

The effect of salinity on larval development of *Uca tangeri* (Eydox, 1835) (Brachyura: Ocypodidae) and new findings of the zoeal morphology

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SUMMARY: In order to evaluate their tolerance to low salinities, zoeae of the fiddler crab *Uca tangeri* from the Rio San Pedro population (southwestern Spain) were reared in the laboratory at 20°C and at three salinities (16, 24 and 32). The zoeal development was completed at 24 and 32 but the crabs died as zoea I or zoea II, and very rarely as zoea III, at 16; tolerance to low salinities varied among clutches produced by different females. The duration of the first zoeal stage and of the complete zoeal development was shorter at 32. Our observations showed that the zoeae of *U. tangeri* could not tolerate retention in the mesohaline water of estuaries, and that export to oceanic waters would be optimal for their successful development. Survival at 24 suggests that larvae could also develop in polyhaline conditions if they were retained in the ocean-estuary interface. The presence of an additional zoeal stage (zoea VI) was observed in some individuals and associated with unfavourable combinations of temperature and salinity. In addition, some previously omitted aspects of zoeal morphology were re-described and illustrated, providing new evidence which supports the basal position of this species in a proto-Atlantic origin of the genus *Uca*. It is proposed that the differences between *Uca tangeri* and the rest of the *Uca* species should be highlighted, and that it should be placed in its own genus as *Afruca tangeri*.

Keywords: larval development, salinity, mortality, duration of development, Ocypodidae, *Uca*, fiddler crab, zoeal morphology.

RESUMEN: EFECTO DE LA SALINIDAD EN EL DESARROLLO LARVARIO DE *UCA TANGERI* (EYDOUX, 1835) (BRACHYURA: OCYPODIDAE) Y NUEVOS HALLAZGOS SOBRE LA MORFOLOGÍA DE LAS ZOEAS. – Se cultivaron zoeas del cangrejo violinista *Uca tangeri* de la población del Río San Pedro (Suroeste de España) en el laboratorio a salinidades de 16, 24 y 32, y una temperatura de 20°C, para evaluar su tolerancia a salinidades bajas. Las larvas completaron el desarrollo como zoeas a 24 y 32 pero murieron como zoea I, como zoea II o, muy raramente, como zoea III a 16; la tolerancia a las salinidades bajas varió entre las camadas producidas por diferentes hembras. La duración del primer estado y del desarrollo completo de las zoeas fue más corto a 32. Nuestras observaciones muestran que las zoeas de *U. tangeri* no tolerarían la retención en las aguas mesohalinas de estuarios y que la exportación hacia aguas oceánicas sería óptima para el éxito de su desarrollo. La supervivencia a 24 sugiere que las larvas también podrían desarrollarse en condiciones polihalinas si fueran retenidas en la interfase océano-estuario. Se observó la presencia de un estadio zoea adicional (zoea VI) en algunos individuos, que se asoció con condiciones desfavorables de temperatura y salinidad. Finalmente, se redescubrieron e ilustraron algunos aspectos previamente omitidos de la morfología de las zoeas, que constituyen nuevas evidencias que favorecen la posición basal de esta especie en el origen Proto-Atlántico del género *Uca*. Se propone la conveniencia de remarcar esta separación entre *Uca tangeri* y las restantes especies de *Uca* colocándola en su propio género y nombrándola *Afruca tangeri*.

Palabras clave: desarrollo larvario, salinidad, mortalidad, duración del desarrollo, Ocypodidae, *Uca*, cangrejo violinista, morfología de zoea.

INTRODUCTION

The ontogeny of most estuarine crabs includes a free larval phase. Two developmental strategies were described in larvae of species whose adults live in brackish waters: retention in the parental habitat, and exportation to the more stable conditions of coastal or oceanic waters (Anger, 2001). When the tolerance range of larval stages to salinity is narrower than that of adults, the exportation strategy allows larvae to develop in a less stressful environment. In addition, once in the ocean, exported larvae may face a reduced predation pressure and play an important role in the dispersal of a species. The effects of salinity on the larval biology of decapods were exhaustively reviewed by Anger (2003). However, the tolerance to reduced salinities is not an “all or not” trait: it has been demonstrated that genetic variability at the clutch level, individual variability in the provisioning of eggs with yolk, and salinity prevailing during embryonic development could affect salinity tolerance during crustacean development (e.g. Lee and Petersen, 2002; Giménez and Anger, 2003; Devreker *et al.*, 2004).

