

Proof Delivery Form

Please return this form with your proof

Article number: S0025315410002055jra

Date of delivery: 26.11.10

Typesetter ref number: MBI1000205

Volume and Issue Number: 0 and 0

Number of pages (not including this page): 7

Journal of the Marine Biological Association of the United Kingdom

Here is a pdf proof of your article for publication in the **Journal of the Marine Biological Association of the United Kingdom**. Please print out the file, check the proofs carefully and answer any queries.

Please return your corrections **via email** (no later than 4 days after receipt) quoting paper number in the header of the email message.

Please also ensure you specify page and line number of each correction required in your email and send to:

Executive Editor JMBA
Email: jmba@mba.eclipse.co.uk

Please return your completed and signed **copyright transfer form** and **offprint form** by post to the addresses given on each form.

Please note:

- You are responsible for correcting your proofs. Errors not found may appear in the published journal.
- The proof is sent to you for correction of typographical errors only. Revision of the substance of the text is not permitted, unless discussed with the editor of the journal.
- Please answer carefully any queries raised from the typesetter.
- A new copy of a figure must be provided if correction of anything other than a typographical error introduced by the typesetter is required

Thank you in advance.

Author queries:

No Queries

Typesetter queries:

Please return this form with your proof

Offprint order form



CAMBRIDGE
UNIVERSITY PRESS

PLEASE COMPLETE AND RETURN THIS FORM. WE WILL BE UNABLE TO SEND OFFPRINTS UNLESS A RETURN ADDRESS AND ARTICLE DETAILS ARE PROVIDED.

VAT REG NO. GB 823 8476 09

Journal of the Marine Biological Association (MBI)

Volume:

no:

Offprints

Offprints may be purchased at the prices shown below. Please complete this form and send it to **the publisher (address below)**. Please give the address to which your offprints should be sent. They will be despatched by surface mail within one month of publication.

Number of offprints required :

Email:

Offprints to be sent to (print in BLOCK CAPITALS):

.....

Post/Zip Code:

Telephone:

Date (dd/mm/yy):

/

/

Author(s):

Article Title:

All enquiries about offprints should be addressed to **the publisher: Journals Production Department, Cambridge University Press, The Edinburgh Building, Shaftesbury Road, Cambridge CB2 8RU, UK.**

Charges for extra offprints (excluding VAT) Please circle the appropriate charge:

Number of copies	25	50	100	150	200	per 50 extra
1-4 pages	£68	£109	£174	£239	£309	£68
5-8 pages	£109	£163	£239	£321	£399	£109
9-16 pages	£120	£181	£285	£381	£494	£120
17-24 pages	£131	£201	£331	£451	£599	£131
Each Additional 1-8 pages	£20	£31	£50	£70	£104	£20

Methods of payment

If you live in Belgium, France, Germany, Ireland, Italy, Portugal, Spain or Sweden and are not registered for VAT we are required to charge VAT at the rate applicable in your country of residence. If you live in any other country in the EU and are not registered for VAT you will be charged VAT at the UK rate.

If registered, please quote your VAT number, or the VAT number of any agency paying on your behalf if it is registered.

VAT Number:

Payment **must** be included with your order, please tick which method you are using:

- Cheques should be made out to Cambridge University Press.
- Payment by someone else. Please enclose the official order when returning this form and ensure that when the order is sent it mentions the name of the journal and the article title.
- Payment may be made by any credit card bearing the Interbank Symbol.

Card Number:

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------

Expiry Date (mm/yy):

/

Card Verification Number:

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
----------------------	----------------------	----------------------	----------------------	----------------------

The card verification number is a 3 digit number printed on the **back** of your **Visa** or **Master card**, it appears after and to the right of your card number. For **American Express** the verification number is 4 digits, and printed on the **front** of your card, after and to the right of your card number.

Signature of card holder:

Amount (Including VAT if appropriate):

£

Please advise if address registered with card company is different from above



Transfer of copyright

Please read the notes overleaf and then complete, sign, and return this form to **The Executive Editor, Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK** as soon as possible.

In consideration of the publication in **JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM**

of the contribution entitled:

by (all authors' names):

1 To be filled in if copyright belongs to you Transfer of copyright

I/we hereby assign to Cambridge University Press, full copyright in all formats and media in the said contribution, including in any supplementary materials that I/we may author in support of the online version.

I/we warrant that I am/we are the sole owner or co-owners of the contribution and have full power to make this agreement, and that the contribution contains nothing that is in any way an infringement of any existing copyright or licence, or duty of confidentiality, or duty to respect privacy, or any other right of any person or party whatsoever and contains nothing libellous or unlawful; and that all statements purporting to be facts are true and that any recipe, formula, instruction or equivalent published in the Journal will not, if followed accurately, cause any injury or damage to the user.

I/we further warrant that permission has been obtained from the copyright holder for any material not in my/our copyright including any audio and video material, that the appropriate acknowledgement has been made to the original source, and that in the case of audio or video material appropriate releases have been obtained from persons whose voices or likenesses are represented therein. I/we attach copies of all permission and release correspondence.

I/we hereby assert my/our moral rights in accordance with the UK Copyrights Designs and Patents Act (1988).

Signed (tick one)

the sole author(s) one author authorised to execute this transfer on behalf of all the authors of the above article

Name (block letters)

Institution/Company

Signature: Date:

(Additional authors should provide this information on a separate sheet.)

2 To be filled in if copyright does not belong to you

a Name and address of copyright holder.....
.....
.....

b The copyright holder hereby grants to Cambridge University Press the non-exclusive right to publish the contribution in the journal and to deal with requests from third parties in the manner specified in paragraphs 4 and 5 overleaf.

