35$ year$^{-1}$) and CA ($0.34$ year$^{-1}$). Similarly to what described for females, CO and VI males exhibited minimum (35.8 mm) and maximum (53.4 mm) values of theoretical maximum length respectively. In AL and CA a more marked difference has been found between males than in females (respectively 49.5 mm and 41.0 mm).

As regards *A. fasciatus* secondary production (Figure 2), in general, artificial habitats resulted to be more productive than natural marshes. In particular, VI presented the highest value ($12.56$ g m$^{-2}$ year$^{-1}$), an order of magnitude higher than that estimated in the other three sites.

The lowest value was calculated for CO ($1.62$ g m$^{-2}$ year$^{-1}$), while AL and CA showed intermediate values (5.32 and 3.10 g m$^{-2}$ year$^{-1}$). Annual standing stock values presented the same pattern. Considering P:B values, CO and VI are located at the extremes again, with the highest (2.05) and lowest (0.87) values respectively, while intermediate values were found in AL (1.86) and CA (1.23).

**Discussion**

This study provides, for the first time, information about secondary production of *A. fasciatus*. Results, obtained through the comparison of four populations from both natural and artificial habitats, suggest a key role of some artificial habitats for the conservation of this species within the Venice lagoon. In the last century coastal areas, and transitional waters in particular, suffered of habitat loss and alteration (Kennish, 2002). This has been considered the major threat for the survival of *A. fasciatus* populations

Table 4 - Von Bertalanffy parameters for the four populations, calculated separately for males and females.

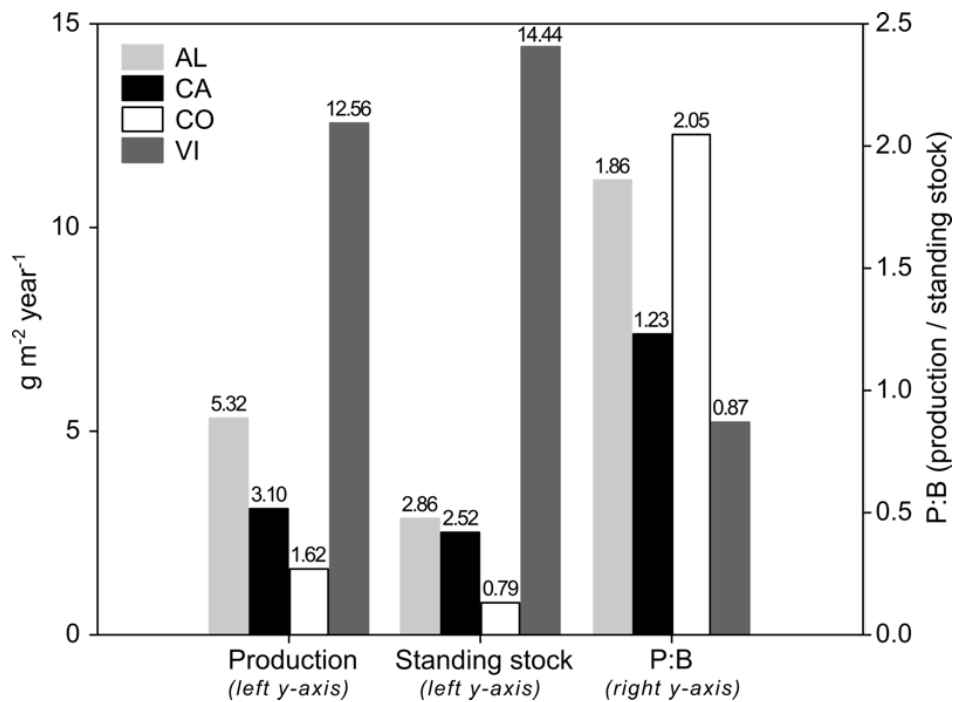|      |     | Female |              |                  |     | Male   |              |                  |
|------|-----|--------|--------------|------------------|-----|--------|--------------|------------------|
| Site | *n* | $t_0$ mm | $L_\infty$ mm | $K$ year$^{-1}$ | *n* | $t_0$ mm | $L_\infty$ mm | $K$ year$^{-1}$ |
| AL   | *38*  | -0.82 | 52.1 | 0.49 | *50* | -0.62 | 49.5 | 0.35 |
| CA   | *135* | -1.15 | 53.9 | 0.35 | *54* | -1.25 | 41.0 | 0.34 |
| CO   | *64*  | -0.91 | 38.3 | 0.73 | *59* | -0.53 | 35.8 | 0.60 |
| VI   | *127* | 0.07  | 60.1 | 0.49 | *63* | -0.56 | 53.4 | 0.29 |

Figure 2. Annual production, standing stock and P:B ratio values for *A. fasciatus* populations in the four sites.