Fiddler crabs (genus *Uca*; Brachyura: Ocypodidae) consist of 94–97 species (depending on authors) of semi-terrestrial intertidal crabs that excavate burrows into muddy or sandy substrates and are characterised by extreme cheliped asymmetry in males (Rosenberg, 2001). They occur in marine and brackish waters of temperate and tropical regions in the western Atlantic (21 species), the eastern Pacific (36 species), the Indo-West Pacific (39 species), and the eastern Atlantic (1 species: *Uca tangeri*). However, adults of at least one species (the western Atlantic *Uca minax*) can live in freshwater habitats as far as 50 km from the sea (Brodie *et al.* 2007).

The geographical distribution of *U. tangeri* ranges from southern Spain and Portugal (~37°N) through the west coast of Africa, down to Angola (~16°S); it is the only species of fiddler crab to be found in Europe and its ecology, physiology, and behaviour have been intensely studied (e.g. Burford *et al.*, 2001; Jordão and Oliveira, 2005; Wolfrath, 1993; Krippeit-Drews *et al.*, 1989; Medina and Rodríguez, 1992; Rodríguez *et al.*, 1997; von Hagen, 1987 and references therein). The complete larval development of *U. tangeri* was first described by Rodríguez and Jones (1993) based on material from Rio San Pedro, an inlet in Cádiz Bay, SW Spain, and it consisted of five zoeae and one megalopa stage. The first zoeal stage had been previously described by Paula (1985), who reported

data missed in the description by Rodríguez and Jones (1993). Later, Rodríguez *et al.* (1997) studied the larval abundance patterns in the same site and suggested that larvae released in the inlet were exported to the bay. However, the tolerance of *U. tangeri* larvae to reduced salinities has not been studied.

The genus *Uca* was divided into nine subgenera by Crane (1975): *Afruca* (with a single species: *U. tangeri*), *Amphiuca*, *Australuca*, *Boboruca*, *Celuca*, *Deltuca*, *Minuca*, *Thalassuca*, and *Uca*. A phylogeny based on a 16S rRNA regrouped the species into 3 clades and suggested that the ancestral clade, which includes *Uca* and *Afruca*, is basal to the Indo-West Pacific clade and the derived American clade (Levinton *et al.*, 1996; Sturmbauer *et al.*, 1996). Later, Rosenberg (2001) reduced the number of genera to seven, unifying *Afruca* + *Uca* and *Boboruca* + *Minuca*, but Beinlich and von Hagen (2006) established eight subgenera: *Australuca*, *Cranuca* subgen. nov., *Gelasimus*, *Leptuca*, *Minuca*, *Paraleptuca*, *Tabuca*, and *Uca* s. str. (which includes former *Uca* and *Afruca*). This schema was followed by Ng *et al.* (2008) in a recent revision of the systematics of crabs. Beinlich and von Hagen (2006) recognised that *U. tangeri* “clearly represents the earliest branch of *Uca* s. str. and is aberrant in its front width and geography”. Recently, molecular studies confirmed the validity of the monotypic subgenus *Afruca*, clearly separated from the other representatives of the subgenus *Uca* (Landstorfer, Felder and Schubart, unpublished).

The original objective of the present study was to evaluate the tolerance of *Uca tangeri* zoeae to low salinities, and to determine whether this tolerance varies among clutches produced by different females. While rearing larvae in the laboratory, we had the opportunity to carefully observe the zoeal morphology; on these grounds, we decided to re-describe and illustrate some previously omitted aspects that may be useful contributions to the knowledge of the phylogeny of *Uca*. Finally, by comparing the results of our larval cultures with those of Rodríguez and Jones (1993), we proposed that the absence of populations on northern Atlantic coasts may be related to a very low tolerance of zoeae to low temperatures.

MATERIALS AND METHODS

Collection

In July 2007, ovigerous females of *Uca tangeri* were collected by hand at low tides in Rio San Pedro,

a shallow inlet of the salt marsh zone in the eastern shore of the Bay of Cadiz, Spain (36°23'–37°N, 6°8'–15'W). Water temperature and salinity of Rio San Pedro in summer vary between 22.2 and 29.6°C and 36.5 and 43.5, respectively (González Gordillo *et al.*, 2003). Four ovigerous females with near-to-hatch embryos (named F1–F4) were carried to the laboratory at the Instituto de Ciencias Marinas de Andalucía. Each female was reared at ambient temperature and photoperiod in individual aquaria filled with water from the inlet until hatching.