(Signature of copyright holder or authorised agent)

3 US Government exemption

I/we certify that the paper above was written in the course of employment by the United States Government so that no copyright exists.

Signature: Name (Block letters):

4 Requests received by Cambridge University Press for permission to reprint this article (see para. 4 overleaf) should be sent to

Name and address (block letters)

Notes for contributors

- 1 The Journal's policy is to acquire copyright in all contributions. There are two reasons for this: (a) ownership of copyright by one central organisation tends to ensure maximum international protection against unauthorised use; (b) it also ensures that requests by third parties to reprint or reproduce a contribution, or part of it, are handled efficiently and in accordance with a general policy that is sensitive both to any relevant changes in international copyright legislation and to the general desirability of encouraging the dissemination of knowledge.
- 2 Two 'moral rights' were conferred on authors by the UK Copyright Act in 1988. In the UK an author's 'right of paternity', the right to be properly credited whenever the work is published (or performed or broadcast), requires that this right is asserted in writing.
- 3 Notwithstanding the assignment of copyright in their contribution, all contributors retain the following **non-transferable** rights:
 - The right to post *either* their own version of their contribution as submitted to the journal (prior to revision arising from peer review and prior to editorial input by Cambridge University Press) *or* their own final version of their contribution as accepted for publication (subsequent to revision arising from peer review but still prior to editorial input by Cambridge University Press) on their **personal or departmental web page**, or in the **Institutional Repository** of the institution in which they worked at the time the paper was first submitted, or (for appropriate journals) in PubMedCentral or UK PubMedCentral, provided the posting is accompanied by a prominent statement that the paper has been accepted for publication and will appear in a revised form, subsequent to peer review and/or editorial input by Cambridge University Press, in **Journal of the Marine Biological Association of the United Kingdom** published by Cambridge University Press, together with a copyright notice in the name of the copyright holder (Cambridge University Press or the sponsoring Society, as appropriate). On publication the full bibliographical details of the paper (volume: issue number (date), page numbers) must be inserted after the journal title, along with a link to the Cambridge website address for the journal. Inclusion of this version of the paper in Institutional Repositories outside of the institution in which the contributor worked at the time the paper was first submitted will be subject to the additional permission of Cambridge University Press (not to be unreasonably withheld).
 - The right to post the definitive version of the contribution as published at Cambridge Journals Online (in PDF or HTML form) on their **personal or departmental web page**, no sooner than upon its appearance at Cambridge Journals Online, subject to file availability and provided the posting includes a prominent statement of the full bibliographical details, a copyright notice in the name of the copyright holder (Cambridge University Press or the sponsoring Society, as appropriate), and a link to the online edition of the journal at Cambridge Journals Online.
 - The right to post the definitive version of the contribution as published at Cambridge Journals Online (in PDF or HTML form) in the **Institutional Repository** of the institution in which they worked at the time the paper was first submitted, or (for appropriate journals) in PubMedCentral or UK PubMedCentral, no sooner than **one year** after first publication of the paper in the journal, subject to file availability and provided the posting includes a prominent statement of the full bibliographical details, a copyright notice in the name of the copyright holder (Cambridge University Press or the sponsoring Society, as appropriate), and a link to the online edition of the journal at Cambridge Journals Online. Inclusion of this definitive version after one year in Institutional Repositories outside of the institution in which the contributor worked at the time the paper was first submitted will be subject to the additional permission of Cambridge University Press (not to be unreasonably withheld).
 - The right to post an abstract of the contribution (for appropriate journals) on the **Social Science Research Network (SSRN)**, provided the abstract is accompanied by a prominent statement that the full contribution appears in **Journal of the Marine Biological Association of the United Kingdom** published by Cambridge University Press, together with full bibliographical details, a copyright notice in the name of the journal's copyright holder (Cambridge University Press or the sponsoring Society, as appropriate), and a link to the online edition of the journal at Cambridge Journals Online.
 - The right to make hard copies of the contribution or an adapted version for their own purposes, including the right to make multiple copies for course use by their students, provided no sale is involved.
 - The right to reproduce the paper or an adapted version of it in any volume of which they are editor or author. Permission will automatically be given to the publisher of such a volume, subject to normal acknowledgement.
- 4 We shall use our best endeavours to ensure that any direct request we receive to reproduce your contribution, or a substantial part of it, in another publication (which may be an electronic publication) is approved by you before permission is given.
- 5 Cambridge University Press co-operates in various licensing schemes that allow material to be photocopied within agreed restraints (e.g. the CCC in the USA and the CLA in the UK). Any proceeds received from such licences, together with any proceeds from sales of subsidiary rights in the Journal, directly support its continuing publication.
- 6 It is understood that in some cases copyright will be held by the contributor's employer. If so, Cambridge University Press requires non-exclusive permission to deal with requests from third parties, on the understanding that any requests it receives from third parties will be handled in accordance with paragraphs 4 and 5 above (note that your approval and not that of your employer will be sought for the proposed use).
- 7 Permission to include material not in your copyright
If your contribution includes textual or illustrative material not in your copyright and not covered by fair use / fair dealing, permission must be obtained from the relevant copyright owner (usually the publisher or via the publisher) for the non-exclusive right to reproduce the material worldwide in all forms and media, including electronic publication. The relevant permission correspondence should be attached to this form.

If you are in doubt about whether or not permission is required, please consult the Permissions Manager, Cambridge University Press, The Edinburgh Building, Shaftesbury Road, Cambridge CB2 8RU, UK. Fax: +44 (0)1223 315052. Email: lnicol@cambridge.org.

The information provided on this form will be held in perpetuity for record purposes. The name(s) and address(es) of the author(s) of the contribution may be reproduced in the journal and provided to print and online indexing and abstracting services and bibliographic databases.

