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Distribution and Biology of *Aphanius fasciatus* (Actinopterygii, Cyprinodontidae) in The Isonzo River Mouth (Friuli Venezia Giulia, Northeast Italy)

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Abstract

The aim of the study is to investigate *Aphanius fasciatus* biology in the northern section of its distribution area. Within the Natural Regional Reserve of the Isonzo River Mouth, 8 sampling sites were monitored between late spring (May) and autumn (November) in 2015 and 2016: 257 specimens were collected, and five age classes were determined by scale reading (0+ to 4+). No significant differences were observed for total length and weight between sexes. Sex ratio was 2.37 and favored females. Seven meristic characters were analyzed on 60 specimens found dead: values showed no significant differences between sexes, except for the number of vertical flank bars in 0+ and 1+ age classes. The gonadosomatic index was analyzed on 33 specimens, showing maximum values in June for females (11.44%) and in July for males (5.74%). Values decrease over following months and reaching a minimum in late August for females (0.42%); no data were available for males in August. Histological analyses suggest that *A. fasciatus* is a multiple serial spawner: the reproductive period starts in June, while the first recruitment phase occurs in August. Female gonads showed asynchronous development, with germinal cells at each maturation stage, but no dominant phases were observed.

Introduction

Mediterranean banded killifish *Aphanius fasciatus* (Valenciennes 1821) is listed among the "Least Concern" species in the IUCN Red List (Rondinini, Battistoni, Peronace & Teofili, 2013) and reported in the Annex II of the European Habitat Directive (92/43/CEE). It is distributed along the coastal waters of the Mediterranean basin except for the Iberian Peninsula, where the congeneric *Aphanius iberus* can be found (García-Alonso, Ruiz-Navarro, Chaves-Pozo, Torralva & García-Ayala, 2009), and in the easternmost part of the Mediterranean basin (in the Middle East), where Frenkel and Goren (1999) reported the presence of *Aphanius dispar*. The distribution area of *A. fasciatus* includes Greece (Leonardos, Sinis & Petridis, 1996; Leonardos & Sinis, 1998; Leonardos & Sinis, 1999), Tunisia (Kraiem, 1983), Malta (Deidun, Arcidiacono, Tigano & Shembri, 2002) and Egypt near the Suez Canal (Villwock, 1985; Lotan & Ben Tuvia, 1996), while Kottelat & Freyhof (2007) indicate populations in several Mediterranean islands, except for Crete (Bianco, Cavraro, Zanatta, Pastres & Malavasi, 1996). In the Adriatic Sea, the species was observed along the coast of Croatia, Slovenia and Albania (Mrakovčić et al., 2006; Jardas, Pallaoro, Vrgoč, Jukić-Peladić & Dadić, 2008; Pano, Lazaridou & Frasheri, 2008; Valdesalici, Langeneck, Barbieri, Castelli & Maltagliati, 2015). The Mediterranean killifish is also present along Italian coastlines, even though populations are isolated and

discontinuously distributed due to paleogeography and habitat suitability (Mordenti, Trentini, Bastoni, Savoia & Scaravelli 2008). Killifish presence is reported in the northernmost part of the Adriatic Sea (Cavraro *et al.,* 2011; Franco *et al*., 2006a; Franzoi, Franco & Torricelli, 2010), the northern and the middle portion of the Tyrrhenian Sea (Valdesalici *et al.,* 2015), Sicily and Sardinia (Ferrito & Tigano, 1996).

Populations of *Aphanius fasciatus* in the Adriatic Sea and in Sicily show a wide range of morphological and functional variability (Tigano & Ferrito, 1984; Ferrito, Mannino, Pappalardo & Tigano, 2007) compared to Tyrrhenian and Sardinian communities, with highly divergent populations. This variability may result from the complete isolation of brackish-water habitats and the highly selective environmental features (Maltagliati, 1999; Cimmaruta, Scialanca, Luccioli, F & Nascetti, 2003). Killifish life cycle develops in lagoon ecosystems and its biology is strictly related to transitional water environments, which allow high trophic resources and protection against predators (Malavasi *et al*., 2004; Franco *et al*., 2006a; Cavraro *et al*., 2011). Even though some populations were also found in riverine ecosystems (Ferrito & Tigano, 1996; Duchi, 2006), *A. fasciatus* lives in saltworks, brackish habitats and lagoons with high salinity levels (up to 65‰) and can tolerate a wide range of temperature variation, between 4 and 40°C (Leonardos & Sinis, 1998). Particularly during the reproductive season, killifish exhibits a gregarious behavior and shows preference for habitats characterized by shallow depths, abundant vegetation and low dissolved oxygen concentration. Overall, *A. fasciatus* appears to have adapted to the high instability and marked spatio-temporal fluctuations of the main environmental parameters (Gandolfi, Zerunian, Torricelli & Marconato, 1991; Cavraro, Daouti, Leonardos, Torricelli & Malavasi, 2014a): it is a eurythermal and euryhaline species and its reproductive biology is strictly related to selected habitats, i.e. emerged shoals bordering coastal saltmarsh (Franco *et al*., 2006b; Cavraro *et al*., 2011; Franzoi *et al*., 2010). Due to the high relevance of brackish environments as biodiversity hot spots and buffer areas against extreme and adverse weather events, *A. fasciatus* is considered as an "umbrella species" for these environments (Simberloff, 1998; Roberge & Angelstam, 2004; Valdesalici *et al*., 2015). Despite its importance, there is still a lack of knowledge about the biology of killifish and in particular of its reproduction, which was previously studied by Leonardos & Sinis (1998) and by Leonardos (2008) in Greece. In this context, it was deemed of interest to study an *A. fasciatus* population in the northernmost section of its distribution area, within a protected zone (Natural Reserve Isonzo River Mouth, Northeast Italy) with the aim to provide useful information for conservation, control and management of populations, especially for protected zones such as the selected study area.