Rearing techniques

Immediately after hatching (F1 and F2: 07/04/2007; F3, 07/05/2007; F4, 07/06/2007), only actively swimming larvae were transferred to individual cultivation vials (10 cm³) with wide-bore pipettes. Three salinities (16, 24, 32) obtained by dilution of filtered seawater (mesh: 0.45 µm) from Rio San Pedro with appropriate amounts of desalinated tap water and checked with a hand refractometer to the nearest PSU were tested. From each female, 24 larvae were placed in each salinity and maintained in cultivation chambers (20 ± 1°C) with a controlled photoperiod of 16:8 h L:D. Larvae were acclimated by transferring gradually (8 h at 24) until the final

salinity to avoid osmotic shock at the lowest salinity (16). Zoeae were fed with newly-hatched *Artemia* nauplii and *Brachionus plicatilis* (previously fed with *Nannochloropsis gaditana*). Water and food were changed daily, and larvae were checked for mortality and moults during each water change.

Statistical procedures

A log-linear model was performed to test interactions between frequency of larval death of each zoeal stage with females and salinity as factors (Norman and Streiner, 1996; Zar, 1996). Number of factors and levels were variable due to the high mortality of larvae at low salinity.

Differences between mean values of development duration of each zoeal stage and the duration of the complete development were tested by a mixed-factor ANOVA model with unequal replicates (Zar, 1996), with salinity as the fixed factor and females as the random factor. This test was used even with non-normal data given that ANOVA is robust to departure from this assumption (Underwood, 1997). Significant differences among treatments after ANOVA were tested with a Student–Newman–Keuls (SNK) test. In all statistical tests, differences were considered significant when $P \leq 0.05$.

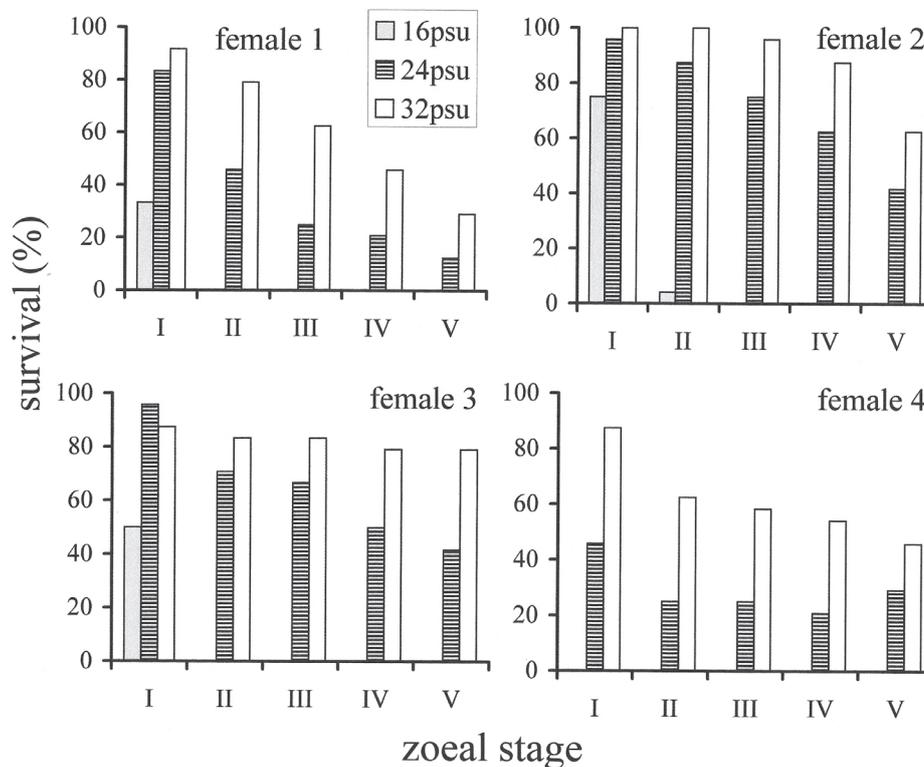


FIG. 1. – *Uca tangeri*. Proportional mortality of larvae from four clutches (F1–F4) reared at different salinities (16, 24, and 32).

TABLE 1. – *Uca tangeri*. Results of a test (log-linear model; Norman and Streiner, 1996; Zar, 1996) applied to detect interactions between proportional mortality of each zoeal stage (Fig. 1) with female and salinity as factors (***: $P < 0.001$; * $P < 0.5$).