Please make a duplicate of this form for your own records

Differential coloration patterns in the intertidal crab *Neohelice* (= *Chasmagnathus*) *granulata*

AGUSTINA MÉNDEZ CASARIEGO^{1,2}, TOMÁS LUPPI^{1,2} AND OSCAR IRIBARNE^{1,2}

¹Departamento de Biología (FCEyN), Universidad Nacional de Mar del Plata, CC 573 Correo Central, B7600WAG, Mar del Plata, Argentina, ²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rivadavia 1917, CP C1033AAJ, Ciudad de Buenos Aires, Argentina

Many crustaceans with spatial segregation may present differential body coloration depending on the habitat they inhabit. Since crustaceans are unable to synthesize carotenoids, individuals must acquire them from their diet, so diet changes can modify individual coloration. The burrowing crab Neohelice (=Chasmagnathus) granulata inhabits the intertidal, from the uppermost parts of salt marshes to the lowest mudflat zones with a spatial segregation of different size-classes. They are primarily deposit feeders in mud flats and herbivorous–detritivorous in the salt marsh. We analysed the effect of intermoult length on the carapace colour of N. granulata, and the changes in carapace colour with crab size and zone. Crab colour varied between unvegetated and vegetated areas. Some of these differences were in part caused by colour changes related to crab size. Larger crabs show less intensive colours, and given that they inhabit the upper part of the intertidal a differential coloration pattern occurred between zones. In field experiments intermoult duration affected crab colour and some body regions analysed showed differences between zones unrelated to size.

Keywords: estuaries, carapace colour, marsh, mudflat, crabs

Submitted 23 June 2010; accepted 2 November 2010

INTRODUCTION

Ontogenetic changes in osmoregulatory ability (Charmantier *et al.*, 2002), air exposure resistance, diet choices, predator avoidance (Hunt & Scheibling, 1997) and reproductive behaviour (Carr *et al.*, 2004) may cause variations in habitat use during the life cycle of organisms (Etherington *et al.*, 2003; Lipcius *et al.*, 2005). In addition, structured habitats may affect these habitat choices influencing possible interactions with other species or ages (Hines *et al.*, 1987). Generally, vegetated intertidal areas, such as mangroves, seagrass beds or marshes, are important refuges or nursery habitats for many species (Beck *et al.*, 2001). Several species with complex life cycles depend upon these areas which enhance survival by diminishing predation and increasing food availability in comparison with non-vegetated areas (Beck *et al.*, 2001; Heck *et al.*, 2003; Minello *et al.*, 2003).

Many crustaceans with spatial segregation of different size-classes may present differential body coloration depending on the habitat they inhabit. Either diet changes or predator avoidance, can promote phenotypic effects upon individuals. Some shrimps, for example, change their colour with different diets (Arredondo-Figueroa *et al.*, 2003). Because there is an ontogenetic diet shift, juveniles and adults are easily recognized (Sagardes *et al.*, 2000). The colour of the green crab *Carcinus maenas* is associated to intermoult duration

because of pigment denaturalization (Reid *et al.*, 1997). There is also spatial segregation in this case, because crabs moulting less often are more tolerant to dissection, inhabiting in higher zones of the intertidal (McGaw & Naylor, 1992; Reid *et al.*, 1997; Styriehave *et al.*, 2004). Colour also plays a key role in social behaviour. In several species, colour is related to reproductive success, directly by female choice, or indirectly as a visual signal of territoriality (Reid *et al.*, 1997; Detto *et al.*, 2004; Styriehave *et al.*, 2004).

Colour in decapod crustaceans is determined by the number, types and distribution of chromatophores and by the carotenoid astaxanthin incorporated into the exoskeleton (Thurman, 1990). Since crustaceans are unable to synthesize carotenoids, they must acquire them from their diet (Sagardes *et al.*, 2000) and thus, a diet change can modify individual coloration (Rao, 1985). Carotenoids in crustaceans are assumed to have various functions being the most common cryptic coloration to avoid predation (Rao, 1985; Thurman, 1988). Several crustaceans incorporate the appropriate colour by changing their diets throughout successive moults (Rao, 1985). This also leads to differential coloration depending on the habitat. Among the functions proposed for carotenoids are thermoregulation (Silbiger & Munguia, 2008) and photoprotection to UV radiation (Moeller *et al.*, 2005), blocking light and the chemical damage to DNA (Edge *et al.*, 1997). For this reason, we hypothesize that crustaceans that inhabit intertidal estuaries and use vegetated zones such as marshes, mangroves or seagrass beds during part of their life cycle may change their coloration either by changes in diet, UV intensity or any physical change that could modify moult rate.