Material and Methods

Study area

The present work was carried out within the Natural Reserve of the Isonzo River Mouth, partially included in the Special Protection Area and Special Area of Conservation IT3330005 (Isonzo River Mouth – Cona Island), where the northernmost wetland system of the Mediterranean basin is placed between the high and rocky coasts of the Karst area and the sandy sedimentary system of the Po/Veneto littoral region (Perco, Simonetti & Venturini, 2006). Within the brackish system near the Isonzo River mouth, eight sampling sites were selected (Fig. 1) on the basis of their biotic and abiotic features. Data regarding salinity (Practical Salinity Units, psu) and water temperature (°C) were used to produce maps in order to identify gradients within the study area (Table 1). QGis software (version 2.18.2) and ordinary Kriging interpolation method were used for this purpose (Oliver & Webster, 2007). Data were provided by the Regional Agency for Environmental Protection of Region Friuli Venezia Giulia (www.arpa.fvg.it). Biotic characterization was considered analyzing different habitats reported for the area in the Habitat Maps of Friuli Venezia Giulia (www.regione.fvg.it-a), based on the habitat classification provided by Poldini *et al.* (2006) (Table 1). The type of substrate was also considered, following the Udden-Wentworth classification (Wentworth, 1922). Ease of access to sampling sites was carefully assessed by the analysis of the bathymetry map (www.regione.fvg.it-b) to ensure the feasibility of sampling operations.

All activities described in the present study were performed by permission of the Safeguard Fisheries Authority of the Region Friuli Venezia Giulia, as requested by local laws. Experimental procedures were carried out according to the Guidelines of the European Directive 2010/63/EU on the protection of animals used for scientific purposes and the principle of the 3Rs was applied. According to the Italian law at the time of this study (D. Lgs. 26/2014), which applies the European Directives, the protocol in use does not require an authorization for the use of animals (art. 2, d).

Fish sampling

Samplings were performed during May, June, August and November of 2015 and 2016. In 2016, an additional sampling was performed in July at site 6, where *A. fasciatus* population was more abundant and always present. Sampling periods were chosen in agreement with a study by Mordenti, Di Biase, Zaccaroni, Bastone and Scaravelli (2010) and Cavraro Zucchetta, Torricelli and Malavasi (2013), which report a higher percentage of adults between April and November. Specimens were collected during the low

tide peak, using a beach seine net (10 m length, 1.2 m height, 2 mm knot to knot) dragged by personnel on an area equal to 300 m^2, allowing a quantitative sampling to obtain density data (ind m^{-2}) (Malavasi *et al*., 2005; Franco *et al*., 2006b). Collected specimens were anesthetized using tricaine methane-sulfonate (MS-222) (Topic Popovic, Strunjak-Perovic, Coz-Rakovac, Barisic & Jadan, 2012) to limit damages during the manipulation, before recording total length (L_T; x ± 0.1 cm) and total weight (*W*; x ± 0.1 g). Three scales were collected from each specimen for age determination. At the end of operations, specimens were released, generally without consequences for their vitality. However, as some individuals were found dead, they were used for meristic investigations, which were preformed analyzing seven meristic parameters suggested for species identification by Gandolfi *et al*. (1991), Doadrio, Carmona and Fernàndez-Delgado (2002) and Teimori, Esmaeili, Gholam, Zarei and Reichenbacher (2012): lateral line scales (*NLLS*), dorsal fin rays (*D*), anal fin rays (*A*), ventral fin rays (*V*), caudal fin rays (*C*), pectoral fin rays (*P*) and number of flank bars (*NRL*). Sex was easily determined due to sexual dimorphism showed during the reproductive period (Gandolfi *et al*., 1991).

At each sampling event, water temperature (°C), pH, conductivity (mS cm^{-1}) and dissolved oxygen (mg l^{-1} and percentage of saturation) were registered using field meters (HI 9033 conductivity meter; HI 9125 pH/ORP meter; HI 9147 dissolved oxygen meter; all instruments are manufactured by Hanna Instruments Inc., Woonsocket, Rhode Island, USA). Each parameter was measured in triplicate and the mean values were reported. In addition, percentage of vegetation cover was recorded.

Reproductive cycle

In order to define the reproductive cycle of *Aphanius fasciatus*, histological analyses were carried out on a subsample of the dead specimens collected in June, July and August 2016. After sampling operations, specimens were immediately brought to the laboratory where the fresh gonad weight (*x* ± 0.0001 g) was measured with an analytical balance immediately after dissection and the gonadosomatic index (*GSI* %) was calculated (Anderson, Richard, Gutreuter &Stephen, 1983):

$$GSI = \frac{gonadal\ weight}{total\ weight} \times 100$$

Gonads were fixed in a solution of 2.5% glutaraldehyde, 0.8% paraformaldehyde and 7.5% saturated aqueous solution of picric acid in 0.15 M PBS, pH 7.4, with 1.5% sucrose. After an overnight fixation samples were washed in 0.15 M PBS, pH 7.4, and post-fixed in 1% osmium tetroxide in the same buffer, serially dehydrated in ethanol and embedded, via propylene oxide, in Embed812/Araldite (Electron Microscopy Sciences, Fort Washington, PA). Cross-sections (2 µm) were stained with toluidine blue and examined with an Olympus BX50; images were acquired with a digital Olympus E-P1 camera. Image analyses were performed with the open-source software ImageJ 1.50i (Schneider, Rasband & Eliceiri, 2012). Oogenesis stages were identified in agreement with Uribe, Grier, Garcia-Alarcon and Parenti (2016) and by Domínguez-Castanedo and Uribe (2019), using terminology reported by authors for other Cyprinodontiformes and by Brown-Peterson *et al*. (2011) for standardized terminology.