Factors	Zoea I		Zoea II		Zoea III		Zoea IV		Zoea V	
	df	χ^2	df	χ^2	df	χ^2	df	χ^2	df	χ^2
Mortality (A)	1	47.69***	1	28.04***	1	9.76*	1	0.50	1	3.93*
Female (B)	3	0.000	3	0.000	3	0.000	3	0.000	3	0.000
Salinity (C)	2	0.000	1	0.000	1	0.000	1	0.000	1	0.000
A x B	3	37.31***	3	31.23***	3	29.60***	3	23.60***	3	17.69***
A x C	2	65.79***	1	12.47***	1	14.42***	1	14.80***	1	9.96*
B x C	6	0.000	3	0.000	3	0.000	3	0.000	3	0.000

RESULTS

Developmental patterns

Most of the reared larvae reached the megalopal stage after passing through five zoeal stages. However, additional zoeae VI were observed in the development of several larvae originating from female 1 (24: 1 case), female 2 (24: 1 case), female 3 (24 and 32: 3 and 1 cases, respectively) and female 4 (24 and 32: 4 and 1 cases, respectively). The proportion of megalopae originating from this “long developmental pattern” was 7%. From 11 zoeae VI obtained in the 4 clutches, 81.8 % were observed at salinity 24.

Mortality

With few exceptions, mortality decreased with increasing salinity, but varied among clutches; it was consistently lower in F2 and higher in F4 (Fig. 1). Larvae reached the megalopa stage at 24 and 32 but died as zoea I (F4), zoea II (F1, F2 and F3) or zoea III (one specimen, F2) at 16. A log-linear analysis showed significant interactions between females and mortality, and between salinity and mortality (Table 1), indicating that both salinity and females affected the survival of zoeae.

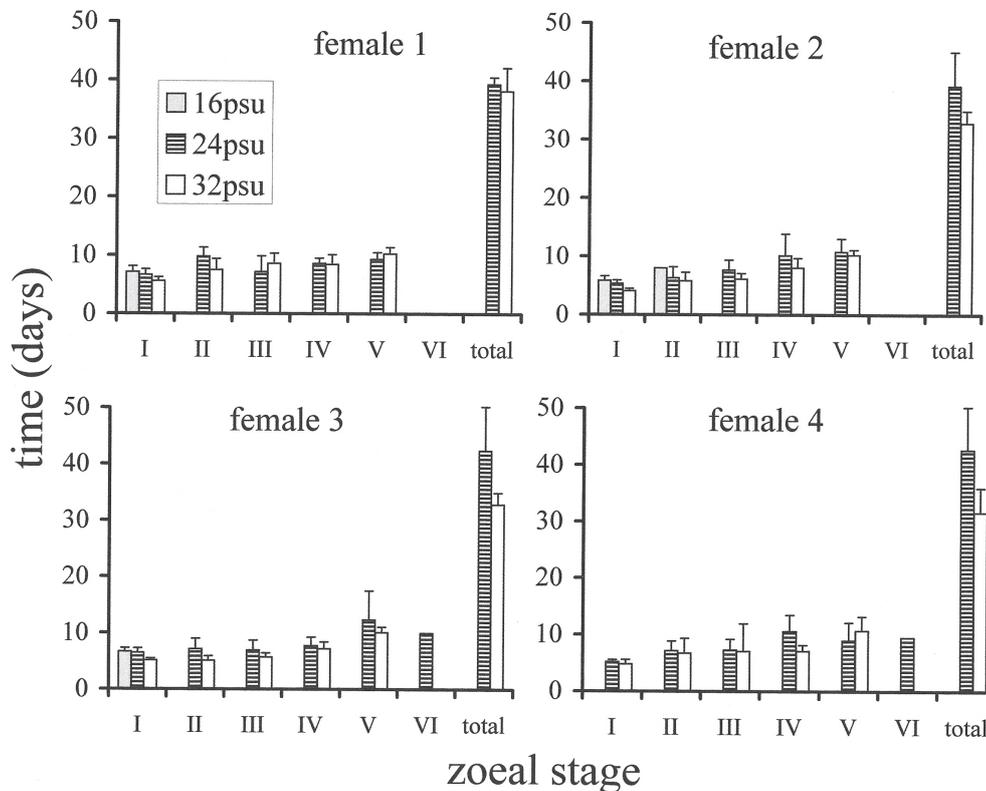


FIG. 2. – *Uca tangeri*. Development duration (d; mean \pm SD) through successive zoeal stages and total time to the megalopa stage from four clutches (F1-F4) reared at different salinities (16, 24, and 32).

