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Morphological and physiological traits of Mediterranean sticklebacks living in the Camargue wetland (Rhône river delta)

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Abstract

Three-spined sticklebacks (*Gasterosteus aculeatus* L.) living at the southern limit of the species distribution range could possess specific morphological and physiological traits that enable these fish to live at the threshold of their physiological capacities. Morphological analysis was carried out on samples of sticklebacks living in different saline habitats of the Camargue area (Rhône delta, northern Mediterranean coast) obtained from 1993 to 2017. Salinity acclimation capacities were also investigated using individuals from freshwater-low salinity drainage canals and from mesohaline–euryhaline lagoons. Fish were maintained in laboratory conditions at salinity values close to those of their respective habitats: low salinity (LS, 5‰) or seawater (SW, 30‰). Fish obtained from a mesohaline brackish water lagoon (BW, 15‰) were acclimated to SW or LS. Oxygen consumption rates and branchial Na⁺/K⁺-ATPase (NKA) activity (indicator of fish osmoregulatory capacity) were measured in these LS or SW control fish and in individuals subjected to abrupt SW or LS transfers. At all the studied locations, only the low-plated “leiurus” morphotype showed no spatial or temporal variations in their body morphology. Gill rakers were only longer and denser in fish sampled from the LS–freshwater (FW) drainage canals. All fish presented similar physiological capacities. Oxygen consumption rates were not influenced by salinity challenge except in SW fish transferred to LS immediately and 1 h after transfer. However, and as expected, gill NKA activity was salinity dependent. Sticklebacks of the Camargue area sampled from habitats with contrasted saline conditions are homogeneously euryhaline, have low oxygen consumption rates and do not appear to experience significantly greater metabolic costs when challenged with salinity. However, an observed difference in gill raker length and density is most probably related to the nutritional condition of their habitat, indicating that individuals can rapidly acclimatize to different diets.

Keywords

osmoregulation, oxygen consumption, salinity, morphometry

Most of the work on rapid evolution in three-spined sticklebacks and adaptation to freshwater has focused on northern populations (DeFaveri et al., 2011; Hohenlohe et al., 2010; Mäkinen et al., 2006; Taugbøl et al., 2014) and very few studies have considered southern populations, notably those of the Mediterranean bioclimatic region which constitutes the southern limit of the species range in the western Palearctic (Crivelli and Britton 1987). Furthermore, some of these southern peripheral populations have steeply declined (Clavero et al., 2009; Foster et al., 2003; Kottelat and Freyhof, 2007). For example, in the Iberian Peninsula, the species is now considered to be endangered following IUCN criteria, after the occurrence of multiple local extinction events from most of its past distribution (Araguas et al., 2012; Clavero et al., 2009; Foster et al., 2003; Reimchen, 1984; Sanz et al., 2015; Vila et al., 2017). Similarly, this species is listed as threatened or endangered in some Neartic locations. For example, the unarmoured three-spined stickleback subspecies *Gasterosteus aculeatus williamsoni*, located in Southern California, has been on the U.S. Fish and Wildlife Services Endangered Species List since 1970. Other stocks of sticklebacks have also been listed as threatened by states such as Massachusetts and Pennsylvania (Williams et al., 1989). However, along the northern coast of the Mediterranean Sea, sticklebacks living in the Camargue area (Rhône delta) are still abundant (Poizat et al., 2002; Rind et al., 2018). The Camargue is an ecological hotspot of diversity and has been a Biosphere Reserve since 1977. It has an exceptional biological richness and covers 13,000 ha between the northern mesohaline, brackish water Vaccares lagoon and the Mediterranean Sea. It is made up of freshwater ponds and shallow water canals with abundant vegetation, freshwater drainage canals with submerged vegetation, mesohaline (brackish water) and euryhaline (brackish to hypersaline) lagoons flooded throughout the year (Crivelli and Britton 1987).

Since the last ice age ended about 10,000 years ago, sticklebacks have repeatedly colonized freshwater habitats because thousands of new streams and lakes formed during glacial retreats (Bell and Foster, 1994; Schluter, 2000). Therefore, postglacial freshwater stickleback populations share a common suite of adaptation throughout the northern hemisphere such as armour reduction (Bell, 1987; Bell and Foster, 1994; Colosimo et al., 2005). They also differ significantly in morphology due to divergent selective pressures that they experience in contrasting freshwater habitats (Baumgartner, 1992; Bell, 1982; Reimchen et al., 1985; Spoljaric and Reimchen, 2007). For example, divergent natural selection for the exploitation of benthic vs. limnetic habitats and associated food items is one of the most common diversifying mechanisms affecting resident freshwater sticklebacks (Walker, 1997; Spoljaric and Reimchen, 2007). Sticklebacks also inhabit a large number of coastal brackish water systems throughout the northern hemisphere and present therefore an extremely wide haloniche (Bell and Foster 1994; Wootton, 1976).

Most fish species living in their native freshwater or marine environments are stenohaline and are not able to survive non-native salinities.

However, for euryhaline fish such as sticklebacks, the

osmoregulatory machinery is adapted to a wide range of salinities. These fish can sustain salinity variations of their environment and move or migrate to non-native salinities (Moyle and Cech, 1996). However, osmoregulation must be costly to maintain (Perry and Fryer, 1997). This cost is expected to be higher in non-native environments compared to native environments due to local acclimation processes. For instance, the expression of genes related to osmoregulation is considered to be reduced in native environments because of the selection for reduced energy expenditure (Aykanat et al., 2011). Although this physiological plasticity facilitates acclimation and the colonization of novel environments (through strong euryhaline capacity), the bioenergetic costs (increased lipid, protein and carbohydrate metabolism) and possible associated physiological trade-offs may, however, lead ultimately to adaptive evolution (Dalziel et al., 2009; Komoroske et al., 2016; Schulte, 2001; Watt, 1985).