Corresponding author:
A.M. Casariego
Email: mendezc@mdp.edu.ar

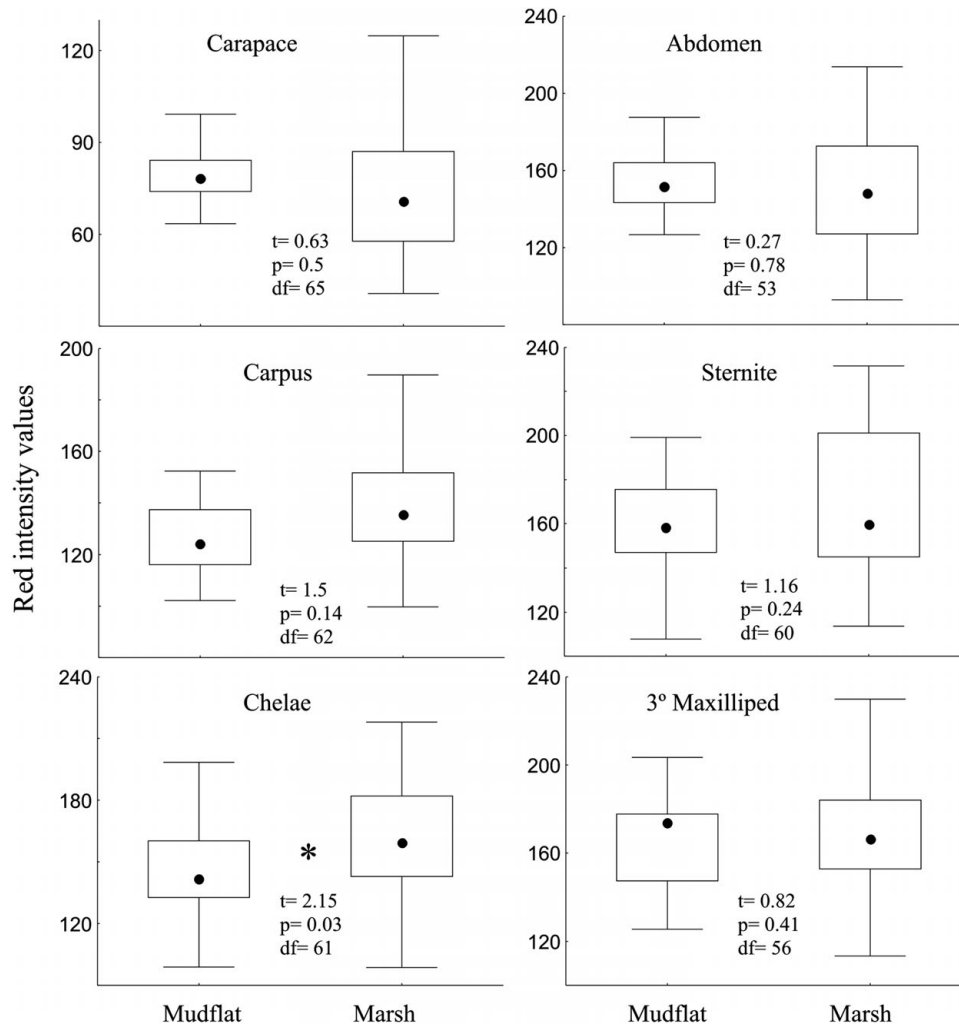


Fig. 1. Box-plots comparing intensity values for the red colour band between marsh and mudflat zones for the 6 body regions analysed. Here and thereafter box-plots are constructed with limits of boxes being the 75th and 25th percentile; lines represent 10th and 90th percentiles and squares inside boxes are medians. Inside: *t*-test results.

Intertidal zones of estuaries and embayments in the southwestern Atlantic (southern Brazil to central Argentina) are dominated by the burrowing grapsoid crab *Neohelice (Chasmagnathus) granulata* (e.g. Boschi, 1964; Spivak *et al.*, 1994; Iribarne *et al.*, 1997) which are characterized by extensive mudflats surrounded by salt marshes dominated by *Spartina* spp. (Isacch *et al.*, 2006). *Neohelice granulata* reaches 40mm carapace width (CW) and their physiological adaptations allow them to occupy the uppermost parts of the intertidal from salt marshes to the lowest mudflat zones in the intertidal (Spivak *et al.*, 1994; Luquet *et al.*, 1998; Halperin *et al.*, 2000). Nevertheless, the environmental conditions in the higher part of the marsh are generally more stressful for these organisms, so densities are usually higher in the lower zones (Spivak *et al.*, 1994; Bortolus *et al.*, 2002). In the high marsh, vegetation ameliorates the physical stress caused by high temperatures allowing crabs to colonize this area during the summer season (Bortolus *et al.*, 2002). Crabs are primarily deposit feeders in mud flats and herbivorous–detritivorous in the salt marsh (Iribarne *et al.*, 1997; Botto *et al.*, 2005), with *Spartina* spp. their main food source (Botto *et al.*, 2005). In addition to these differences between habitats and diet crab changes, the

size-dependent spatial segregation present in this species it is common to see crabs with coloration brighter in the marsh than in the mudflat. In this research we analyse the relationship between size, zone, and intermoult length and the colour of *Neohelice granulata*.

MATERIALS AND METHODS

Study area

Experiments and field samples were performed in Mar Chiquita coastal lagoon (37° 46'S 57° 27'W, Argentina). This is a body of brackish water (46 km²) of low tidal amplitude (~1 m) permanently connected to the sea (Reta *et al.*, 2001). The main habitats around the lagoon are intertidal mudflats and large tidal plains irregularly flooded (10 to 15 times per month) dominated by the cordgrass *Spartina densiflora* (Isacch *et al.*, 2006). The crabs *Neohelice granulata* are distributed in both the *S. densiflora* salt marsh and the intertidal mudflats generating large burrowing beds (Spivak *et al.*, 1994; Iribarne *et al.*, 1997; Botto *et al.*, 2005).