TABLE 2. – *Uca tangeri*. Results of a test (mixed-factor ANOVA model with unequal replicates; Zar, 1996) applied to detect differences between mean values of duration of each zoeal stage (Fig. 2), developed in 16 (zoea I), 24 and 32. Female and salinity were random and fixed factors, respectively, and stage duration the variable response.

	Zoea I			Zoea II			Zoea III			Zoea IV			Zoea V			Total		
	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Female (A)	3	20.457	0.0025	3	6.259	0.0831	3	1.012	0.4962	3	1.497	0.3742	3	0.555	0.6799	3	0.380	0.7761
Salinity (B)	2	33.567	0.0010	1	7.149	0.0692	1	0.400	0.5678	1	5.064	0.1022	1	0.000	0.9835	1	13.860	0.0296
A*B	5	2.185	0.0574	3	2.362	0.0744	3	2.042	0.1122	3	2.561	0.0595	3	2.371	0.0777	3	2.719	0.0521

Duration of development

The duration of zoeal stages increased with development from 5.59 ± 1.09 to 10.48 ± 2.40 (all females pooled). Individual larvae moulted after 4–9 days in zoea I, 4–13 days in zoea II, 4–23 days in zoea III, 6–17 days in zoea IV, 6–20 days in zoea V and 9–10 days in the few larvae that passed through a zoea VI stage. The average values of each stage duration in larvae from the four clutches (F1–F4) reared at different salinities are summarised in Figure 2. A two-way ANOVA showed that salinity and females had a highly significant effect on the duration of zoea I development (Table 2) but no interactions appeared between factors. A post-hoc comparison allowed the duration to be ranked among salinities ($16 > 24 > 36$) and females (F1 and F3 > F2 and F4).

The complete zoeal development of individual larvae lasted from 23 to 51 days. The minimum and maximum average values of different clutches (F1–F4) and salinities (24 and 32 psu) ranged from 31.6 ± 4.4 to 42.7 ± 7.6 days (F4, 32 and 24, respectively, Fig. 2). The duration of the complete development was significantly affected by salinity (ANOVA, Table 2).

Zoeal morphology

The morphology of the 5 zoeal stages and megalopa is basically equal to those described by Rodríguez and Jones (1993), with the exception that the telson of zoea I to zoea V had a small dorsal spine, 2 pairs of minute lateral spines, and 2–3 minute ventral spines on each furcal arm (Figs. 4, 5). Two patterns were observed regarding the morphology of the few zoeae VI obtained. Some individuals showed the typical traits of an “advanced” developmental stage, combining zoeal and megalopal characters (i.e. dorsal setae on the telson plaque, natatory setae on abdominal pleopods), as well as a general increase in size and number of setae on the appendages (i.e. 14 natatory setae on exopods of first and second maxillipeds; 10 (4+4+2) aesthetascs and 2 setae on anten-

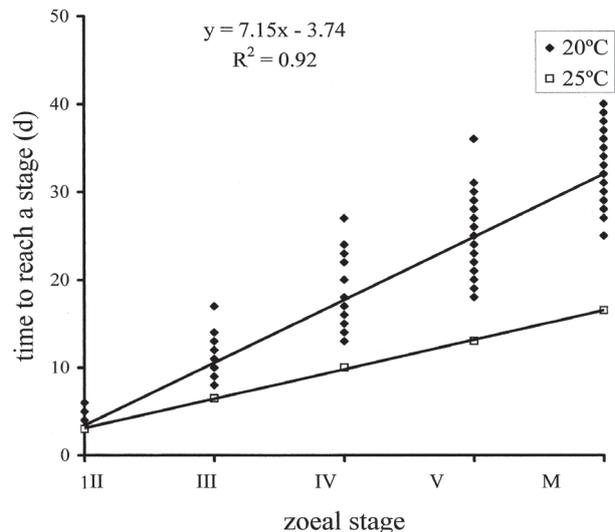


FIG. 3. – *Uca tangeri*. Cumulative development duration from hatching to the onset of successive larval stages (zoea II, III, IV, V and megalopa) of larvae cultured at 20 (this study, salinity 32) and 25°C (Rodríguez and Jones, 1993).

