SCIENTIA MARINA 72(3)
September 2008, 469-476, Barcelona (Spain)
ISSN: 0214-8358

Osmoregulation as a potential factor for the differential distribution of two cryptic gobiid species, *Pomatoschistus microps* and *P. marmoratus* in French Mediterranean lagoons

FRANÇOIS RIGAL [1,2], THIBAUD CHEVALIER [1,2], CATHERINE LORIN-NEBEL [2], GUY CHARMANTIER [2], JEAN-ANTOINE TOMASINI [1], FABIEN AUJOULAT [2] and PATRICK BERREBI [1,3]

[1] Laboratoire Ecosystèmes Lagunaires, UMR 5119, Université Montpellier II, F-34095 Montpellier cedex 05, France.
[2] Equipe Adaptation Ecophysiologique et Ontogenèse, UMR 5171 GPIA, Université Montpellier II, F-34095 Montpellier cedex 05, France.
[3] Present address: Institut des Sciences de l'Evolution, UMR5554, cc065, Université Montpellier II, F-34095 Montpellier cedex 05, France. E-mail: berrebi@univ-montp2.fr

SUMMARY: This study was aimed at the detection of potential differences in the osmoregulatory capacity of two cryptic species of gobies, *Pomatoschistus microps* (Krøyer, 1838) and *P. marmoratus* (Risso, 1810), that have different distributions in French Mediterranean lagoons characterised by different salinity regimes. Specimens of both species were experimentally exposed to different salinities, their salinity tolerance was evaluated and their blood osmolality was measured. Both species are strong osmoregulators over a wide range of salinities but *P. microps* showed higher performances of hyper-regulation at very low salinities (10 and 40 mosm/kg, i.e. freshwater 0.3 and 1.4) and of hypo-regulation at high salinities (1500 mosm/kg, 51). Only *P. microps* was able to tolerate freshwater exposure over 4 days. We conclude that the high osmoregulatory capacity found in *P. microps* is linked to its better survival at very low salinities and is a physiological requirement for living in areas such as the Mauguio lagoon where salinity is highly variable. In contrast, as osmoregulation of *P. marmoratus* is less efficient at extreme salinities, this species cannot colonise such environments and is restricted to habitats where salinity is more stable, such as the Thau lagoon.

Keywords: competition, lagoons, osmoregulation, Pomatoschistus, salinity tolerance.

RESUMEN: LA OSMOREGULACIÓN COMO FACTOR POTENCIAL DE LA DISTRIBUCIÓN DIFERENCIAL DE DOS ESPECIES CRÍPTICAS DE GÓBIDO, *POMATOSCHISTUS MICROPS* Y *P. MARMORATUS*, EN LAGUNAS MEDITERRÁNEAS FRANCESAS. – Este estudio tuvo por objeto la detección de diferencias potenciales en la capacidad osmorreguladora de dos especies crípticas de góbido, *Pomatoschistus microps* (Krøyer, 1838) y *P. marmoratus* (Risso, 1810), que presentan diferentes áreas de distribución en lagunas mediterráneas francesas caracterizadas por regímenes de salinidad distintos. Especímenes de ambas especies fueron expuestos experimentalmente a diferentes salinidades, evaluándose su tolerancia a la salinidad y midiéndose su osmolalidad sanguínea. Ambas especies tienen una amplia capacidad osmoreguladora en un amplio rango de salinidades. Sin embargo, en comparación con *P. marmoratus*, *P. microps* mostró mayores rendimientos hiper-regulatorios a salinidades muy bajas (10 y 40 mosm/kg, es decir agua dulce 0.3 y 1.4) e hipo-regulatorios a salinidades altas (1500 mosm/kg, 51). Sólo *P. microps* fue capaz de tolerar la exposición al agua dulce durante 4 días. Podemos concluir que la gran capacidad osmoreguladora encontrada en *P. microps* está ligada a su mayor supervivencia a salinidades muy bajas, siendo un requerimiento fisiológico para vivir en áreas tales como la laguna de Mauguio, donde la salinidad es muy variable. Por el contrario, puesto que la osmoregulación de *P. marmoratus* es menos eficiente en salinidades extremas, esta especie no puede colonizar tales ambientes y se ve restringida a hábitats donde la salinidad es más estable, como la laguna de Thau.

Palabras clave: competición, lagunas, osmoregulación, *Pomatoschistus*, tolerancia a la salinidad.