(Bianco, 1995). Thus, artificial creeks, with structural features resembling the natural habitats of the species, may play an important function in maintaining healthy and abundant populations. This has been evaluated through the study of secondary production, obtaining comprehensive information about population status, since secondary production represents a composite measure of ecosystem function (Benke, 1993).

A great number of studies concern killifishes, but only a few deals with population productivity in estuarine habitats. Those available (Valiela *et al*., 1977; Meredith and Lotrich, 1979; Perez-Espana *et al*., 1998; Teo and Able, 2003) concern Atlantic populations, thus it is difficult to compare results of this study with other similar situations within the Mediterranean basin.

As set forth above, coastal lagoons, and salt marsh habitats in particular, are particularly productive ecosystems. The values of

secondary production calculated for *A. fasciatus* in the four sites are comparable with those found for the killifish *Fundulus heteroclitus* (Valiela *et al*., 1977; Teo and Able, 2003) and *F. Parvipinnis* (Perez-Espana *et al*., 1998), but they are well below the 40.7 g m$^{-2}$ y$^{-1}$ calculated for *Fundulus heteroclitus* in Delaware by Meredith and Lotrich (1979). Considering the same geographical area, slightly lower values were found by Franco *et al*. (2012) for two small gobies in the lagoon of Venice (0.6 - 1.0 g m$^{-2}$ y$^{-1}$). Nevertheless, the two natural marshes were still less productive than the two artificial habitats, perhaps for the non-continuous habitat use of the species, due to tidal cycles and seasonal rhythms, that influence also fish mortality. Natural creeks are, in fact, intertidal habitats, forcing *A. fasciatus* to move towards deeper waters during low tides, where larger piscivorous fish are present. On the contrary, in the two

artificial sites fish find refuge from aquatic predation even during low tide, as confirmed by a higher survival rate (Cavraro *et al.*, unpublished data).

The values calculated are however consistent with population structures and dynamics estimated for the four populations. A population composed of small-sized, fast-growing individuals, as that of CO, presented in fact the lowest values of production but the highest P:B ratio. On the other side, in CA production value was double than in CO, while P:B was lower, according with a bigger size and a slower growth rate. The same pattern shown between CA and CO was found in AL and VI, but in the artificial sites a lower mortality and a continuous use of the habitat led to higher secondary production, even of an order of magnitude higher in VI than in the other sites. Water chemico-physical characteristics may not be determinant in influencing the dynamics of *A. fasciatus* populations, due to the high tolerance of the species (Tryantafyllidis *et al.*, 2007). Despite this, the mean lower values of salinity in the two natural sites could determine more stressful conditions for the species, that seems to be adapted to high salinity habitats. Also the lower content of dissolved oxygen could contribute to the differences observed. Anyway, differences in productivity between similar habitats (natural or artificial) may be explained by their trophic richness. The higher production found in CA, respect to CO, and in VI, compared to AL, reflect the differences in the percentage of organic matter within the sediment. CA and VI showed a content of sediment organic matter double than AL and CO. Considered the importance of the benthic compartment among energy fluxes in shallow-water transitional habitats, habitat trophic richness could be measured by the content of organic matter within the sediment (Howes *et al.*, 1984; Odum, 1988; Mitsch and Gosselink, 2000). Furthermore, *A. fasciatus*

feeds mainly on small benthic invertebrates (Leonardos, 2008), whose abundance depends on sediment organic content (Kevrekidis 2005, Figuereido-Barros *et al.* 2006). Thus, the level of environmental basal resources, as sediment organic matter, may constraint the higher trophic levels represented by fish populations.

Thus *A. fasciatus* secondary production, estimated in four sites of the Venice lagoon, reflected the differences found in population structure and in the local environmental pressures, suggesting the importance of artificial habitats for the preservation of the species.

Furthermore, production values estimated in this study highlight the role of *A. fasciatus* within the estuarine food web. Due to its local high abundances, this species could play a key role within the energy fluxes of the lagoon, connecting some highly productive areas with other habitats, and exporting the energy produced by the benthic compartment in these sites to higher trophic levels.

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