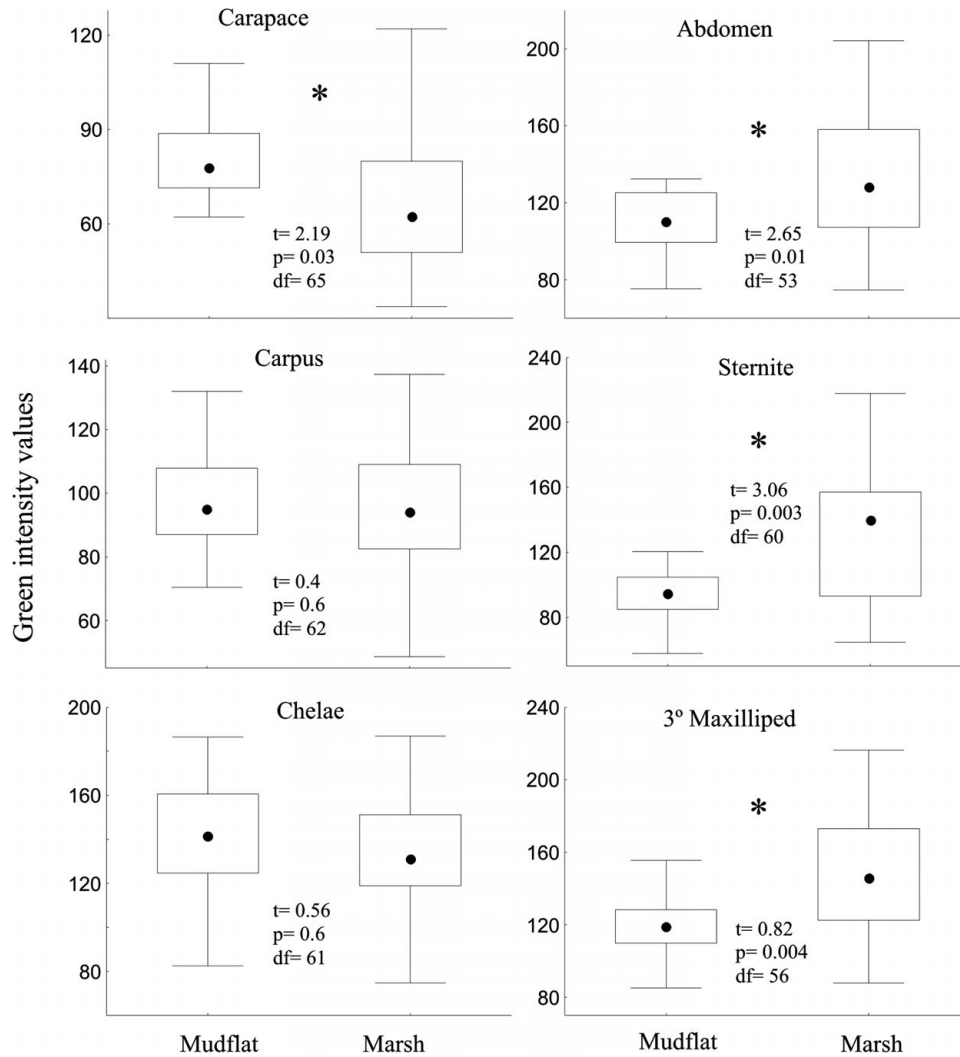


Fig. 2. Box-plots comparing intensity values for the green colour band between marsh and mudflat zones for the 6 body regions analysed, with *t*-test results inside.

Differences in crab coloration

To investigate differences in *N. granulata* coloration between marsh and mudflat zones, crabs were collected randomly by hand at both zones and photographed with a digital camera (following Tlustý & Hyland, 2005), analysed with Adobe Photoshop program. Each individual was dorsally and ventrally photographed with a white reference square in order to standardize colour values of the photos. Pictures were opened in red–green–blue (RGB) colour mode, and a part of the crab could be selected giving the average value for red, green and blue from a histogram plot provided by the program. Each colour value ranged from 0 to 255, where 0 is black. Values of 255 in the 3 bands represent white and 0 black, while a pure colour (red, green or blue) is 255 for that particular colour and 0 for the other two. A total of 6 crab regions were analysed, 3 ventral zones, abdomen (Ab), sternite (St), 3rd maxilliped (Mx) and 3 dorsal zones, chela (Q), carapace (Cap) and carpus (Car). Each region of the crab was compared between marsh and mudflat zones with a *t*c test (*t*-test corrected) for unequal variances (Welch approximation *t*c; Zar 1999) separately by colour bands (RGB). The *t*c is equal to the *t*-value when sample sizes are the same, but degrees of freedom decrease as the difference between variances of the 2 groups increases

(Zar, 1999). To analyse if crab size is related to coloration, the 6 regions were analysed with correlation analysis (Zar, 1999) between colour values and crab size, separately by colour band.

Differences in crab colour between zones may be related to differences in crab size. To avoid this confounding factor, crabs from the same size-range (22–36 mm) were compared between zones. The 6 regions were compared for each colour band with *t*c tests (Zar, 1999).

Intermoult length can also affect crab coloration patterns. To analyse changes in crab colour through intermoult an individual inclusion experiment was performed with cylindrical boxes (6 replicates, 40 cm diameter, 1 mm mesh size). In each cage, 1 recently moulted adult crab was included. The different crab regions were compared with *t*c test for dependent samples at the beginning and 15 days after the crabs moulted.

RESULTS

All crab regions analysed had differences in colour at least in one of the colour bands. The red value for the chela was highest in the marsh (Figure 1) while the carapace showed highest green values at the mudflat (Figure 2). The 3 dorsal