Statistical analyses

The Spearman rank correlation coefficient (ρ_s) was used to relate the densities of *A. fasciatus* observed in the sampling sites with abiotic features. Significant differences for biometric and meristic parameters between males and females of different age classes were identified using the non-parametric Mann–Whitney U test. The relationship between total length (L_T) and total weight (*W*) was examined by non-linear regression for both males and females (Ricker, 1975).

$$W = a\ {L_T}^b$$

The difference was tested by ANCOVA application after log-transformation to compare slopes of linearized regressions (Zar, 1984; Leonardos & Sinis, 1999). A pairwise comparison with Wilcoxon rank sum test and Bonferroni's correction was used to investigate differences in *GSI* values among sampling months. Analyses were carried out using StatSoft STATISTICA 7.1 software, with a $P< 0.05$ (α=0.05) used to interpret significance for all tests.

Results

A total of 257 specimen of *Aphanius fasciatus* were found; density values are reported in Table 2. Killifish was observed only at sites 2, 4, 6 and 8 during the 2015 campaign, but only in site 6 population was numerically consistent and always present. At each site, killifish was not observed in May. During 2016, *A. fasciatus* was collected exclusively at site 6 and was also absent in May.

Values of chemical and physical parameters recorded during the two years of the study are reported in Table 3. Data regarding July 2016 (all sites) and November 2016 (site 1) are missing, due to instruments malfunctioning. Site 6 showed the widest range in temperature values, both during 2015 and 2016 (ΔTemp=14.7 and 14.5°C, respectively). The same site showed a vegetation cover between 45 and 75% and a finer substrate granulometry than other sites (Table 1). Spearman rank correlation coefficient (ρ_s) highlighted

positive correlation between *A. fasciatus* densities and percentage of vegetation cover (ρ_s = 0.272; *n*=64; *P* < 0.05) while a negative correlation was observed with substrate granulometry (ρ_s = –0.453; *n*=64; *P* < 0.001).

Biometric and Meristic Analysis

Sex was determined for 253 specimens (178 females and 75 males) belonging to five age classes (from 0+ to 4+). Classes 0+ and 1+ showed the highest number of individuals (Table 4). Females were higher in number in each age class and notably the oldest recovered specimen (class 4+) was a female (Table 4). Within each age class, L_T and *W* values did not differ significantly between males and females (Table 4), as confirmed by the length-weight regressions, which show clearly overlapped curves with similar trend (Table 5; Fig. 2).

Data regarding the main meristic characteristics obtained from dead specimens (*n*=60) were reported in Table 6. Once again, the values did not differ significantly between the two sexes for each considered parameter within each age class, except for the number of flank bars (*NRL*), which ranged from 7 to 11 in males and between 7 and 13 for females, only for 0+ and 1+ age classes (*P* <0.002).

Reproductive Cycle

Histological analyses were carried out on 33 dead specimens collected in June, July and August 2016. The gonadosomatic index (*GSI*) values for females and males are shown in Figure 3. In June females showed the maximum median *GSI* (6.61%), decreasing slowly in July (4.37%) and plummeting in August (0.94%). Pairwise comparisons of *GSI* over June, July and August using Wilcoxon rank sum test with Bonferroni's correction revealed that the *GSI* observed for August was statistically different from the one observed for June (*P*<0.05) but not from the one for July (*P*=0.1439).

The two ovaries appeared as elongated saccular organs, surrounded by the ovarian wall, located in the abdominal cavity dorsally to the intestine and having the same size and degree of maturation. The section of the ovary appeared circular, with a central lumen bridged by an irregular profile of lamellae on which the oocytes develop. Ovaries in August contained primary growth oocytes (Fig. 4a) while ovaries from June showed larger secondary growth oocytes (Fig. 4b). Primary growth oocytes were characterized by a numerical increase of the nucleoli, the appearance of cortical alveoli and lipid droplets. At the multiple nucleoli step, oocytes showed a mean diameter of 68±18µm (*n*=15) with a maximum of 104 µm. The cytoplasm was rather homogeneous and basophilic and small lipid droplets of up to 6µm in diameter began to appear in the perinuclear area (Fig. 4d). The follicle cells formed a squamous layer with a maximum thickness of 3 µm. At the perinucleolar step and cortical alveoli step, the mean diameter of oocytes was 127±18µm (*n*=26), with a maximum of 165µm. The perinucleolar step oocytes had a few scattered nucleoli in the circular nucleus, with a diameter of about 10µm (Fig. 4c). Only occasionally they were located in the periphery of the nucleus. At this stage, an initial reduction in the number of large light cortical alveoli in an intermediate position between the lipid droplets in the perinuclear area and the oolemma was observed, followed by a progressive increase (Fig. 4c). The oocytes at the circumnuclear oil droplets step showed a mean diameter of 233±38µm (*n*=28) with a maximum of 308 µm. The cytoplasm showed lipid droplets around the nucleus and, more externally, large cortical alveoli up to 30µm in diameter (Fig. 4d). During secondary growth stage, the oocytes completed the vitellogenesis phase, depositing gradually large amounts of yolk granules and increasing the lipid that was homogeneously distributed in the ooplasm. Additionally, at this stage of development the cubic epithelial layer of the granulosa and the squamous theca layer were structured, and the deposition of the chorion was evident. The early secondary growth step oocytes showed a mean diameter of 297±58.9µm (*n*=18) with a maximum of 377µm (Fig. 4e). The nucleus had an irregular profile due to the large accumulation of reserve material in the cytoplasm. Although the lipid droplets were still distinguishable to some extent, coalescence into larger oil droplets with a diameter of up to 60µm in was clearly observed (Fig. 4e). The lighter cortical alveoli also merged into larger vesicles of up to 40µm in diameter. The periphery of the cytoplasm was basophilic and small yolk globules were present (Fig. 5a). The chorion thickness was 1.5 µm, while the granulosa cells layer reached a height of about 12 µm. The formation of attachment filaments among the former and the latter was clearly observed (Fig. 4e). The late secondary growth step oocytes showed a mean diameter ranging from 554.9±91.9 µm (*n*=16) to a maximum of 697 µm (Fig. 4d, e; 5a). The most relevant aspect was the progressive fusion of cortical granules, the yolk globules and lipid droplets into a homogeneous mass. The chorion thickness was 4 µm. In sections almost tangential to the chorion, the formation of the attachment filaments about 0.8 µm thick among granulosa cells was evident (Fig. 5b). They emerged from the chorion at a distance of about 3.5 µm and they presented a length of about 35 µm (Fig 5b). The full growth oocyte step reached a mean diameter of 782±97 µm (*n*=16) with a maximum of 889µm (Fig. 4f). The profile was circular and the most of its volume was occupied by a large yolk mass. Some cortical alveoli remained at the vegetal pole whilst the remaining cytoplasm and the nucleus was located at the animal pole. Large oil droplets were still present between the cytoplasm and the yolk mass (Fig. 4f).