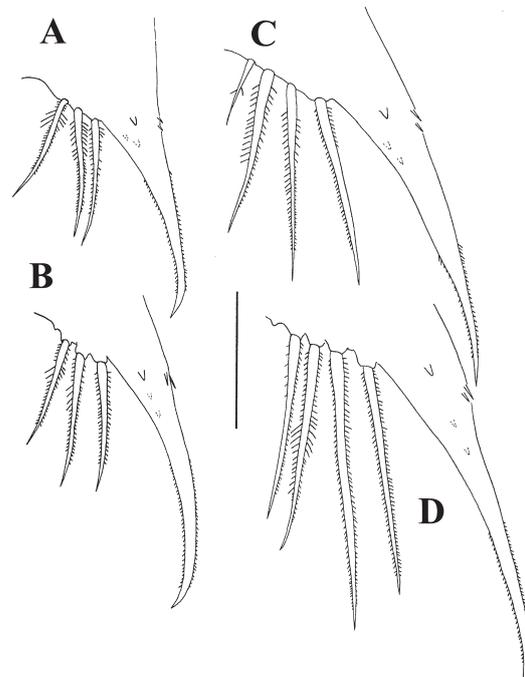


FIG. 4. – *Uca tangeri*. Dorsal view of the posterior and right region of the telson of zoeal stages. A, zoea I; B, zoea II; C, zoea III; D, zoea IV. Scale bar = 0.1 mm.

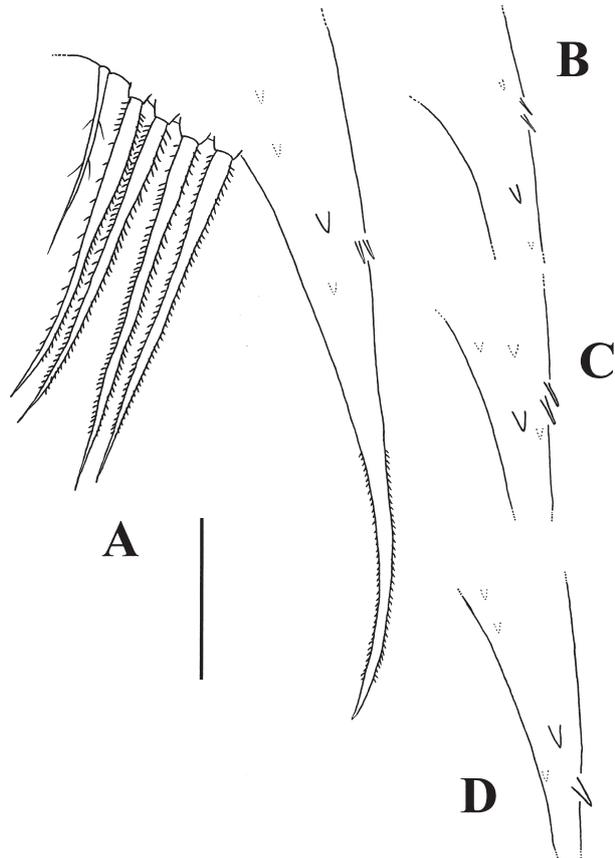


FIG. 5. – *Uca tangeri*. Dorsal view of the posterior and right region of the telson of zoeal stages. A, zoea V; B-D, zoea VI. Scale bar = 0.1 mm.

nule; 16–18 setae on ventral margin of cephalothorax. Further features observed were: endopod of antenna 3-segmented (2-segmented in zoea V), terminal segment of the endopod of first maxilliped with 6 setae (5 setae from zoea I to V). Other individuals were morphologically similar to a “normal” zoea V; in fact, they originated from an “intermediate” previous stage (zoea V) that had some features of a zoea IV. No significant differences in morphology were found between megalopae originating from 6th or 5th zoeal stages.

DISCUSSION

Larval tolerance to low salinities

Larvae of decapod crustacean usually tolerate narrower salinity ranges than adults of the same species (Charmantier, 1998). Adults of *Uca tangeri*, including the ovigerous females, can live in diluted warm or temperate waters, as Ewa-Oboho (1993) reported for populations of Port Harcourt (southern Nigeria),

which live in habitats with salinities ranging from 10.8 to 16.2. In addition, adults are hyper-hyporegulators (Krippeit Drews *et al.*, 1989). However, larval development is strongly affected by low salinities. In our study, larvae of *Uca tangeri* from the Rio San Pedro population completed the development at 24 and 32 but died as zoea I or zoea II at 16. The highest survival was observed at 32, but even in this optimal condition differences between clutches from different females were observed. The duration of the first zoeal stage and of the complete zoeal development was shorter at 32. A similar pattern was observed in larvae of *Uca pugnax* from the northwestern Atlantic salt marshes reared at salinities of 10, 20 and 30: they were unsuccessful in moulting at 10 and had a slight delay in moulting at 20; the megalopal stage, however, was shorter at 20 (O’Connor and Epifanio, 1985). On the contrary, salinity-induced mortality was not detected in the zoea I of *U. minax* originating from a freshwater population during their long trip (>50 km, several days) to the sea. These larvae survived longer than those spawned by females from a brackish water population; a physiological acclimation during embryogenesis may explain the observed differences (Brodie *et al.*, 2007).