INTRODUCTION

Lagoons are critical transition zones between the land, freshwater habitats and the sea, providing essential ecological functions and ecosystem services (Levin *et al*., 2001). The fish fauna inhabiting these very productive zones exploits the abundant biological resources in different ways, through temporary occupation for feeding and growth only, or by spending their entire life in these habitats (Knoppers, 1994).

Among the non-migratory fish species living in the lagoons of southern France, gobiids, especially those belonging to the genus *Pomatoschistus*, play an important role in the food web (Doornbos and Twisk, 1987). Due to their great abundance and their trophic position, they provide a link between benthos production and carnivorous fish and fish-eating birds.

The genus *Pomatoschistus* is represented in the western Mediterranean by several species. Among them, *P. microps* (Krøyer, 1838) and *P. marmoratus* (Risso, 1810) spend their whole life cycle in lagoons. Both species are cryptic (Berrebi *et al*., 2005) because they are very difficult to differentiate in the field, though some morphological characters such as genipores on the head (Sanzo, 1911), the pelvic disc and scale distribution (Miller, 1986) can be used to differentiate them under the microscope. *Pomatoschistus marmoratus* has been scarcely recorded in the study region (Crivelli, 1981) because of its morphological similarity with *P. microps.* Using molecular markers (allozymes and introns), recent studies have demonstrated that the lagoons of the French Mediterranean coast are inhabited by the unexpected species *P. marmoratus*, together with the well-known species *P. microps* (Berrebi *et al*., 2005).

These species show an unusual distribution, since lagoons generally harbour only one species (Rodriguez, 2002). In particular, *P. marmoratus* is found in the Thau lagoon (Fig. 1), and *P. microps* in the Mauguio lagoon (Fig. 1). However, when both species are in sympatry or parapatry, as in the Vaccarès lagoon, they can hybridise (Calatayud, 2003; Berrebi *et al*., 2005). These observations raise the question of the differential ecological preferences of each species and of the causes of their differential distribution.

The Thau and Mauguio lagoons (Fig. 1) differ in several ways, among them their size and salinity regime. The Thau lagoon is large (7500 ha, 19 km long), relatively deep (average depth 4.5 m, maximum 10 m) and open to the sea through two permanent channels at each of its ends. Consequently, variations in temperature and salinity are limited and buffered by the volume of water and the communication with the sea. Salinity is generally close to that of seawater (36), with a range of variation limited to 31-38 (Plus *et al*., 2001), except on northern shores where freshwater arrives from rivers or resurgences. In contrast, in the Mauguio lagoon, the area (3166 ha), water volume (eight times less than in Thau) and depth (average 0.8 m, maximum 1.3 m) are much lower than in the Thau lagoon (SMGO, 2005). Its communication with the sea is limited to one narrow opening (Fig. 1). All these factors contribute to much higher seasonal vari-

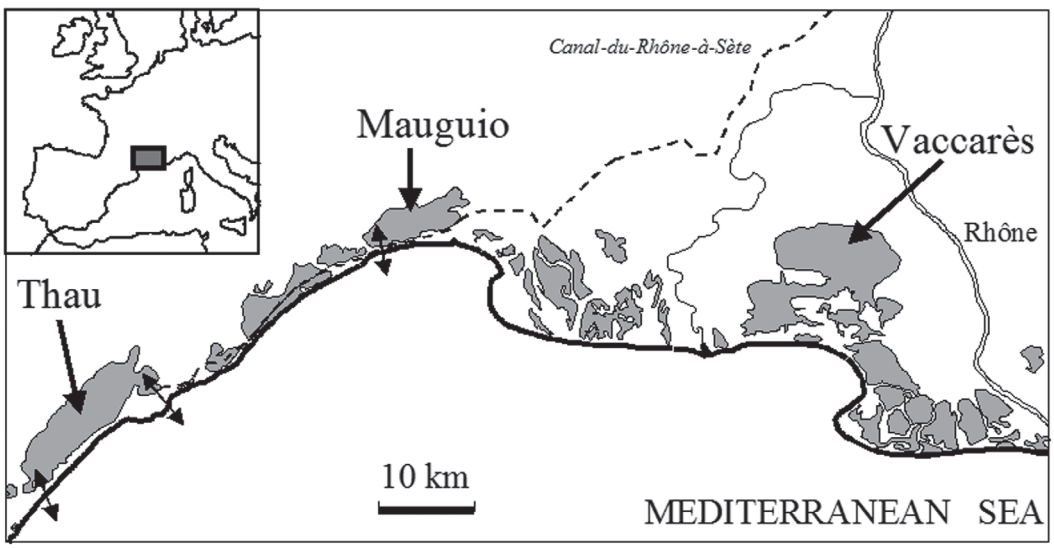

FIG. 1. – Map of lagoons along the French Mediterranean coast, including the Thau and Mauguio lagoons.