Discussion

Observed densities of *A. fasciatus* within the study area are related to the species behavior and ecology, as killifish prefers shallow waters and still/low running waters zones, with abundant vegetation (Gandolfi *et al*., 1991; Zerunian, 2004). Moreover, benthic submerged vegetation represents an elective spawning habitat for *A. fasciatus* (Kottelat & Freyhof, 2007) and provides protection against predators/competitors. In addition, some degree of protection may also stem from the harsh environmental conditions of some sites, as *A. fasciatus* shows high tolerance against wide variations of chemical-physical parameters (Valdesalici *et al*., 2015). The maximum age recorded in the present study (4+) is lower than that reported by Leonardos & Sinis (1999) (6+) and by Cavraro, Varin and Malavasi (2014b) (7+). On the other hand, and in agreement with the Authors, older specimens were always females, indicating a higher longevity. However, female L_T values were not higher than those observed for males within the same age class, in contrast with Mordenti *et al.* (2008), Annabi, Said and Messaoudi (2013), Rinaldi *et al*. (2014) and Brigolin, Cavraro, Zanatta, Pastres and Malavasi (2016). L_T values showed wide overlapping ranges among age classes, in agreement with Leonardos & Sinis (1999), probably due to the *A. fasciatus* reproduction strategy, whose phases occur several times over the summer months. With regards to the growth of *A. fasciatus* within the studied area, regression models obtained for males and females did not differ significantly, in contrast with Penaz & Zaki (1985), Annabi *et al*. (2013) and Rinaldi *et al*. (2014). On the other hand, coefficients reported in the present study are in agreement with ranges proposed for *A. fasciatus* by Leonardos & Sinis (1999). As reported by Leonardos *et al*. (1996), Leonardos & Sinis (1999), Cavraro *et al*. (2014a) and Rinaldi *et al*. (2014), a pronounced energy investment in somatic growth is observed in young organisms, followed by a decrease after the first age when specimens reach sexual maturation and slow down growth to cope with reproduction.

Sex ratio (2.37) favors females and was similar to values observed for killifish during the reproductive period by Penaz & Zaki (1985), Leonardos & Sinis (1999) and Annabi *et al*. (2013); interestingly, Mordenti *et al*. (2008) and Alcaraz, Gholami, Esmaeili and García-Berthou (2015) found lower values, although the sex ratio was again in favor of females. Higher number of females could be related to the male predation rates, because they could be more easily identified by predators due to bright sexual coloration (Leonardos & Sinis, 1999); in addition, male samplings could be more difficult than female collection, due to territorial behavior of males during the reproductive period (Gandolfi *et al.* 1991). Limited presence of adult male specimens could also reduce intraspecific competition between adults and new recruits for the use of resources (Leonardos & Sinis, 1999; Mordenti *et al*., 2010; Cavraro *et al*., 2011; Brigolin *et al*., 2016).

The results of the meristic analyses are in agreement with the ranges reported by Gandolfi *et al*. (1991); observed values did not show significant differences between males and females, except for the number of flank bars for 0+ and 1+; this result is likely due to high number of specimens recorded for these age classes. Number of flank bars is often used in studies about congeneric species (Ferrito *et al*., 2007) and about the same species (Cavraro *et al*., 2011), because sexes exhibit different colors: dark bars on a light background in females and light bars on a silvery background in males (Gandolfi *et al*., 1991; Kottelat & Freyhof, 2007). However, Ferrito *et al*. (2007) and Teimori *et al*. (2012) stated that this character does not allow to recognize without error the species of the genus *Aphanius*.

The morphology of the different oocyte stages of *A. fasciatus* generally matches the description found in the recent literature for the Cyprinodontidae (García-Alonso, Ruiz-Navarro, Chaves-Pozo, Torralva & García-Ayala, 2009; Monsefi, Shiva & Esmaeili, 2009; Uribe *et al*., 2016). However, the presence in *A. fasciatus* (Fig. 4c) of numerous nucleoli, typically located at the periphery of the nucleus at the perinuclear step, has previously never been observed. Semi-thin sections of ovaries post-fixed in osmium tetroxide allow to easily observe the presence of small lipid droplets already in the cytoplasm of oocytes at the multiple nucleoli step with a diameter of about 100 µm (Fig. 4d). Moreover, this methodology allows us to appreciate the formation of numerous attachment filaments that begin to be distinguishable from the early secondary growth step. These structures develop among the granulosa cells and emerge from the chorion regularly arranged over the entire oocyte surface (Fig. 5a, b). The filaments were never characterized in this genus of Cyprinodontidae (García-Alonso *et al*., 2009; Monsefi *et al*., 2009) and their presence was documented in the cyprinodontoid flagfish *Jordanella floridae* (Uribe *et al*., 2016). Attachment filaments were described in a range of teleosts (Giulianini & Ferrero, 2001; Riehl & Patzner, 1998) and they were described as structures that become sticky after contact with water, allowing the eggs to be fixed on the substrate (Riehl & Patzner 1998).