The physiological mechanisms involved to maintain ion and water homeostasis are coordinated between the gills, the kidney and the gut (Eddy and Handy, 2012). In fish, the ionocytes, also known as the chloride cells or mitochondria rich cells (MRCs), are crucial cells to facilitate ion transport (Laurent, 1984; Jurss and Bastrop 1995; Rind et al., 2018). The internal structure of these cells is rich in mitochondria and also possesses a tubulovesicular system composed of basal membrane invaginations containing the sodium pump, Na⁺/K⁺-ATPase, or NKA (Evans and Claiborne, 2009; Dymowska et al., 2012). This key enzyme involved in salinity tolerance (Herrera et al., 2009; Sampaio and Bianchini, 2002; Yang et al., 2009) is responsible for the exclusion of three sodium ions from the cell in exchange for two potassium ions, requiring the hydrolysis of one ATP molecule. Its activity maintains cell membrane potential, facilitates ion transport via channels and transporters that are localized in the apical and basolateral membranes, and thus maintains osmotic equilibrium and cell volume. NKA is mostly located in gill ionocytes, the intestinal absorbing enterocytes and ionocytes found along the tubules of the kidney (Evans and Claiborne, 2009). NKA activity has already been measured in the gills and kidney of sticklebacks kept at different salinities (Divino et al., 2016; Judd, 2012; Jurss et al., 1982; Schaarschmidt et al., 1999). Also, changes in salinity and the coordinated physiological response are often reflected by different oxygen consumption rates (Gracia-Lopez et al., 2006; Morgan et al., 1997). This measurement can be used as an indicator of fish metabolism (Cech, 1990) and to assess the osmoregulatory cost (da Silva Rocha et al., 2005; Morgan and Iwama, 1998; Tsuzuki et al., 2008). This cost can impact growth rate (Boeuf and Payan, 2001, Imsland et al., 2003, Woo and Kelly, 1995) with also a minimal osmoregulatory energy demand in an environment that is natural for a particular species, therefore indicating some fitness-related advantages of being adapted to local environmental salinities (Morgan and Iwama, 1991).

The aim of this study was to determine whether Mediterranean sticklebacks living in different saline environments (freshwater–low salinity drainage canals and euryhaline–seawater lagoons) of the Camargue area have different morphological and physiological traits. It is hypothesized that this coastal population is morphologically homogenous. However, fish sampled from contrasted saline water

bodies could respond differentially when challenged with abrupt salinity transfers. It is predicted that oxygen consumption rates, as a measure of metabolic rate, could reflect the key functional osmoregulatory changes that occur with salinity changes, such as NKA activity modulations. To test these hypotheses, we analysed the morphology of fish sampled directly from the different aquatic environments. Acclimated sticklebacks were challenged with abrupt salinity transfers in controlled experimental conditions to evaluate whether some phenotypic traits could be habitat specific at different physiological levels (gill NKA activity and individual oxygen consumption).

2 MATERIALS AND METHODS

2.1 | Fish sampling and experimental design

In the Camargue region, a protected area located within the Rhone river delta in Southern France, subadult three-spined sticklebacks (*Gasterosteus aculeatus*) were fished from 1993 to 2017 and from different water bodies (Table 1) using fixed hoop nets of decreasing mesh size from 13 to 6 mm at the end of the hoop net ("capêchade"). This sampling was carried out to determine how homogenous the population had been over the last 24 years and to determine whether migration and mixing could occur in this coastal wetland area. Immediately after fishing, some of the collected fish were directly fixed in 10% formalin and kept in alcohol until use. For the physiological study, fish [fresh body weight (FBW) $0.56 \text{ g} \pm 0.18$] were collected in December 2016 and February 2017 from three different areas. One sampling site corresponds to a freshwater–low salinity (FW–LS) drainage canal (Versadou canal, salinity values $<5\text{‰}$) running from the Rhône river and loading FW into a brackish (BW)/seawater (SW) lagoon (La Contesse lagoon). The other two sites correspond to the constantly BW Vaccarès lagoon (salinity of $20\text{--}25\text{‰}$) and the BW/SW Marteau lagoon (salinity ranging from 20 to 50‰). These salinities recorded during fish sampling correspond to the salinities

typically measured during that period of the year (winter). However, the highest salinities up to 50‰ were only be recorded during the summer period. These sites are partially interconnected, but their topography could limit migration and mixing among sticklebacks living in these contrasted habitats. Typically, dams, "salt dams" and the numerous locks along the drainage canals and between lagoons could constrain fish dispersal.

Captured fish were directly transported to the laboratory facility at the University of Montpellier, France in separate large oxygenated containers filled with water from the respective sampling sites. Fish were then kept at room temperature (21°C) and under a natural photoperiod in separate tanks containing 100 l of recirculated and filtered water (12 fish per tank with an approximate 1:1 sex ratio) for an acclimation period of 2–3 weeks. Fish were then transferred to 40 l tanks and maintained at different salinities for another 2 weeks. Fish from the BW Vaccarès lagoon were acclimated to 30‰ or 5‰ , those from the BW/SW Marteau lagoon were kept at 30‰ and those from the FW–LS Versadou canal at 5‰ . Fish were fed three times a day with chironomid larvae (EXOMARC, Lormont, France), excluding the last day before experimentation. Salinity and temperature levels were recorded using a YSI 30 Conductivity/Salinity/Temperature instrument (Yellow Springs, Ohio). Fish behaviour was also checked daily. After this acclimation period, fish were directly used for respirometry measurements, with or without salinity transfers from SW to LS or from LS to SW (Figure 1a–c). For gill sampling, fish were sacrificed with an overdose of anaesthetic (2-phenoxyethanol, 500 mg l^{-1}), and then dissected on ice. Gills were either directly frozen in liquid nitrogen or kept at -80°C for NKA activity measurements.