ations in environmental parameters, including salinity ranges from 0.1 (our observation in March 2004) to 37. Salinity is one of the main external factors that exert a selective pressure on aquatic animals including fish (Poizat *et al*., 2004).

Osmoregulation is the physiological response to salinity variations. In most invertebrates, e.g. in crustaceans, salinity tolerance is linked to the pattern of osmoregulation (Péqueux, 1995; Charmantier, 1998). In contrast, teleosts, as vertebrates, regulate their blood osmolarity at an almost constant value of about 280-350 mosm/kg (Evans, 1979). The differences in salinity tolerance between fish species are therefore based on their abilities to osmoregulate at different salinities (high, low, or both), particularly at the extremes of the salinity range. This has been elegantly shown in several species of blennies (Plaut, 1998).

The aim of this study was to estimate the differences in salinity tolerance and osmoregulatory capacity of *P. microps* and *P. marmoratus*, and to evaluate the link between this capacity and the differential distribution of the two gobiid species.

MATERIALS AND METHODS

Biological materials

Pomatoschistus microps and *P. marmoratus* were collected in February and March 2004 respectively. *Pomatoschistus microps* were captured in the Mauguio lagoon by local fishermen using fixed fyke nets. Because the capture took place during the rainy season, the salinity was lower than 2. *Pomatoschistus marmoratus* specimens were caught with a beach seine in the Thau lagoon at two locations: Balaruc-le-Vieux (north-east of the lagoon) and Bouzigues (further south on the north coast). The salinity was about 20, due to a river influence. The specimens captured were transported to the laboratory in cool boxes containing water from the fishing site in order to limit osmotic and thermal stress. In both species, the specimens captured were adult fish, measuring from 33 to 46 mm for *P. microps* and from 37 to 55 mm for *P. marmoratus*. Following preliminary measurements showing no difference in blood osmolarity between males and females, both sexes were used for the experiments.

The water was progressively brought over a day to a salinity of 20 and a temperature of 19°C. Since the two fish species were caught under different environmental conditions, they were then maintained for two weeks in 200 L tanks at 20 salinity (mixture of sea water and dechlorinated tap water), at a constant 19°C temperature and a 12 h dark: 12 h light photoperiod. This period was long enough to allow for complete acclimatisation of the fish to the same salinity (Evans, 1979). All experiments were conducted after this two-week period. Rocks and *Cerastoderma sp.* shells were provided as shelters. Fish were fed every two days (commercial Biomar® pellets) and this timing was also retained for exchanging a third of the water volume, which was continuously filtered using Eheim™ filters. The experiments were conducted according to the French law on scientific animal experimentation (law 2001-131, 6 February 2001).

Experiments on osmoregulation

For all experiments, nine experimental media were prepared in 30 L aerated tanks and adjusted to the following salinities: 10 mosm/kg (freshwater, 0.3), 40 mosm/kg (1.4), 150 mosm/kg (5.1), 300 mosm/kg (10.2), 500 mosm/kg (17), 750 mosm/kg (25.5), 1000 mosm/kg (seawater, 34), 1200 mosm/kg (40.8) and 1500 mosm/kg (51). The media were prepared by mixing freshwater and seawater and by adding Instant Ocean® marine salts for the high salinities.

The osmotic pressure of the media was measured with a micro-osmometer Model 3300 (Advanced Instruments, Needham Heights, MA, USA).

Salinity tolerance and osmoregulation

A preliminary experiment (EXP1) was conducted to determine the acclimatisation time, i.e. salinity tolerance and time necessary for the blood osmolality to stabilise. For this, a group of 65 *P. microps* individuals was transferred from the holding tanks which were at 20 to 5.1. The blood osmolality of the investigated fish was measured in the original medium at T0 (start of the experiment) then at 8, 18 and 48 h and 3, 5, 8 and 16 days following the transfer. Because of the similar sizes of the two species, the results obtained in *P. microps* were considered to be applicable to *P. marmoratus*.