The development of *U. tangeri* larvae hatched in Rio San Pedro is likely to occur in Cádiz Bay, and also in the open sea, via larval exportation from parental waters (Rodríguez *et al.*, 1997). The export strategy may be a consequence of different selective forces: physiological (i.e. low tolerance to diluted water) and ecological (i.e. predator avoidance). Our observations show that the zoeae of *U. tangeri* could not tolerate retention in the mesohaline water of estuaries, and that export to oceanic waters should be optimal for their successful development. However, the larval export from Rio San Pedro may be better explained by ecological factors since water is euhaline or hyperhaline in this particular habitat, at least during summer (González Gordillo *et al.*, 2003). The small and vulnerable (i.e. with small dorsal and rostral cephalothoracic spines) zoeae of several *Uca* species emigrate from estuaries, usually with large-amplitude nocturnal ebb tides; this strategy allows them to escape from planktivorous fish, which are especially abundant in estuaries and near-shore areas and less common in the coastal ocean (“predator avoidance hypothesis”, Christy, 2003 and references therein). In fact, high densities of *Uca tangeri* zoeae I were found during nocturnal ebb tides (Rodríguez *et al.*, 1997).

Survival at 24 suggests that *U. tangeri* zoeae could also develop in polyhaline conditions, if they were retained in the ocean-estuary interface. Eventually, retention of some larvae in low estuarine waters may compensate for an excessive larval dispersal due to the export strategy.

Variability in survival and development duration among clutches from different females has been observed in other intertidal and estuarine crabs, such as *Armases rubripes* (Luppi *et al.*, 2003). A variation among clutches in optimal salinity was also observed in the calanoid copepod *Eurytemora affinis* (Lee and Petersen, 2002; Devreker *et al.*, 2004) and it has been proposed that “the variability at the clutch level could be a ‘reservoir of genetic diversity’ to respond either to short- or long-term changes in salinity (i.e. in the case of fresh- or seawater inflows)” (Lee, 1999). Crabs, in fact, have been used as models to examine the complex physiological relationships between phases of the life cycle. For example, tolerance of *Neohelice granulata* larvae to low salinities depend on two factors: 1) the salinity prevailing during the embryonic development and 2) the initial larval biomass (Giménez and Anger, 2003).

The number of larval stages is variable in many decapod species but has usually been considered as constant and species-specific in Brachyura (Anger, 2001). However, there are reports of additional zoeal stages in Brachyura, specially in estuarine intertidal species, belonging to Portunidae, Xanthoidea, Calappidae, Parthenopidae, Grapsoidea and Ocypodidae (see Montú *et al.*, 1990; Anger, 1991, 2001; Rieger, 1996, 1997 and 1998 and references therein).

Additional zoeal stages were observed in *Uca tangeri* and in three other fiddler crabs (*U. uruguayensis*, *U. mordax* and *U. burgersi*; Rieger, 1996, 1997 and 1998) but not in the few other species that have been studied: *U. pugilator*, *U. annulipes*, *U. vocans*, *U. triangularis*, *U. arcuata*, *U. thayeri*, and *U. lactea* (Ko and Kim, 1989; Rieger, 1998, and references therein); *U. subcylindrica* has an abbreviated development (Rabalais and Cameron, 1983). The proportion of “normal” and “longer” pathways differs among species. The frequency of long pathways was low in *Uca tangeri* (7%) and *U. uruguayensis* (4.8%), but high in *U. mordax* and *U. burgersi* (57% and 60%, respectively) (Rieger, 1996, 1997 and 1998). The presence of additional zoeal stages in crabs has been associated with unfavourable combinations of temperature and salinity (Anger, 2001) and this seems to be the case in *U. tangeri*. However, *U. mordax*, *U. burgersi*, *U.*

uruguayensis were cultured under favourable salinity and temperature (34 and 25 °C; Rieger, 1996, 1997 and 1998), suggesting that other factors must be responsible for the long pathways.