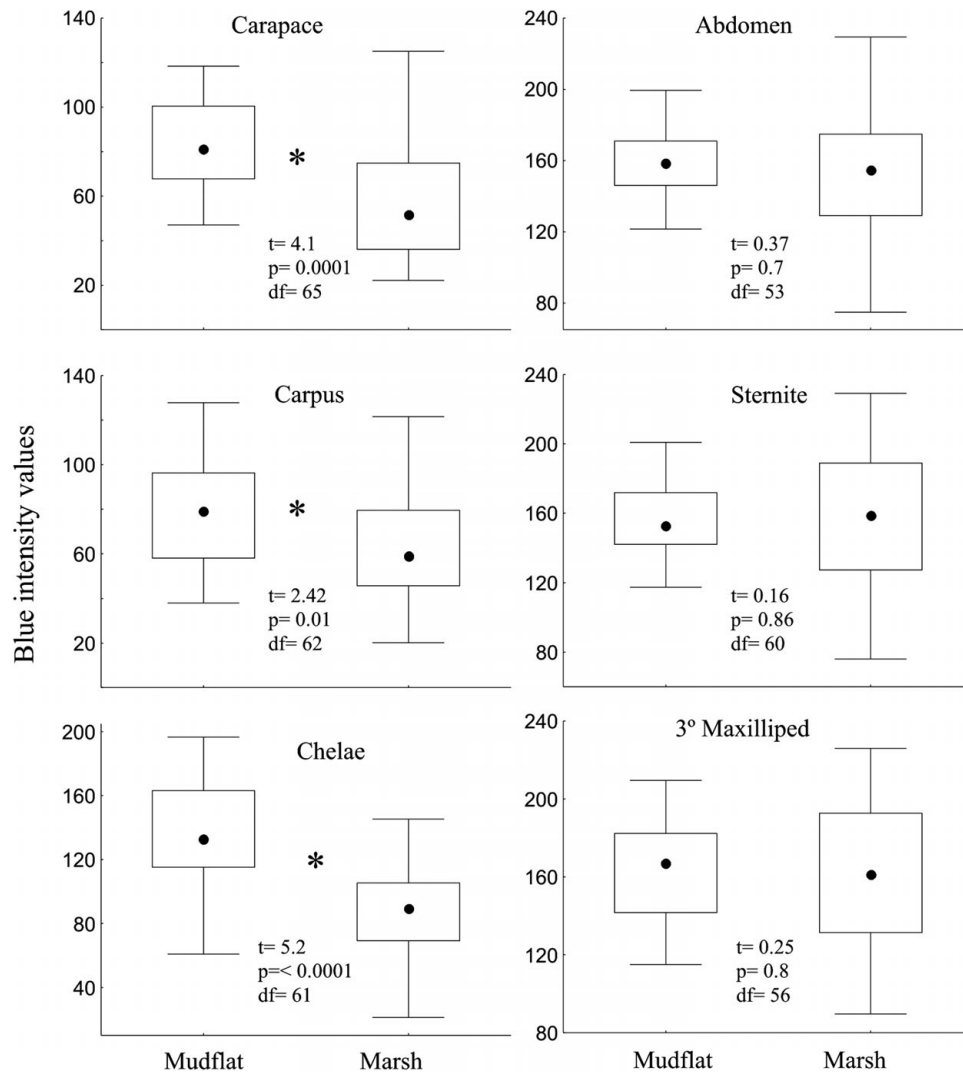


Fig. 3. Box-plots comparing intensity values for the blue colour band between marsh and mudflat zones for the 6 body regions analysed, with *t*-test results inside.

regions had differences for the blue band with higher values in the mudflat than the crabs in the marsh (Figure 3) and the ventral regions had differences for green values being higher in the marsh than in the mudflat (Figure 2).

Correlation analysis between colour values and size showed relationships (Table 1) for the same bands that showed differences between zones. Ventral regions showed a decrease in green values with size (Figure 4), and dorsal regions showed an increase in blue values with crab size (Figure 5). Nevertheless, the r^2 values are moderate or low, explaining between 20 to 40% of the variability in colour.

When the same size-range was considered, some of the colour differences between zones were eliminated. Green values for the abdomen and carapace regions had no differences in their values (t_c Ab = 0.79, df_{29} , $P = 0.4$; t_c Cap = 0.44, $df = 39$, $P = 0.6$). Carapace and carpus had no differences for the blue values (t_c Cap = 1.66, $df = 39$, $P = 0.1$; t_c Car = 1.02, $df = 39$, $P = 0.3$). Green values for the sternite and the 3rd maxilliped had differences between zones (t_c St = 2.65, $df = 37$, $P = 0.01$; t_c Mx = 2.27, $df = 35$, $P = 0.03$) as well as differences in blue values for the chela ($t_c = 2.3$, $df = 40$, $P = 0.02$).

Crabs recently moulted and those 15 days postmoult had differences in colour. Green ($t_c = 2.16$, $P = 0.05$) and red

($t_c = 2.3$, $P = 0.05$) values of the carapace diminished during intermoult stage, while the sternite showed an increase in green value ($t_c = 2.9$, $P = 0.05$). The other regions showed no differences along this period (all $P > 0.01$).

DISCUSSION

Coloration of *Neohelice granulata* showed differences between the low unvegetated intertidal and the high vegetated intertidal areas. Some of these differences were caused by colour changes related with crab size. There was a moderate relationship between crab size and colour; because of the spatial size segregation present in this species, this affects the coloration patterns between zones. Nevertheless, ventral zones and chela had differences between zones unrelated to size. Intermoult duration also affected crab colour in field experiments.

Colour associated with social interactions is often related to claws. Many male crabs have bright chelae which are used in aggressive interactions with other males and waving displays to attract females (Crane, 1975; Christy & Salmon, 1984). *Neohelice granulata* showed differences in chelae colour between marsh and mudflat zone, and chelae were the body region with the highest association with size. If chelae are

253 **Table 1.** Correlation analysis between colour values and crab size for the
 254 six crab parts analysed. St, sternite; Ab, abdomen; 3° Mx, 3° maxilliped;
 255 Cap, carapace; Car, carpus; Ch, chela; r2, coefficient of determination
 256 ****P* < 0.0001; ***P* < 0.01; **P* < 0.5.