In the main experiment (EXP2), groups of 10-16 fish were directly transferred from 20 to each experimental salinity (0.3 to 51), where they were main-

tained for 4 days. During this period, they were fed once on the second day, and half of the water was changed on that day. Survival rates were estimated by removing and counting the dead fish 3 times a day. The media osmolalities were checked on days 1, 2 and 4 (in the last case, along with the blood osmolality). For blood sampling conducted on the surviving fish on day 4, each fish was anaesthetised using phenoxy-ethanol (0.3 mL/L), quickly rinsed in deionised water and then superficially dried on filter paper. The gill chambers were gently and carefully dried with a small piece of filter paper under a dissecting microscope in order to avoid water and sampled blood mixture. The blood was then sampled with a fine hand-made glass micropipette (using Drummond microcaps, Bioblock) inserted into the heart and quickly transferred into mineral oil to prevent evaporation. The blood osmolality was measured with reference to a 300 mosm/kg standard solution on a Kalber-Clifton nanolitre osmometer (Clifton Technical Physics, Hartford, N.Y., USA) measuring the freezing point depression and requiring a volume of about 20 nL. A piece of caudal fin was taken and preserved in ethanol for genetic analyses.

Molecular analyses

DNA was extracted from fin tissues using the Chelex 100 Resin (Biorad) method (Walsh *et al.*, 1991). PCR reactions in order to amplify the sixth creatine kinase intron system (CK6) were carried out in an Eppendorf Mastercycler® in a 10 μL volume of a mix containing 1 μL of 10x buffer (Promega), 2.5 mmol $MgCl_2$, 0.2 mmol of each dNTPs (Invitrogen), 0.5 μmol of each primer (CK6f labelled with CY5 and CK7r, provided by MWG-Biotech-AG) of the intron system CK6 (Chow and Takeyama, 1998), 0.3 U of Taq polymerase (Sigma) and 1 μL of DNA template. A first denaturation at 94°C for 3 min was followed by 35 cycles of denaturation for 1 min, annealing at 48°C for 1 min and extension at 72°C for 1 min 20 sec, and completed by a final extension at 72°C for 10 min.

Only the length polymorphism of intron amplification was analysed. One μL of PCR mixture of each individual was loaded onto an 8% denaturing polyacrylamide gel (Biorad). The PCR products were visualised with a FMBIO II fluorescent imaging system (Hitachi). Allele sizes were determined using a fluorescence labelled ladder of known size (Promega) with the FMBIO Analysis 8.0 image analyser program.

According to Calatayud (2003), the CK6 system, composed of three loci (CK6-1, CK6-2 and CK6-3), is diagnostic between the two species analysed, providing a sure specific a posteriori identification of each fish. In this system, the non-diagnostic locus CK6-1 presents the shared allele 302 (= number of bp); CK6-2 is diagnostic with the 278 bp allele for *P. microps* and 266 and 272 for *P. marmoratus*; finally, CK6-3 provides complementary information, with the alleles 203 and 216 for *P. marmoratus* and only 216 for *P. microps*.

Statistical analyses

Because of the low number of individuals in each experimental sample, non-parametric tests were used for statistical analysis. To compare survival between *P. microps* and *P. marmoratus* for each salinity, Fisher's exact method (Zar, 1999) was used. For the intraspecific comparison of average blood osmolalities between the different salinities, the Kruskal-Wallis (KW) test and the multiple comparison post-hoc test suggested by Noether (1976) were used. For each salinity, interspecific comparisons of average blood osmolalities were tested by the Mann-Whitney (MW) test.

RESULTS

Species identification

All fish used in the interspecific osmolality comparison were identified to species level using the CK6 intron system. This molecular analysis showed that all specimens from the Mauguio lagoon were *P. microps* and all those from the Thau lagoon were *P. marmoratus*. No hybrids such as those recorded in other lagoons (Berrebi *et al.*, 2005) were found.

Osmoregulation acclimatising time (EXP1)

Following the direct transfer of *P. microps* from 20.2 to 5.1, the blood osmolality decreased in the first 18 hours then increased and stabilised within two days slightly below (by 8%) the original value (Table 1). In the light of these observations, an exposure time of 4 days at each salinity was used in all further experiments, in order to ensure complete osmotic stabilisation before the blood sampling.