Conclusions

The aim of the present study was to improve the knowledge about some aspects of the biology of *Aphanius fasciatus* (listed in the Habitat Directive) in the northernmost portion of its distribution area. Results could provide useful information to better understand colonization behavior and reproductive cycle of *A. fasciatus* in transitional habitats, where correct survival strategies and tolerance against wide parameter variations (both biotic and abiotic) are of pivotal

importance. The killifish colonize habitats with complex ecological characteristics, likely because it finds refuge from predators, trophic resources in absence of competitors and elective spawning sites. Higher densities of *A. fasciatus* observed in sites with similar features highlight the importance of these habitats for the conservation of natural resources, as reported by Leonardos & Sinis (1998) and by Cavraro *et al.* (2011). These areas clearly require a careful management and a constant monitoring activity.

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Table 1. Characteristics of the sampling sites: geographical coordinates, habitats (MI1: well-graded fine sand biocenosis; MI5: surface or shallow waters fine sand biocenosis; MI6: muddy sand surface biocenosis), ranges for annual salinity and water temperature (Temp). Values of salinity and water temperature were obtained with ordinary Kriging interpolation method (Oliver & Webster, 2007), using QGis software.

Site	Coordinates	Habitat	Substrate	Salinity (psu)	Temp (°C)
1	45°42'54.22"N - 13°31'10.36"E	MI1	mud	35.83-36.29	≤16.5
2	45°43'44.71"N - 13°33'58.93"E	MI5	sand	34.92-35.38	>17.3
3	45°43'41.98"N - 13°33'52.08"E	MI5	sand + gravel	34.92-35.38	>17.3
4	45°43'35.78"N - 13°33'32.72"E	MI1	sand	34.92-35.38	>17.3
5	45°44'12.54"N - 13°32'48.23"E	MI1	sand	35.38-35.83	16.9-17.3
6	45°44'52.51"N - 13°31'5.97"E	MI6	mud	35.38-35.83	≤16.5
7	45°45'56.10"N - 13°31'40.26"E	MI5	sand	35.38-35.83	≤16.5
8	45°45'57.30"N - 13°31'30.74"E	MI6	mud + sand	35.38-35.83	≤16.5

Table 2. Densities (ind m^{-2}) of *A. fasciatus* within the study area. Missing sites mean that the species was absent at every sampling event.

	2015				2016
	2	4	6	8	6
May	-	-	-	-	-
June	-	-	0.037	-	0.310
July	-	-		-	0.170
August	0.020	-	0.130	0.023	0.033
November	-	0.003	0.123	-	0.007

Table 3. Values of chemical and physical parameters and vegetation covers recorded at every sampling event during 2015 and 2016. Annual excursions of temperature (ΔTemp) and conductivity (ΔCond) observed for each site are reported at the bottom of the table (Temp= water temperature; DO=dissolved oxygen; Cond=conductivity; Veg. cover= vegetation cover; Jun=June; Aug=August; Nov=November; - = missing value).

		2015								2016							
Site:		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
May	Temp (°C)	20.7	20.3	20.3	20.7	19.4	27.9	21.4	21.4	19.5	18.3	18.1	16	18.7	19.8	19	19
	DO (mg l^{-1})	11.2	9.3	9.3	7.6	9.9	10.5	9.8	9.8	10.8	10.2	10.1	10	12.1	9.8	8.8	8.8
	DO (%)	150	131	131	105	143	163	126	126	144	134	133	125	161	142	117	117
	pH	8.4	8.3	8.3	8.3	8.3	8.6	8.3	8.3	8.4	8.3	8.5	8.7	8.4	7.7	8.1	8.1
	Cond (mS cm^{-1})	49.5	47.5	47.5	13.4	52.9	39.2	33	33	34.8	38.4	33.5	15	35.4	35.5	32.1	32.1
	Veg. cover (%)	30	0	25	0	35	45	15	60	30	0	25	0	35	45	15	60
Jun	Temp (°C)	25.3	19.7	19.7	16	21	29.5	18.7	18.7	28.1	24.3	24.4	19.4	26.6	26.5	24.3	24.3
	DO (mg l^{-1})	9.4	6.6	6.6	5.8	6.7	6.4	6.1	6.1	7.8	6.9	6.9	6.7	8.5	8.5	7.2	7.2
	DO (%)	116	150	150	55	122	110	106	106	128	104	104	92	132	132	107	107
	pH	8.8	8.2	8.2	8.5	8.4	8.6	8.1	8.1	8.7	8.3	8.3	8.6	8.6	8.5	8.4	8.4
	Cond (mS cm^{-1})	16.3	40.1	40.1	3.9	33	30.1	22.7	22.7	39.8	40.8	40.8	7.8	43.4	32.1	39.4	39.4
	Veg. cover (%)	40	0	35	0	45	75	35	80	40	0	35	0	45	75	35	80
Aug	Temp (°C)	26.1	24.9	24.9	20.5	23.7	24.4	24	24	26.4	21.3	21.5	19.2	20.5	28.1	24.7	24.9
	DO (mg l^{-1})	9.7	8.5	8.5	8.3	9.8	6.2	10.5	10.5	7.9	6.9	6.9	6.9	6.7	6.8	6.7	6.7
	DO (%)	150	128	128	112	144	92	155	155	123	97	97	93	94	106	99	99
	pH	8.5	8.3	8.3	8.4	8.5	8.3	8.6	8.6	8.6	8.3	8.3	8.3	8.6	8.7	8.6	8.6
	Cond (mS cm^{-1})	36.2	32.8	32.8	11.6	40.1	35.2	39.5	39.5	51.4	46.9	46.9	12.7	46.4	46.1	52.9	52.9
	Veg. cover (%)	40	0	35	0	45	75	35	80	40	0	35	0	45	75	35	80
Nov	Temp (°C)	13.7	13	13	10.5	14.2	14.8	11.8	11.8	-	10.3	11	10.4	12.3	13.6	12.2	12.4
	DO (mg l^{-1})	15	11.9	11.9	11.1	13.5	13.9	11.8	11.8	-	8.7	8.7	9.2	8.6	8.8	8.9	8.9
	DO (%)	172	136	136	121	155	167	131	131	-	84.9	85.4	88.5	85.7	88.8	92.4	92.1
	pH	8.3	8.1	8.1	7.8	8.3	8.3	8.2	8.2	-	8.56	8.6	8.7	8.3	7.9	6.9	7.9
	Cond (mS cm^{-1})	44.4	44.6	44.6	12.7	42.2	40.7	37.4	37.4	-	1.3	1.4	1.5	7.7	19.9	10.5	10.7
	Veg. cover (%)	30	0	25	0	35	45	15	60	30	0	25	0	35	45	15	60
	ΔTemp (°C)	12.4	11.9	11.9	10.2	9.5	14.7	12.2	12.2	8.6	14	13.4	9	14.3	14.5	12.5	12.5
	ΔCond (mS cm^{-1})	33.2	14.7	14.7	9.5	19.9	10.6	16.8	16.8	16.6	45.6	45.5	13.5	38.7	26.2	42.4	42.2