2.2 Morphometry

For bone and lateral plate staining, fish kept in alcohol were stained overnight with 0.008% Alizarin Red S in 1% potassium hydroxide, destained in water overnight, then lightly cleared in a 0.25% potassium hydroxide/50% glycerol solution. The number of lateral plates

TABLE 1 Habitat characteristics, location names, GPS positions and average plate number of the different sticklebacks sampled from these sites

Habitats	Location name	GPS position	1993	Mean number of plates/year					Mean number of plates \pm S.E.M.	
				1994	1995	1996	2001	2016/17		
Freshwater (drainage canals)	Fumemorte	43°31'43,2"N 4°42'10,9"E							4,5 \pm 0,97	4,5 \pm 0,97
	Baisse sa'ée	43°29'15,0"N 4°39'02,7"E		4,7 \pm 0,67	4,3 \pm 0,67		4,7 \pm 0,9			4,5 \pm 0,77
	Giraud	43°29'28,8"N 4°41'42,1"E				4,5 \pm 0,73				4,5 \pm 0,73
	Versadou	43°27'15,2"N 4°35'22,6"E							3,9 \pm 0,73	3,9 \pm 0,73
Mesohaline	Vaccarès	43°32'16,0"N 4°37'54,0"E			4,1 \pm 0,99		4,3 \pm 1,15		3,5 \pm 0,52	3,9 \pm 0,96
	La Capelière	43°32'26,0"N 4°38'33,9"E						4,4 \pm 0,66		4,4 \pm 0,66
	Fournelet	43°29'37,7"N 4°37'24,9"E					4,8 \pm 0,63			4,8 \pm 0,63
Euryhaline	Marteau	43°27'36,4"N 4°34'58,3"E							4,8 \pm 0,63	4,8 \pm 0,63
	Malagroy	43°30'41,6"N 4°29'16,6"E					4,7 \pm 0,94			4,7 \pm 0,94

were counted from 10 fish collected from the different sampling sites. In addition, head height, body height, dorsal fin length and gill raker length were measured using the ImageJ image processing program (National Institute of Health, Bethesda, MD, U.S.A.). For gill rakers, only the first gill from the left branchial arch was considered. Gill raker lengths were measured from the base to the tip of the rakers. Gill raker density was measured as the number of gill rakers positioned along the gill arch along a distance of 100 μm . The ratio of these values with the fish length was also calculated. Digital images of fish viewed laterally and dissected gills were acquired on a (Leica DM2500, Germany). Fixed gills were also observed with a FEI Quanta 200 electron optics international B.V., Netherlands using the conventional mode (high vacuum) and a Thornley-Everhart secondary electron detector. Prior to SEM observations of the gill rakers, samples were dehydrated, bathed in 1,1,1,3,3,3-hexamethyldisilazane, air-dried, glued to specimen stubs and coated with palladium for 180 s ($\sim 40 \text{ mA}$) using an Edwards Sputter Coater (BOC Edwards, UK).

Fish morphology was analysed using landmark-based geometric morphometrics. Seventeen landmarks were digitized on the right side of the body using TpsDIG (Rohlf, 2005) in order to get the X and Y coordinates (Figure 1d). Digitized landmarks were then transferred to MorphoJ (Klingenberg, 2011). Data were analysed through a principal component analysis (PCA) for shape variance, then principal component coefficient scores were transferred to Excel (2013) to visualize the possible variations between fish sampled from the FW-LS habitats and those from the mesohaline-SW lagoons.

2.3 Oxygen consumption rate

Respiration measurements were conducted in 73 ml transparent metabolic chambers, equipped with an oxygen optode (OXSP5, sensor code SD7-545-214) (Pyro-Science GmbH, Aachen, Germany) previously glued to its inner wall. Optodes were calibrated to 100% (using air-saturated water) and 0% air saturation (using a freshly made