TABLE 1. – Changes in blood osmolality in *Pomatoschistus microps* after rapid transfer from an original medium (594 mosm/kg, 20.2) to a diluted medium (150 mosm/kg, 5.1) at 20°C (EXP1). Data are given as mean ± SE; n = 5 except at 5 days where n = 9; h = hours and d = days. Letter of the last line: different letters indicate significant differences in blood osmolality.

Time	0	8h	18h	2d	3d	5d	8d	16d
External medium (‰)	20.2	5.1	5.1	5.1	5.1	5.1	5.1	5.1
(mosm/kg)	594	150	150	150	150	150	150	150
Blood (mosm/kg)	342±5	294±5	298±4	314±2	312±2	313±2	316±2	316±2
p	a	b	b	c	c	c	c	c

TABLE 2. – Survival rate (%) of *Pomatoschistus microps* and *P. marmoratus* after 4 day-exposure to different media (EXP2), with 10-16 individuals at the start of each experiment and significance of the inter-species differences (Fisher's exact method). FW = freshwater; SW = sea water.

	Medium salinity 0.3(FW)	1.4	5.1	10.2	17	25.5	34(SW)	40.8	51
	(mosm/kg) 05-10	40	150	300	500	750	1000	1200	1500
P. marmoratus	alive 0	10	13	13	13	13	13	13	14
	dead 15	4	0	0	0	0	0	0	0
	total 15	14	13	13	13	13	13	13	14
	% survival *0*	*71.4*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
P. microps	alive 13	10	12	13	13	12	11	13	7
	dead 3	0	3	2	2	3	4	0	8
	total 16	10	15	15	15	15	15	13	15
	% survival *81.3*	*100*	*80*	*86.7*	*86.7*	*80*	*73.3*	*100*	*46.7*
phi	0.823	0.378	0.322	0.258	0.258	0.322	0.380		0.596
p	<0.0001	0.1140	0.2262	0.4841	0.4841	0.2262	0.1016	>0.9999	0.0022

Salinity tolerance (EXP2)

Following the 4-day exposure to different salinities, the survival rate varied according to the species (Table 2). Within a salinity range of 1.4-40.8 (40-1200 mosm/kg), survival was 75-100% in both species. At the highest salinity of 51 (1500 mosm/kg), the survival rate was 50% in *P. microps* and 100% in *P. marmoratus*. In freshwater, all exposed *P. marmoratus* died, but survival was over 80% in *P. microps*. At extreme salinities, the differences in survival between the two species were significant (Fisher's exact method, $p<0.05$) (Table 2).

Interspecific osmoregulation comparison

Pomatoschistus microps and *P. marmoratus* hyper-osmoregulated (Fig. 2) at salinities below 10.2 (300 mosm/kg) and hypo-osmoregulated at higher salinities. Over the entire range of salinities tested, the blood osmolality changed in relation to the medium concentration in both species (KW: $p<0.0001$). However, for each species, the blood osmolality did not change significantly (KW: $p>0.05$) at salinities ranging from 10 to 40 (300-1200 mosm/kg) in *P. microps* and from 10 to 35 (300-1000 mosm/kg) in *P. marmoratus*. At these salinities, both species kept their blood osmolality almost constant at 340-350 mosm/kg. But, at extreme salinities (low and high), the osmoregulatory ability was stronger in *P. microps* than in *P. marmoratus*. At 40.8 and 51 (1200 and 1500 mosm/kg, respectively), the blood osmolality was kept significantly lower in *P. microps* (MW:

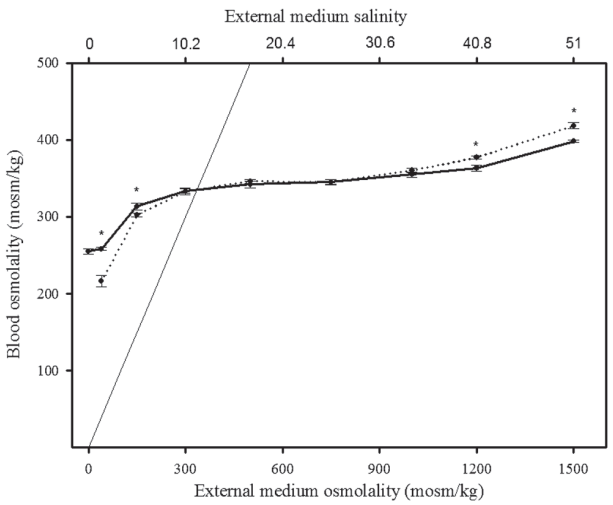

FIG. 2. – Variations in blood osmolality in *Pomatoschistus microps* (plain line) and *P. marmoratus* (broken line) in relation to the osmolality/salinity of the external medium, following 4 day-exposure at 20°C. Data are presented as means ± standard deviation (n = 9-10). As a reference, the isoconcentration line is drawn, passing by the origins of both axes. *: significant differences between the two species at this salinity.