The differences between zoea V and zoea VI in *Uca tangeri* were the number of aesthetascs and setae of the antennule, the number of setae of the maxillule, maxilla and maxillipeds 1 and 2, and, to a lesser extent, the number of dorsal setae of the first abdominal segment, as was observed in *U. mordax*, *U. burgersi* and *U. uruguayensis* by Rieger (1996, 1997 and 1998). However, the zoea VI of *Uca tangeri* had an additional seta in the distal segment of the first maxilliped endopod (setation: 2,3,2,2,6), while that of *U. mordax*, *U. burgersi*, *U. uruguayensis* remained 2,3,2,2,5, as in zoea V (Rieger, 1996, 1997 and 1998).

Larval morphology and *Uca* phylogeny

It has been suggested that *U. tangeri* should belong to a separated and monospecific subgenus on the basis of adult morphology (Crane, 1975; Rosenberg, 2001); this subgenus has been named *Afruca*. Molecular studies also support a differentiation between *Uca (Afruca) tangeri* and the *Uca (Uca) s. str.* clade (Levinton *et al.*, 1996; Sturmbauer *et al.*, 1996). The spines observed in the telson of all *U. tangeri* zoeae (Paula, 1985; this paper), as well as the presence of lateral spines in the cephalotorax (Paula, 1985; Rodríguez and Jones, 1993), have not been found in any other *Uca* species described up to this moment. These are new evidences that support the *Afruca* hypothesis as well as its basal position in a proto-Atlantic origin of the genus *Uca* suggested by molecular data (Levinton *et al.*, 1996; Sturmbauer *et al.*, 1996). Recent molecular data also point out the separation of this species in a monotypic subgenus of *Uca* (Landstorfer, Felder and Schubart, unpublished). The spermatozoal ultrastructure, however, did not allow *U. tangeri* to be separated from the other *Uca* species: centrioles were present in adult spermatozoa of the American species *U. maracoani*, *U. thayeri* and *U. vocator*, and absent in both *U. tangeri* and the Indo-Pacific *U. dussumieri* (Benetti *et al.*, 2008). Putting together morphological (adult and larval) and molecular evidences, we suggest that *Uca tangeri* should be separated from the rest of the *Uca* species and placed in its own genus as *Afruca tangeri* by moving the present status of the subgenus *Afruca* to the genus level.

Paula (1985) mentioned the dorsal spine on furcal arms of the telson of zoea I, but not the lateral and ventral ones. None of these spines was observed by Rodríguez and Jones (1993), probably because of their small size. Likewise, similar small lateral spines of the furcal arms, which had also been overlooked in previous descriptions, have been recently recognised as a typical character of sesarimid zoeae (Cuesta *et al.* 2006a, 2006b). However, the spines described in *U. tangeri*'s telson are very small and their presence in zoeae of other *Uca* species, including those already described, should not be discarded. In the case of *Uca speciosa* zoea I, only a minute scale-like lateral spine is present on each furcal arm, and dorsal and ventral spines are absent (Cuesta and Schubart, unpublished data); also, only 1 or 0-2 minute lateral spines have been described in the zoea I and II of *Uca annulipes* (Hashmi, 1968) and zoea I of *Uca subcylindrica* (Rabalais and Cameron, 1983), respectively.

Geographical distribution: Does low tolerance of zoeae to cold water explain the northern limit of *U. tangeri*?

Temperatures also affect crustacean larval development and *U. tangeri* is not the exception. It has been widely demonstrated that development duration increases at lower temperatures in crustaceans (see Ismael *et al.*, 1997, and references therein). Comparing our data (salinity 32) with those by Rodríguez and Jones (1993) who reared *U. tangeri* at 25°C, we observed that a temperature decrease of 5°C caused a duplication of the larval span by increasing the duration of each stage (Fig. 3). Although it was often suggested that delayed development increased the possibility of dispersal, a longer larval development is correlated with a rise in mortality so its advantages are not obvious.

The northern population of *U. tangeri* inhabits the Mira estuary (37°43'N 8°47'W) (Costa *et al.*, 2001). Mortality associated with an increase in duration of development at low temperatures and the latitudinal temperature diminution could limit the larval dispersal of this species, and consequently its northern distribution. Also, the northern limit of *Uca pugnax* in the northwestern Atlantic is likely maintained by the influence of cooler water temperatures on the larval phase, as reported by Sanford *et al.* (2006), who found that there are suitable northern habitats for this species (extensive salt marshes) and that trans-

planted adult crabs could resist severe winter conditions. However, few larvae completed development at temperatures that they would regularly encounter north of their range boundary. The present distribution of *U. tangeri* in Europe could be explained by a combination of ecological and physiological experiments and knowledge of local hydrographical processes; this multidisciplinary study would contribute to the prediction of changes arising from an increase in sea temperature.

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