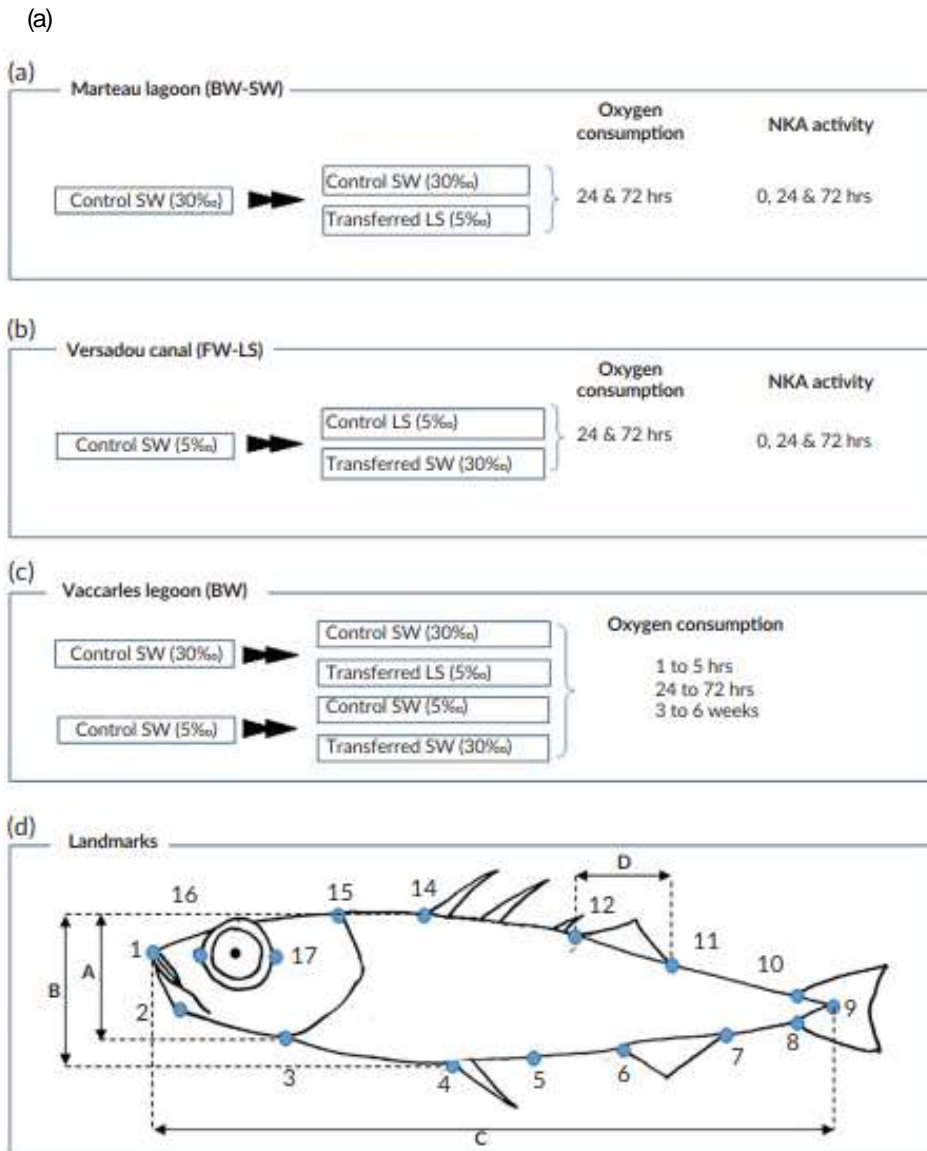


FIGURE 1 Experimental set-up for sticklebacks collected from (a) the Marteau lagoon (SW), (b) the Versadou canal (FW-LS) and (c) the mesohaline/brackish water Vaccarles lagoon (BW). (d) Linear measures and positions of the 17 landmarks used for the morphogeometric analysis. Morphological characters were measured from the left side of each fish

80 mM Na₂SO₃ solution). Each metabolic chamber was equipped with a magnetic stirrer (to ensure correct O₂ mixing in the water column) and partially immersed in a 2 l tank. This tank contained a water pump connected to the metabolic chamber through a hole on the lid of the chamber also allowing water overflow and was supplied with an aeration system to maintain water fully oxygenated. A structure of 1 mm² mesh was installed at the bottom of the chamber above the magnetic stirrer in order to keep the fish away from the stirrer. Fish were introduced in each of the chambers containing filtered (0.2 µm Whatman) medium at the salinity values at which the animal was acclimated 24 h before salinity transfer. After this time, the water of each tank and metabolic chamber was quickly siphoned and replaced with water at the desired salinity.

The rates of oxygen consumption were measured at room temperature (21°C) and at different salinities (freshwater 0,5‰ and sea-water 30‰). To ensure that fish were consuming O₂ differentially

at different temperatures, the effects of SW to LS transfers on oxygen consumption were analysed at 21 and 23°C. Similarly, the rate of oxygen consumption was also assessed with LS to SW transfers monitored at 25°C. Change in oxygen concentration over time within each of the chambers was measured at different times before and after water transfer (1 h before, immediately after transfer, 1, 2, 3, 5, 24, 72 h after transfer and up to 3 and 6 weeks after salinity transfer) using 20 min periods during which the water pump and aeration system were shut off. Oxygen concentration was measured using a four-channel fiberoptic oxygen meter (FireSting, Pyro-Science GmbH, Aarhen, Germany) and recorded through Pyro Oxygen Logger software (Pyro-Science GmbH, Aarhen, Germany). All measurements started in fully oxygenated water (>98%) and respiration was recorded as a function of declining pO₂ over time. Parallel measurements were carried out at a time (including a blank) and data were recorded using 5 s. intervals. Animals were then weighed

TABLE 2 Body morphology of sticklebacks sampled from FW-LS and SW habitats

	Head height/ FL (A)	Head length/ FL (1-15)	Body height/ FL (B)	Eye diameter (16-17)	Pelvic girdle/ FL (4-5)	Dorsal Fin/ FL (D)	Jew length/ FL (1-2)	Snout length/ FL (1-3)	Caudal height (8-10)
FW-LS	20,5 ± 0,50	23,65 ± 1,65	23 ± 1,30	10,24 ± 0,53	9,4 ± 0,21	23,6 ± 0,20	6,35 ± 1,31	3,47 ± 0,75	9,11 ± 1,70
SW	20,3 ± 0,60	22,97 ± 1,48	22,8 ± 0,90	9,45 ± 1,05	10,9 ± 0,20	23,1 ± 0,28	6,22 ± 0,83	3,75 ± 0,63	11,08 ± 4,12

Note: Data are presented as ratios of different measures: means ± S.E.M. × 100/fish length (FL). Elements in brackets refer to the landmarks illustrated in Figure 1d. Body parameters were obtained from 10 fish collected from the FW-LS Versadou canal and 12 from the SW Marteau lagoon.