Table 4. Values of total length L_T (cm) and weight W (g) of *A. fasciatus* males and females for each age class observed at the sampling sites during the study period.

Age class		Females		Males		Mann - Whitney test (Females *vs* Males)		
		L_T	W	L_T	W		L_T	W
0+	Mean	22.42	0.14	24.02	0.17			
	Median	23	0.14	24	0.15	*Z*	0.525	0.386
	SD	3.74	0.07	2.02	0.06			
	Min	15	0.04	20	0.07	*P*	0.524	0.386
	Max	32	0.49	29	0.32			
	CV%	16.67	50.29	8.42	37.51			
	n	99		51				
1+	Mean	30.2	0.36	28.89	0.31			
	Median	30	0.33	29	0.28	*Z*	1.188	1.554
	SD	3.16	0.14	3.33	0.12			
	Min	23	0.15	23	0.15	*P*	0.235	0.120
	Max	39	0.88	35	0.51			
	CV%	10.46	38.89	11.52	38.37			
	n	61		18				
2+	Mean	36.62	0.66	38.33	0.77			
	Median	36.5	0.65	40	0.81	*Z*	-0.768	-1.008
	SD	2.85	0.23	2.89	0.17			
	Min	32	0.36	35	0.59	*P*	0.442	0.313
	Max	42	1.17	40	0.92			
	CV%	7.78	34.62	7.53	21.61			
	n	15		5				
3+	Mean	45	1.21	40	0.67			
	Median	45	1.21	-	-	*Z*	-	-
	SD	4.24	0.58	-	-			
	Min	42	0.8	-	-	*P*	-	-
	Max	48	1.63	-	-			
	CV%	9.43	48.1	-	-			
	n	2		1				
4+	Mean	53	1.95	-	-			
	Median	-	-	-	-	*Z*	-	-
	SD	-	-	-	-			
	Min	-	-	-	-	*P*	-	-
	Max	-	-	-	-			
	CV%	-	-	-	-			
	n	1		0				

Table 5. Non-linear regression performed on total length (L_t) and total weight (W) of male and female specimens of *A*. *fasciatus* collected during the study period (2015-2016) and ANCOVA results.

		Estimation	SE	d.f.	*T*	*P*	Confidence interval
Females (*n*=178)	*a*	0.0106	0.0056	177	18.871	<0.001	0.0952 - 0.0117
	b	3.1484	0.03952		79.6842	<0.001	3.0704 - 3.2264
Males (*n*=75)	*a*	0.0102	0.0010	74	10.650	<0.001	0.0089 - 0.0129
	b	3.1114	0.0793		39.247	<0.001	2.9532 - 3.2696

ANCOVA

	Sum of Squares	d.f	Mean square	*F*	*P*
Adjusted mean	0.001	1	0.001	0.226	0.885
Adjusted error	9.098	241	0.004		
Adjusted total	0.911	242			

Table 6. Values of the analyzed meristic characters of *A. fasciatus* within the study area during the study period (*n*=60) (*NLLS*=lateral line scales; *D*=dorsal fin rays; *A*=anal fin rays; *V*=ventral fin rays; *C*=caudal fin rays; *P*=pectoral fin rays; *NRL*=number of flank bars).