p=0.0032 and p=0.0407, respectively) than in *P. marmoratus* (while the survival of this species was better). At 5.1 (150 mosm/kg) and 1.4 (40 mosm/kg), the blood osmolality was maintained significantly higher in *P. microps* (MW: p=0.0142 and p=0.0014, respectively). For instance, in the 40 mosm/kg medium, the values for blood osmolality were 258±15 mosm/kg in *P. microps* and 216±23 mosm/kg in *P. marmoratus*. In freshwater, the difference was even clearer: among the 81.2% of surviving *P. microps*, the blood osmolality was 255±22 mosm/kg, while all the *P. marmoratus* were dead (n=15).

DISCUSSION

Among the various ecological parameters influenced by weather conditions, salinity and temperature are the most important for fish adaptation (Poizat *et al*., 2004). Several physico-chemical parameters in coastal lagoons depend on the proportion of seawater entering from the adjacent sea and of freshwater originating from inland water inputs and precipitation, subject to meteorological changes.

Non-migratory lagoon fish such as *P. microps* and *P. marmoratus*, and also *Salaria pavo* (Plaut, 1998), must react rapidly to such variations. Their capacity for adaptation is a condition for their survival because there is no real option of leaving the brackish water lagoons.

Interspecific differences in osmoregulation capacity

The two species investigated showed a wide salinity tolerance. The osmoregulatory capacities of both species were very similar at medium salinity values, i.e. between 300 and 1000 mosm/kg, which represents a salinity of 10 to 35, frequently encountered in all French Mediterranean coastal lagoons. However, the range of tolerance was larger in *P. microps* than in *P. marmoratus*, particularly toward low salinities. This was demonstrated by the fact that, in freshwater, the majority of *P. microps* survived and were able to efficiently hyper-osmoregulate, while all *P. marmoratus* died. It is worth noting that the lowest blood osmolality measured in *P. marmoratus*, at 1.4, was close to 220 mosm/kg. This value is considered to be the minimum value compatible with survival in several teleosts, including salmon (Franklin *et al.*, 1992) and sea bass (Nebel *et al*., 2005).

A similar study has been conducted on the blennies *Salaria pavo* and *S. fluviatilis* (Plaut, 1998). *S. fluviatilis*, which lives in both fresh and brackish water habitats, is able to osmoregulate in media ranging from freshwater to seawater. *S. pavo,* restricted to marine habitats, can tolerate freshwater during brief exposure but is not able to maintain constant blood osmolality at very low salinities.

Ecological significance

Under natural conditions, the two *Pomatoschistus* species investigated show a differential range of distribution. *P. microps* lives in, among others, the Mauguio lagoon, which is relatively small and therefore exposed to weather-related changes. *Pomatoschistus marmoratus* is found in the Thau lagoon, which is much larger, more open to the sea and therefore less influenced by the weather. Salinity variations are thus much higher in the Mauguio lagoon than in the Thau lagoon, a fact confirmed by our own observations. This raises the question of the link between the osmoregulatory capacity of the fish and the salinity regime of each lagoon.

In lagoons in southern France, most of the time salinity ranges between 10 and 34; such values seem fully tolerable for both *P. microps* and *P. marmoratus*. Under these conditions, both species appear equally fit to live in lagoons, at least in terms of salinity.

Hypersalinity is a rare phenomenon, always limited to a part of the lagoon during the hot weather, and probably easily avoided by gobiids. On the other hand, low salinities are common during the rainy seasons typical of the Mediterranean climate, mainly in autumn and spring. Salinities close to freshwater have been frequently observed in small lagoons, such as during our sampling in the Mauguio lagoon. We thus hypothesise that low salinity values are the main challenge faced by gobiids in their environment. Therefore, their osmoregulatory abilities at low salinities down to freshwater constitute the most informative data reported in this study, coupled with the survival rates. Among the two species, *P. microps* has clearly better osmoregulatory capacities at 1.4 and can tolerate freshwater, in which *P. marmoratus* dies.