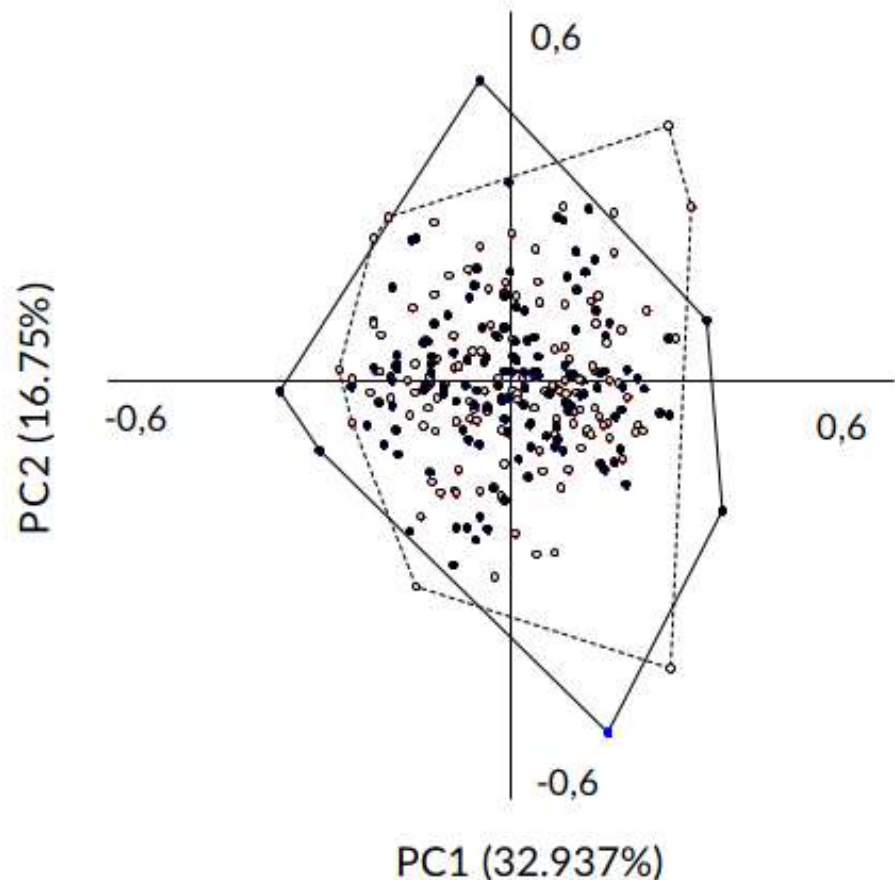


FIGURE 2 First and second principle components of morphological variance between FW-LS and SW sticklebacks. Body parameters were obtained from fish collected from the FW-LS habitats (Baisse Salée and Giraud lagoons, Fumemorte and Versadou canals) and the mesohaline/SW lagoons (Malagroy and Marteau lagoons)

From each fish, four gill arches (from the same side of the fish) were dissected and homogenized in pairs in sucrose–EDTA–imidazole (SEI) buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.4) using a manual Potter–Elvehjem tissue homogenizer. The specific, Na⁺- and K⁺-dependent, ouabain-sensitive ATPase activity was measured in these homogenates using 1.4 mM of ouabain as described in detail by Metz et al. (2003). Aliquots (10 μ l in triplicate) of homogenate (protein content of 1 mg ml⁻¹) were incubated in assay medium for 20 min at 25°C in microplate wells. Sample protein content was estimated using the protocol originally described by Bradford (1976) using a commercially prepared reagent (Sigma-Aldrich, St Louis, U.S.A.), and bovine serum albumin as standard. Initially, two different substrate concentrations were checked (dilution at 1:10 and 1:20). Optical densities were recorded using a spectrophotometer (Tecan Infinite M200, Switzerland). Only the linear part of the slopes for the optical densities read at 630 nm were considered. The specific activity was calculated by subtracting the K⁺-independent, ouabain-insensitive ATPase activity from total ATPase activity. ATP hydrolysis was

assessed by the amount of inorganic phosphate formed per minute per milligram of protein.

2.5 Statistical analysis

Data normality validation was checked using a Shapiro test. Homoscedasticity was tested by the Bartlett test, and the residual independence was checked with the Durbin–Watson test allowing parametric tests to be used. Results were evaluated by one-way analysis of variance (ANOVA), followed by Dunn's test or Tukey post hoc test (honestly significant difference) with a significance level of $P < 0.05$. t-tests were also performed in order to compare the different ratios. A one-way ANOVA was used to test gill raker data. All statistical analyses were conducted using the R package, IBM SPSS Statistics 23 and Microsoft Excel (2013). All results are presented as means \pm S.E.M.