	Mean ± SD	Median	Min	Max	CV%
NLLS	27 ± 2.23	27	15	30	8.23
D	11 ± 1.13	11	9	14	10.22
A	10 ± 0.99	10	8	13	9.76
V	7 ± 0.26	7	7	8	3.64
P	12 ± 1.37	12	10	15	11.33
C	22 ± 1.74	22	18	26	8.01
NRL	9 ± 1.46	9	7	13	15.48

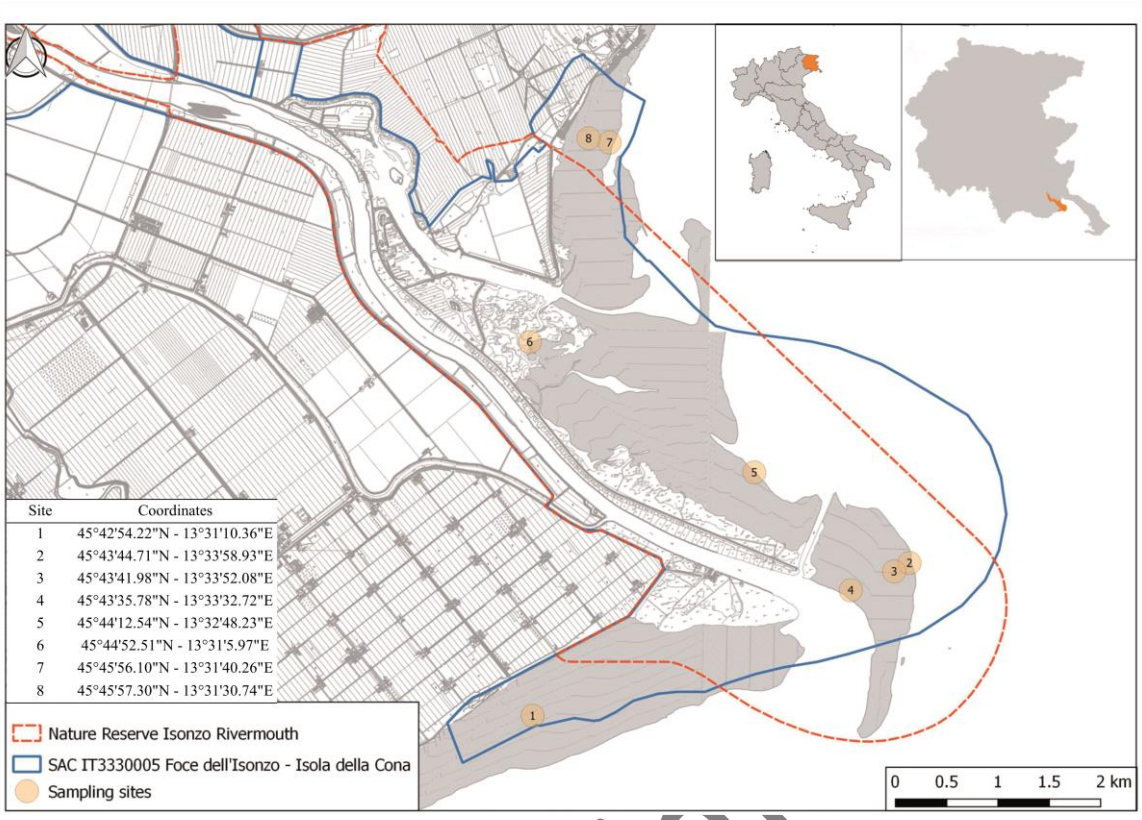

Figure 1. Sampling sites and study area

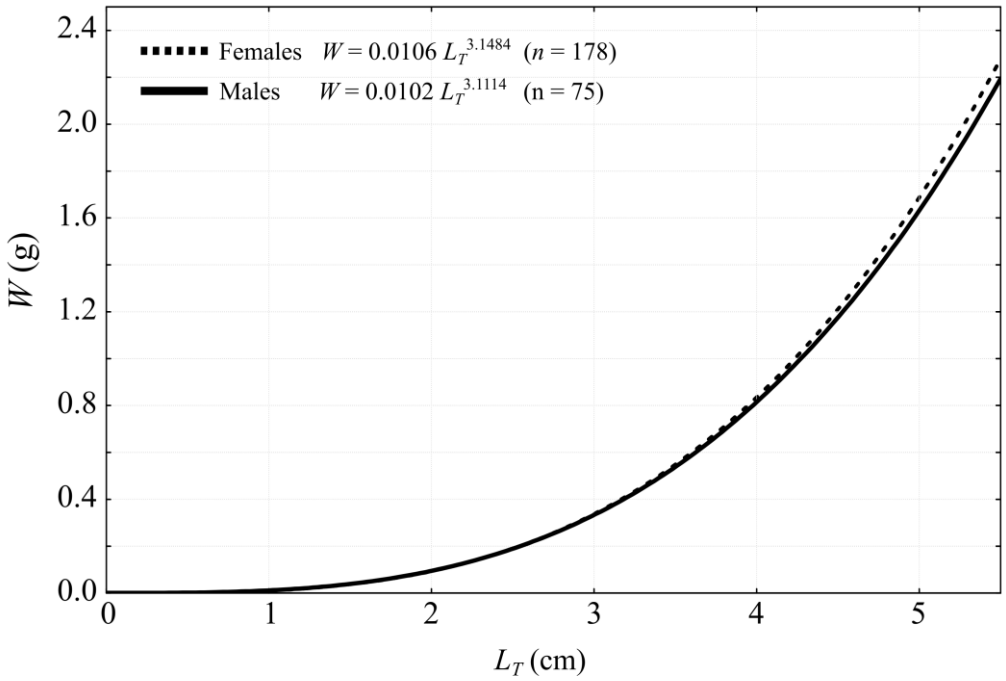

Figure 2. Relationship between total length (L_T) and total weight (W) obtained from *A. fasciatus* specimens collected during the two-year sampling activity for females and males.