The size and depth of the Thau lagoon, and its permanent links with the sea, buffer it against wide variations in salinity. However, during a rainy period, most of the Mauguio lagoon reaches a salinity

close to 0. For example, when the Mauguio specimens of *P. microps* were captured, the salinity was 1-2 over the whole period from February to March. Thus, the very wide euryhalinity of *P. microps*, based on a strong capacity to osmoregulate, even in freshwater, appears to be a preadaptation that enables this species to spend its entire life cycle in the Mauguio lagoon. In contrast, the more limited osmoregulatory ability of *P. marmoratus*, particularly when confronted with freshwater that it cannot tolerate, restricts this species to areas such as the Thau lagoon where the amplitude of salinity variations is more limited than in the Mauguio.

In others terms, we hypothesise that, if founding individuals of *P. marmoratus* are present in the Mauguio lagoon (and probably in other similar small lagoons which are numerous along the French Mediterranean coast), they cannot survive when salinity decreases sharply, or they escape, and it is impossible for a stable population to establish. This hypothesis implies that, though we know that complex ecological phenomena occur, the decrease in salinity in spring and autumn is one of the main factors that explain the absence of *P. marmoratus* in several Mediterranean lagoons similar to Mauguio.

The interpretation of the situation in the Thau lagoon is more complex. Here, extreme values of salinity, and especially very low salinities, do not occur except in very limited marginal areas. Given its physiological abilities, the occurrence of *P. marmoratus* under these ecological conditions is acceptable, but these conditions do not justify the absence of *P. microps*. Past statistics on the captures of gobiids in these two lagoons cannot be used, since prior to the application of molecular tools, fisheries scientists considered that only *P. microps* lived along this coast, including the Thau lagoon (Bach, 1985; Zainuri, 1993). Molecular data on non-migratory *Pomatoschistus* in the two lagoons of interest report that *P. microps* has been detected in the Thau lagoon (maximum 10% in one sample, unpublished data) but *P. marmoratus* has never been found in the Mauguio lagoon (Berrebi *et al*., 2005). These records are in agreement with the "one parameter" hypothesis concerning the exclusive occurrence of *P. microps* in the Mauguio lagoon. Based on its high euryhalinity, the occurrence of *P. microps* in Thau was expected, but it occurs in very low proportions in comparison with *P. marmoratus*. In order to explain the near-absence of *P. microps* in the Thau lagoon, other hypotheses should be proposed, such as competition or the distribution of suitable spawning areas. The data of the present study do not allow any hypotheses to be made concerning competition.

REFERENCES

Bach, P. – 1985. *La pêche dans l'étang de Thau. Application de quelques notions d'écologie théorique aux communautés de poissons et à leur exploitation. Stratégie de quelques populations ichthyologiques capturées.* PhD thesis, Univ. Montpellier 2 France.

Berrebi, P., P. Rodriguez, J.-A. Tomasini, G. Cattaneo-Berrebi and A.J. Crivelli. – 2005. Differential distribution of the two cryptic species, *Pomatoschistus microps* and *P. marmoratus*, in the lagoons of southern France, with an emphasis on the genetic organisation of *P. microp*s. *Estuar. Coast. Shelf Sci.,* 65: 708-716.

Calatayud, R. – 2003. *Coexistence et hybridation de deux espèces cryptiques de gobies sédentaires (Pomatoschistus microps et P. marmoratus). Suivi spatio-temporel de l'introgression et adaptations aux lagunes languedociennes*. Master thesis, Univ. Montpellier 2 France.

Charmantier, G. – 1998. Ontogeny of osmoregulation in Crustacean: a review. *Invertebr. Reprod. Dev.,* 33: 177-190.

Chow, S. and H. Takeyama. – 1998. Intron length variation observed in the creatine kinase and ribosomal protein genes of the swordfish *Xiphias gladius*. *Fish. Sci.,* 64: 397-402.

Crivelli, A.J. – 1981. Les peuplements de poissons de la Camargue. *Rev. Ecol. (Terre Vie),* 35: 617-671.