3 RESULTS

3.1 Fish morphometry

Sticklebacks fished since 1993 from different aquatic habitats of the

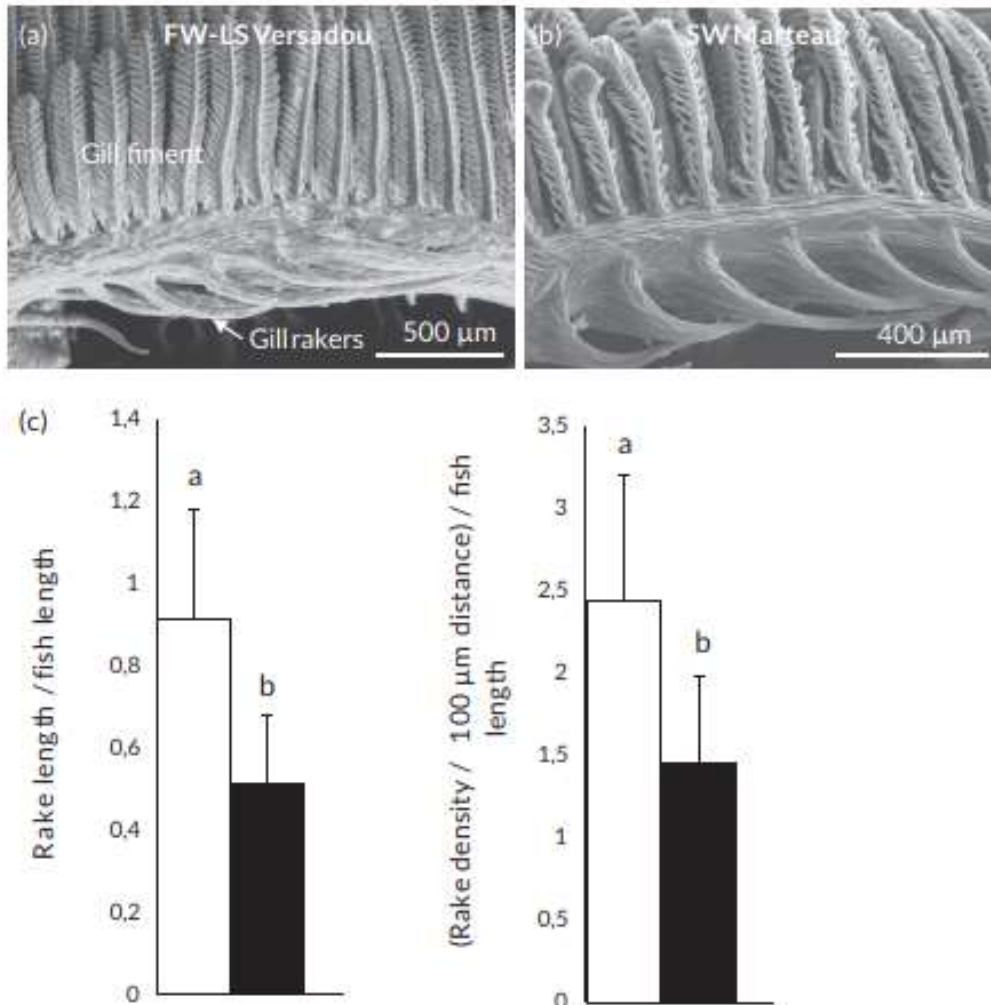


FIGURE 3 Representative SEM images of gill arches from one stickleback collected from the Versadou canal (FW–LS) (a) and one from the Marteau lagoon (SW) (b). Gill raker length according to fish length and gill raker density along 100 μ m of the gill arch according to fish length for FW–LS (Versadou) and SW (Marteau) sticklebacks (c). Results are presented as means \pm S.E.M. Data were obtained from 10 fish collected from the FW–LS Versadou canal and 12 from the SW Marteau lagoon

minimum of two and a maximum of six lateral plates observed for some of these fish. No statistical differences in lateral plate number between the different locations and salinities could be identified (Table 1). Also, no statistical differences were observed in terms of body morphology between sticklebacks sampled in 2016–17 (Vaccares and Marteau lagoons, Fumemorte and Versadou canals) and those sampled since 1993 (Malagroy, La Capelière, Fournelet, Baisse Salée and Giraud lagoons) (Tables 1 and 2).

Morphogeometric analysis using 17 different landmarks from fish sampled from the FW–LS habitats (Baisse Salée and Giraud lagoons, Fumemorte and Versadou canals) and the mesohaline–SW lagoons (Malagroy and Marteau lagoons) revealed that 32.9% of the variance

is explained by the first component while the second component accounts for 16.7% of the variance (Figure 2). The scattered plot for the first and second components indicates limited morphological variations between sticklebacks from the two contrasted habitats. However, a significant difference is observed for the morphology of the gill rakers between sticklebacks sampled from these two habitats (Figure 3). Gill raker length and gill raker density appeared significantly higher in sticklebacks living in the FW–LS drainage canals compared to the fish living in the mesohaline–SW lagoons.

3.2 Oxygen consumption rate

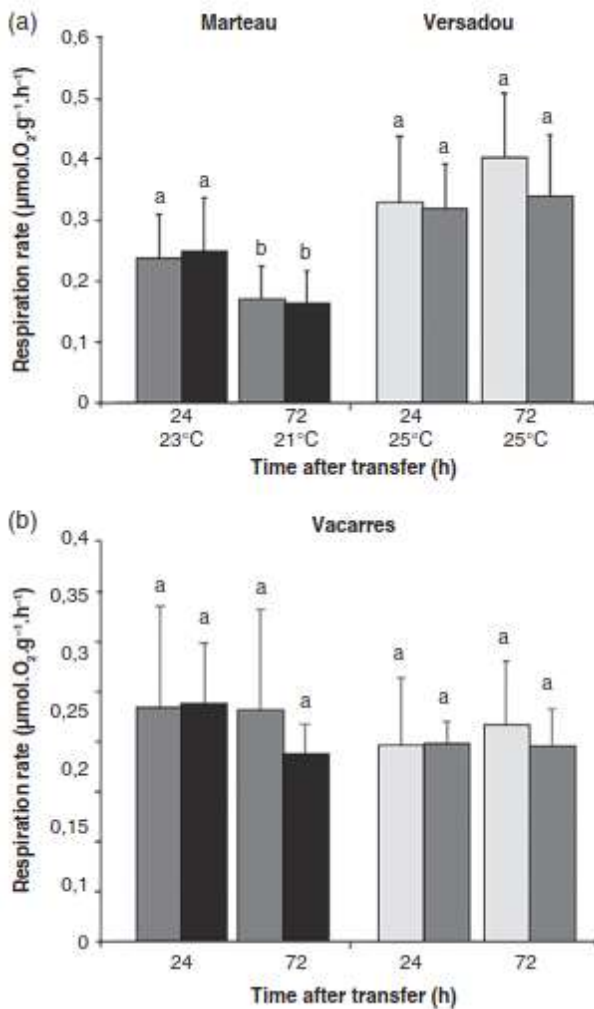


FIGURE 4 (a) Oxygen consumption rate for sticklebacks sampled from the FW–LS Versadou canal (FBW 0.57 ± 0.18) and the SW Marteau lagoon (FBW 0.54 ± 0.18). Fish were maintained at different temperatures and transferred 24 and 72 h after from SW to LS and from LS to SW. (b) Oxygen consumption rates for sticklebacks maintained at 21°C collected from the mesohaline Vaccares lagoon (FBW 0.52 ± 0.13) and maintained in SW or LS and transferred for 24 and 72 h to LS and SW, respectively. Results are presented as means \pm S.E.M., $10 < n < 12$