257	Red			
258		r2	F	P
259				
260	St	0.001	0.09	0.7
261	Ab	0.01	0.64	0.42
262	3°Mx	0.02	1.26	0.26
263	Cap	0.09	6.78	**
264	Car	0.0001	0.01	0.91
265	Q	0.0007	0.04	0.8
266				
267	Green			
268	St	0.25	19.6	***
269	Ab	0.21	13.8	***
270	3°Mx	0.08	5.12	*
271	Cap	0.17	12.76	***
272	Car	0.008	0.51	0.47
273	Q	0.02	1.53	0.22
274	Blue			
275	St	0.0001	0.008	0.9
276	Ab	0.004	0.2	0.6
277	3°Mx	0.01	0.64	0.42
278	Cap	0.23	19.42	***
279	Car	0.09	6.57	*
280	Q	0.39	41.6	***

281
 282
 283 used in social interactions such as courtship or agonistic
 284 encounters as reported for other crab species (*Uca*: Crane,
 285 1975), colour can be a useful sign to predict the outcome of
 286 competitive disputes (Detto *et al.*, 2004). Body size generally
 287 is a good indicator of the outcome (Huntingford *et al.*,
 288 1995), but when size differences are small, claw size is a
 289 more reliable indicator of fight outcome than body size
 290 (Sneddon *et al.*, 1997). In some species, claw colour is the pre-
 291 dominant morphological feature in identifying conspecific
 292 mates from a distance (*Uca mjoebergi*: Detto *et al.*, 2008). In
 293 *Heloecius cordiformis* colour is also an important signal
 294 during courtship or aggressive interactions (Detto *et al.*,
 295 2004). In consequence, colour claw could reduce the need to
 296 approach rivals or mates to determine the likely outcome of
 297 a fight for a male or a female.

298 Ventral regions had colour differences between marsh and
 299 mudflat zones independently from the crab size. Since crus-
 300 taceans are unable to synthesize carotenoids *de novo*, individ-
 301 uals must acquire them from their diet (Chien & Jeng, 1992).
 302 Because *N. granulata* change their diet from deposit feeder in
 303 the mudflat to herbivore in the marsh (Iribarne *et al.*, 1997),
 304 this diet change could be affecting pigment incorporation,
 305 resulting in a differential colour. Pigments can be extracted
 306 by crabs from both zones, since algae in the mudflat and
 307 plants in the marsh can synthesize precursors (Davenport
 308 *et al.*, 2004) and stable isotopes were used to determine
 309 that their main source of food of crabs in mudflat areas is
 310 also *Spartina* (Botto *et al.*, 2005). Physical and biological con-
 311 ditions in each zone might affect crab colour. Plant cover in
 312 the marsh can modify colour by providing pigments through
 313 consumption and by modifying the UV intensity which alter
 314 pigments change of colour (Davenport *et al.*, 2004). Plant
 315 canopy also reduces temperature by shading (Bortolus

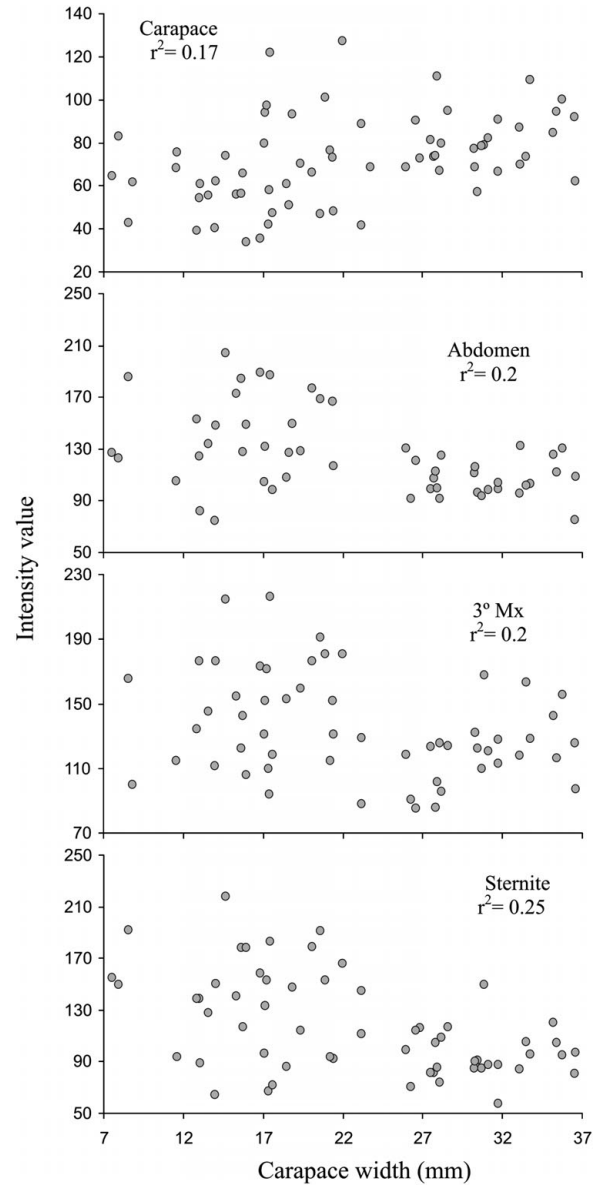


Fig. 4. Correlation analysis between green colour values and crab size for the crab parts with a significant relationship.

et al., 2002). Higher temperatures generate shorter inter-
 moult lengths by enhancing growth rates (Hartnoll, 2001).
 Mudflats are more frequently flooded by tides than the
 marsh zone, so burrow temperatures are more variable in
 the mudflat and more stable for longer periods in the
 marsh (Silva *et al.*, 2009).