Doornbos, G. and F. Twisk. – 1987. Density, growth and annual food consumption of gobiid fish in the saline Lake Grevelinngen, The Netherlands. *Neth. J. Sea Res.,* 21: 45-74.

Evans, D.H. – 1979. Fish. In: G.M.O. Maloiy, (ed.), *Comparative physiology of osmoregulation in animals*, pp. 305-390. Academic Press, London.

Franklin, C.E., M.E. Forster and W. Davison. – 1992. Plasma cortisol and osmoregulatory changes in sockeye salmon transferred to sea water: Comparison between successful and unsuccessful adaptation. *J. Fish Biol.,* 41: 113-122.

Knoppers, B. – 1994. Aquatic primary production in coastal lagoons. In: B. Kjerve, (ed.), *Coastal Lagoon Processes*, pp. 243-286. Elsevier Science, Amsterdam.

Levin, L.A., D.F. Boesch, A. Covich, C. Dahm, C. Erséus, K.C. Ewel, R.T. Kneib, A. Moldenke, M.A. Palmer, P. Snelgrove, D. Strayer and J.M. Weslawski. – 2001. The function of marine critical transition zones and the importance of sediment biodiversity. *Ecosystems,* 4: 430-451.

Miller, P.J. – 1986. Gobiidae. In: P.J.P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J.L. Nielsen and E. Tortonese (eds.), *Fishes of the north-eastern Atlantic and the Mediterranean*, pp. 1019-1085. Unesco, Paris.

Nebel, C., B. Romestand, G. Nègre-Sadargues, E. Grousset, F. Aujoulat, J. Bacal, F. Bonhomme and G. Charmantier. – 2005. Differential freshwater adaptation in juvenile sea-bass *Dicentrarchus labrax*: involvement of gills and urinary system. *J. Exp. Biol.,* 208: 3859-3871.

Noether, G.E. – 1976. *Introduction to statistics. A nonparametric approach*. 2d ed. Houghton Mifflin Co, Boston.

Péqueux, A. – 1995. Osmotic regulation on Crustacean. *J. Crust. Biol.,* 15: 1-60.

Plaut, I. – 1998. Comparison of salinity tolerance and osmoregulation in two closely related species of blennies from different habitats. *Fish Physiol. Biochem.,* 19: 181-188.

Plus, M., J.M. Deslous-Paoli, I. Auby and F. Dagault. – 2001. Factors influencing primary production of seagrass beds (*Zostera noltii* Hornem.) in the Thau lagoon (French Mediterranean coast). *J. Exp. Mar. Biol. Ecol.,* 259: 63-84.

Poizat, G., E. Rosecchi, P. Chauvelon, P. Contournet, and A.J. Crivelli. – 2004. Long-term fish and macro-crustacean community variation in a Mediterranean lagoon. *Estuar. Coast. Shelf Sci.,* 59: 615-624.

Rodriguez, P. – 2002. *Génétique des populations de trois espèces de*

Pomatoschistus (Poissons Gobiidés) des lagunes corses et continentales. Allozymes et RFLP de l'ADN mitochondrial. Master thesis, Univ. Corsica, Corte, France.

Sanzo, L. – 1911. Distrubutione delle papille cutanee (organi ciatiform) e suo valore sistematico nei gobi. *Mitt. Zool. Stat. Neapel,* 20: 251-328.

SMGO. – 2005. L'étang de l'Or (34) in La surveillance FOGEM des zones humides côtières du Languedoc-Roussillon 2000-2005. Information available online at http://www.languedoc-roussillon.ecologie.gouv.fr/eau/fogem/bilan_2000_2005/Partie-1/40-Etang-Or.pdf

Walsh, P.S., D.A. Metzger and R. Higushi. – 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques,* 10: 506-513.

Zainuri, M. – 1993. *Structure des peuplements ichtyologiques d'une zone d'herbiers à Zostera marina de l'étang de Thau (France). Etude de la composition alimentaire des juvéniles de loup (Dicentarchus labrax, Linnaeus, 1758), de la daurade (Sparus aurata, Linnaeus, 1758) et du muge (Chelon labrosus, Risso, 1826) par des approches expérimentales*. PhD thesis, Univ. Montpellier 2 France.

Zar, J.H. – 1999. *Biostatistical analysis*. 4th ed. Prentice-Hall Inc, New Jersey.

Scient. ed.: J.B. Company.
Received September 7, 2007. Accepted March 4, 2008.
Published online July 3, 2008.