Sticklebacks collected from the FW–LS Versadou canal, the BW Vaccares lagoon and the mesohaline–SW Marteau lagoon, kept at

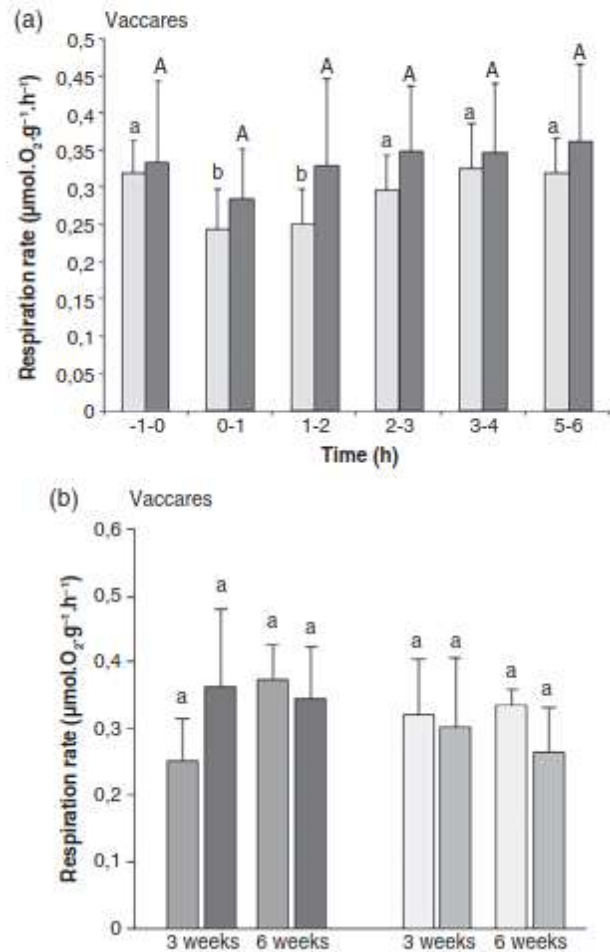


FIGURE 5 Oxygen consumption rate for sticklebacks collected from the mesohaline Vaccares lagoon maintained in SW or LS at 21°C and abruptly transferred up to 5 h to either LS or SW (a). Oxygen consumption rate for sticklebacks from the Vaccares lagoon maintained in SW or LS at 21°C and transferred to LS and SW, respectively for 3 and 6 weeks (b). Results are presented as means \pm S.E.M. (a) $6 < n < 8$, (b) $10 < n < 12$, FBW 0.52 ± 0.13

different temperatures (21, 23 and 25°C) in LS or SW and after salinity transfers presented significantly different oxygen consumption rates (P value = 0.0104) (Fig. 4). Oxygen consumption increases with temperature. However, salinity transfer from LS to SW or SW to LS did not induce any change in the rate of oxygen consumption when recorded 24 and 72 h after transfer (P value = 0.283) (Figure 4a,b).

Sticklebacks collected from the BW Vacarres lagoon and kept in SW presented a significant reduction in oxygen consumption rate immediately and up to 1 h after abrupt transfer to LS (Figure 5a). However, fish collected from the same environment and kept in LS prior to SW transfer did not modify their oxygen consumption rate during this 6 h experiment.

A longer acclimation to SW or LS of up to 3 and 6 weeks did not induce any change in oxygen consumption rate compared to fish kept in LS and SW (Figure 5b).

3.3 Branchial Na⁺/K⁺-ATPase activity

Following direct transfer from SW to LS, branchial Na⁺/K⁺-ATPase activity of sticklebacks sampled from the SW Marteau lagoon significantly increased by 28% and 40% within 24 and 48 h post transfer, respectively (P value = 0.0009) (Figure 6). However, when sticklebacks sampled from the LS Versadou canal were transferred from LS to SW, gill Na⁺/K⁺-ATPase activity was significantly decreased by 36% within 24 h (P value = 0.0127), resulting in a 1.65-fold change in gill Na⁺/K⁺-ATPase activity when fish were abruptly transferred to a different salinity. Furthermore, gill Na⁺/K⁺-ATPase activity of fish acclimated or

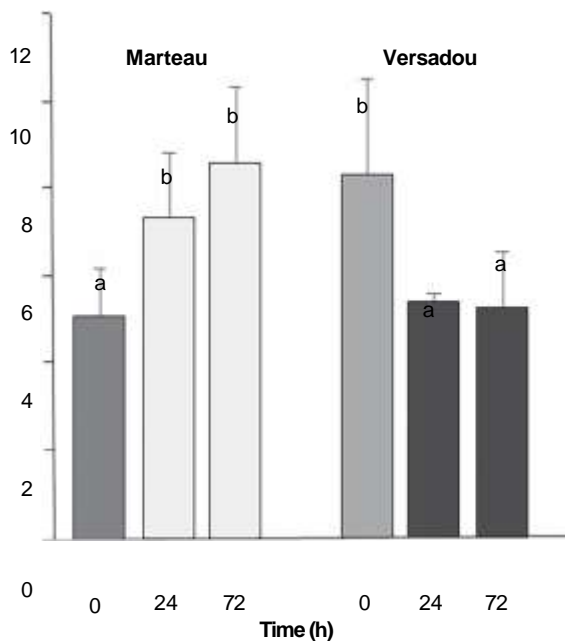


FIGURE 6 Branchial Na⁺/K⁺-ATPase activity for fish collected from the SW Marteau lagoon (FBW 0.54 g ± 0.18) and the FW-LS Versadou canal (FBW 0.57 g ± 0.18) maintained at 21°C and transferred for 24 and 72 h from SW to LS and from LS to SW. Results are presented as means ± S.E.M., 10 < n < 12

transferred to SW or LS appeared to be similar despite the fact that these fish originated from two contrasting habitats.