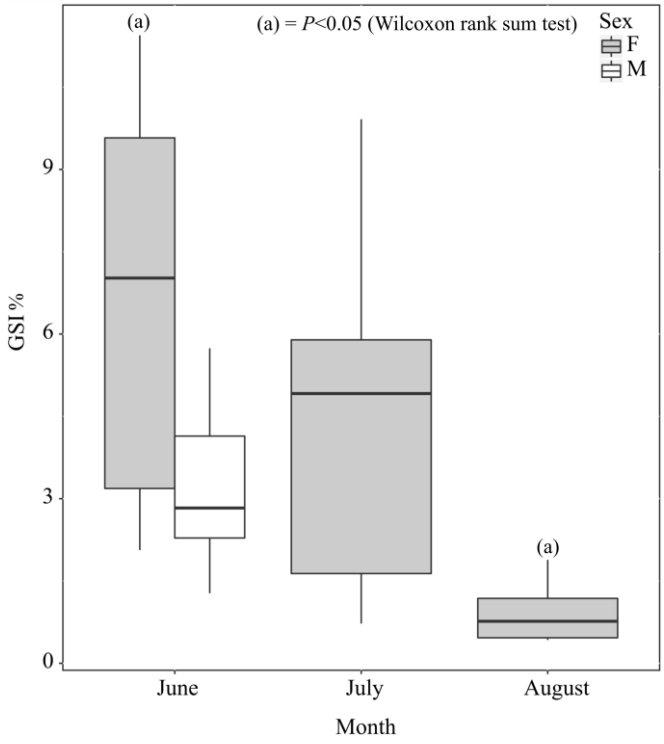

Figure 3. Values of gonadosomatic index (GSI) obtained for females (F) and males (M) of *A. fasciatus* during the sampling period (June, July and August 2016).

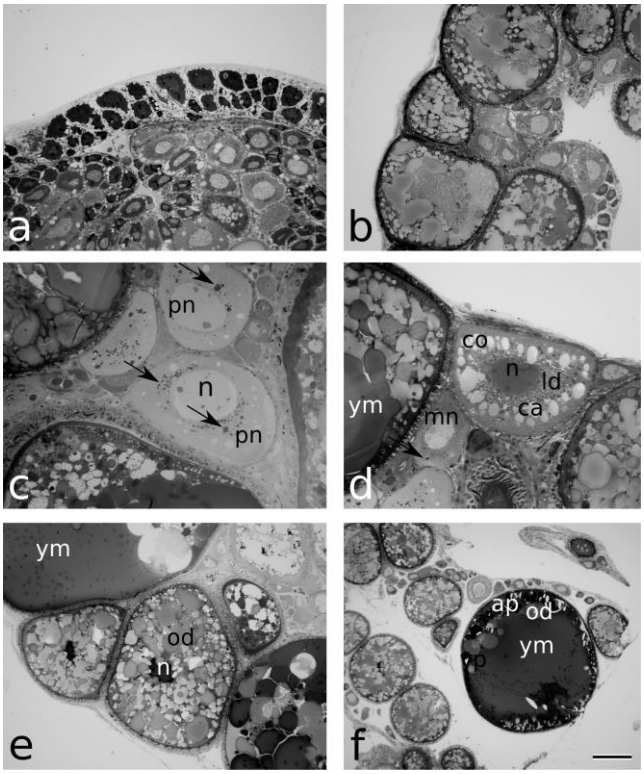

Figure 4. Cross-sections of ovaries from a female sampled in August (a) and from a female sampled in June (b). Cross-sections of oocytes at perinuclear step (c, d), circumnuclear oil droplets step (d), early secondary growth step (e), full growth oocyte step (f). Scale bar=100 µm (a, b, e); scale bar=50 µm (c, d); scale bar=200 µm (f). ap=animal pole; ca=cortical alveoli; co=circumnuclear oil droplets step oocyte; ld=lipid droplets; mn=multiple nucleoli step oocyte; n=nucleus; pn=perinuclear step oocyte; od=oil droplets; ym=yolk mass; arrows=small lipid droplets.

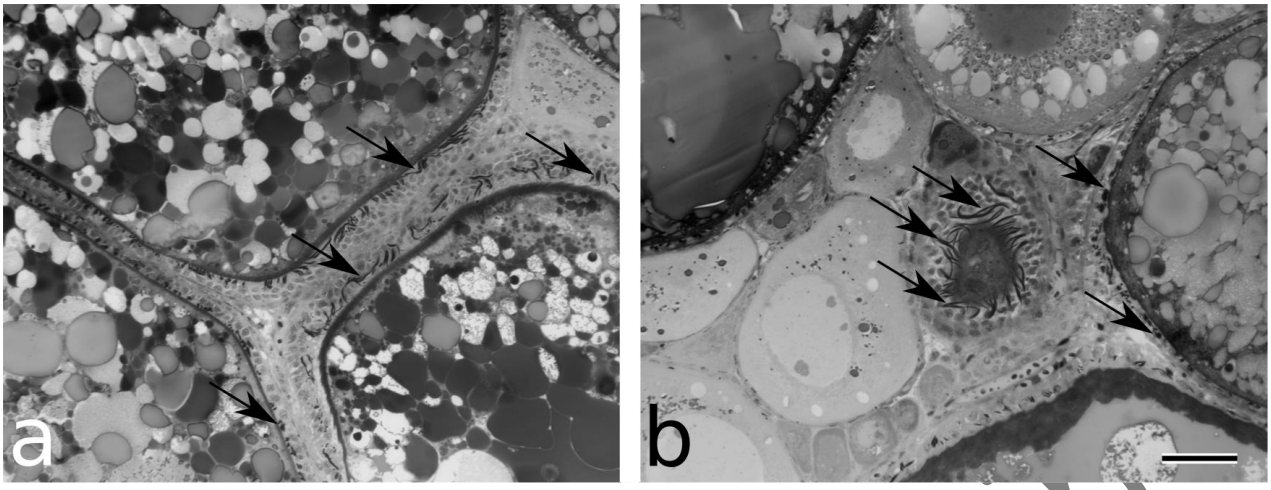

Figure 5 Cross-sections of follicular layers in transversal plane (a) and almost tangential one (b) showing the growing attachment filaments (arrows) in secondary growth step oocytes. Scale bar=50 µm.