4 DISCUSSION

Three-spined sticklebacks of the Camargue area living within the Rhone river delta in SW, BW or LS-FW habitats have similar body morphologies. Habitat and salinity have no effect on the number of lateral plates. This is consistent with what has already been observed for other southern peripheral populations: freshwater and coastal sticklebacks of the Mediterranean bioclimatic region are all low-plated, with fewer than five plates or indeed none at all (Münzing, 1963). Lateral plate number is genetically determined, with a high heritability across all the different morphs, although there can be substantial within-morph variation (Hansson et al., 2016). Usually, marine sticklebacks are predominantly fully plated with more than 30 plates while freshwater fish have fewer than 15 plates, typically between six and 10 (Bell and Foster, 1994; Hansson et al., 2016; Wiig et al., 2016; Østbye et al., 2018). The three-spined stickleback is well known for having undergone adaptive radiation following the end of the last glaciation and is well adapted to the freshwater environment (Bell and Foster, 1994). FW populations are less armoured and smaller than the marine ancestral populations. These typical morphological traits are due to parallel genetic adaptations to a novel environment (Schluter, 1996; Foster and Baker, 2004; Colosimo et al., 2005). Many potential selective agents have been proposed to explain the morphological parallel evolutionary changes across stickleback populations (MacColl and Aucott, 2014; Smith et al., 2014; Spence et al., 2013; Voje et al., 2013). It has been suggested, for example, that the nature and intensity of predation (Hagen and Gilbertson 1973; Reimchen 1980; Bell et al. 1993), buoyancy (Myhre and Klepaker 2009), salinity and calcium availability (Giles 1983; Spence et al., 2012, 2013) can act as selective factors in plate morph evolution for sticklebacks. Consequently, fitness advantages could be related to differences in predator regimes or intensity between different habitats (Reimchen, 1983, 2000). A salinity effect on body shape has also been demonstrated (McCairns and Bernatchez, 2012). However, these authors tracked morphological changes exclusively via partial warp scores but not in terms of physical shape differences, making their results difficult to interpret (Mazzarella et al., 2015). Also, sticklebacks of the Camargue area have specific life history traits compared to northern populations. They have a shorter life expectancy (1–2 years), a high growth rate, early sexual maturity and a long reproduction period (May to November), reflecting a high metabolism (Crivelli and Britton, 1987). A summer pause in somatic growth has also been detected in sticklebacks living in intermittent Mediterranean streams (Clavero et al., 2009). This highlights harsh environmental conditions for these Mediterranean fish with an annual life cycle compared to those living further north.

Although collected from contrasted saline environments, sticklebacks of the Camargue area may still have the possibility to migrate locally between these different coastal habitats, especially between

COMPLIANCE WITH ETHICAL STANDARDS

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published (Jurss et al., 1982; Judd, 2012), hypo-osmoregulating fish in SW typically relying on less and smaller ionocytes compared to hyper-osmoregulating fish in LS or FW conditions. An increased gill NKA activity has been documented when LS sticklebacks are transferred to SW (Schaarschmidt et al., 1999). In their experiment, fish were transferred to a higher salinity level (35‰) compared to the one tested in this study (30‰). As already observed in fish such as the European seabass and the marbled spinefoot, *Siganus rivulatus*, NKA activity over a large salinity gradient typically presents a U shape curve with maximal activity levels at extreme salinities (Lasserre, 1971; Saoud et al., 2007). Therefore, the hypersaline condition used in the study from Schaarschmidt et al. (1999) could explain the elevated NKA activity they measured.

In conclusion, the low-plated stickleback population sampled from euryhaline, mesohaline and freshwater habitats in the Mediterranean Camargue area are morphologically and physiologically homogenous with efficient osmoregulatory capacities that do not impair their overall energetic balance. However, some habitat-related differences were observed, especially at the gill level. It would be interesting to better characterize the energetic cost of this functional remodelling along the gills and to identify how individual fish can cope with differential, organ-specific energy use and maintain an overall low energy use. Also, it is known that peripheral populations of wide-ranging species have less genetic variation compared to core populations (Lesica and Allendorf, 1995). Isolated populations that inhabit marginal environments tend to be more genetically differentiated from central populations than isolated populations that do not live in extreme environments (Bouza et al., 1999). Furthermore, local extinction events recently occurred for sticklebacks living at the southern edges of the species distribution (Foster et al., 2003) and particularly in freshwater habitats of Mediterranean climate areas in Europe (Clavero et al., 2009; Kottelat and Freyhof, 2007; Vila et al., 2017). Therefore, there is a strong need for further analysis of the salinity-induced physiological adjustments of these southern peripheral stickleback populations. This could shed light on specific physiological traits and trade-offs limiting the overall energy use despite a highly demanding environment. This knowledge could efficiently contribute to a better protection of these populations and conserve their evolutionary heritage.

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CONFLICT OF INTEREST

The authors declare that they do not have a conflict of interest.

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