There were also changes in coloration during moulting. In
 experiments, crabs changed coloration after moulting. The
 average intermoult period for the size of crab range selected
 for the experiment (22–24 mm) is about 150 days (ambient
 temperature; Luppi *et al.*, 2004). After this selection 15 differ-
 ences occurred in crab colour suggesting that intermoult
 length could be an important factor determining crab coloration.
 In crustaceans, colour changes can be affected by inter-
 moult length (*Carcinus maenas*: Reid *et al.*, 1997; Styri-
 shave *et al.*, 2004), by individual age or size (*Panulirus* spp.:
 Melville-Smith *et al.*, 2003; Wade *et al.*, 2005). Smaller
 crabs moult more frequently than large crabs (Luppi *et al.*,
 2004). Thus, in *N. granulata* the colour may be a

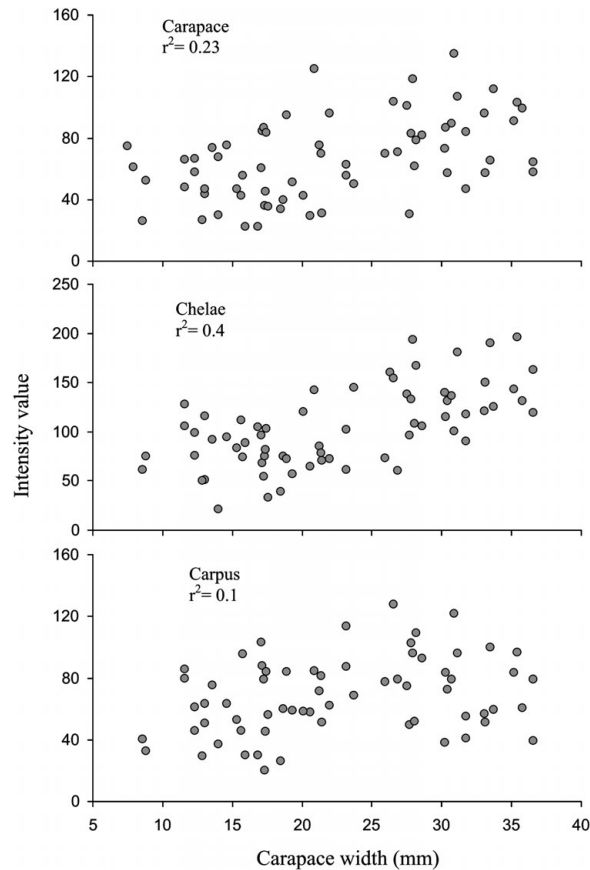


Fig. 5. Correlation analysis between blue colour values and crab size for the crab parts with a significant relationship.

combination of these factors. In other species (e.g. *Uca capricornis*: Detto *et al.*, 2008) carapace coloration is determined by ontogenetic colour changes during moulting. In addition to individual identity, the ontogenetic colour changes in *U. capricornis* provide information about the sex, size, and reproductive status of the individual and also have the potential to signal competitive ability. Rapid colour changes in crustaceans have been emphasized in several publications (Hultgren & Stachowicz, 2008; for a review: Stuart-Fox & Moussalli, 2009) while slow changes have received relatively little attention. Even the effects of colour change were not evaluated in this work; the potential social significance of ontogenetic colour changes in *N. granulata* compared with other well-studied species such as *Uca* spp., highlights the importance of studying the effects of ontogenetic colour changes. This research demonstrates that colour in *N. granulata* is related to size, zone and intermoult duration, especially in the chela, suggesting that this body part could be an intraspecific signal.

ACKNOWLEDGEMENTS

We thank two anonymous referees and the Executive Editor, Dr Ann Pulsford for helpful comments on the manuscript. This project was supported by Universidad Nacional de Mar del Plata, ANPCyT (01272) and CONICET (PIP5669 all granted to O.I.). A.M.C. was supported by a Doctoral scholarship from CONICET.

REFERENCES

- Arredondo-Figueroa J., Pedroza-Islas R., Ponce-Palafox J.T. and Vernon-Carter E. (2003) Pigmentation of the pacific white shrimp (*Litopenaeus vannamei*, Boone 1931) with esterified and saponified carotenoids from red chili (*Capsicum annuum*) in comparison to astaxanthin. *Revista Mexicana de Ingeniería Química* 2, 101–108.
- Beck M.W., Heck Jr K.L., Able K.W., Childers D.L., Eggleston D.B., Gillanders B.M., Halpern B., Hays C.J., Hoshino K., Minello T.J., Orth R.J., Sheridan P.J. and Weinstein M.P. (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51, 633–641.
- Bortolus A., Schwindt E. and Iribarne O. (2002) Positive plant–animal interactions in the high marsh of an Argentinean coastal lagoon. *Ecology* 83, 733–742.
- Boschi E.E. (1964) Los crustáceos decápodos Brachyura del litoral bonaerense. *Boletín del Instituto Biología Marina* 6, 1–99.
- Botto F., Valiela I., Iribarne O., Martinetto P. and Alberti J. (2005) Impact of burrowing crabs on C and N sources, control, and transformations in sediments and food webs of SW Atlantic estuaries. *Marine Ecology Progress Series* 293, 155–164.
- Carr S.D., Tankersley R.A., Hensch J.L., Forward R.B. and Luettich R.A. (2004) Movement patterns and trajectories of ovigerous blue crabs *Callinectes sapidus* during the spawning migration. *Estuarine, Coastal and Shelf Science* 60, 567–579.
- Charmantier G., Giménez L., Charmantier-Daures M. and Anger K. (2002) Ontogeny of osmoregulation, physiological plasticity and larval export strategy in the grapsid crab *Chasmagnathus granulata* (Crustacea, Decapoda). *Marine Ecology Progress Series* 229, 185–194.
- Chien Y.-H. and Jeng S.C. (1992) Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources and levels and feeding regimes. *Aquaculture* 102, 333–346.
- Christy J.H. and Salmon M. (1984) Ecology and evolution of mating systems of fiddler crabs (genus *Uca*). *Biological Reviews* 59, 483–509.
- Crane J. (1975) *Fiddler crabs of the world. Ocypodidae: genus Uca*. Princeton, NJ: Princeton University Press.
- Davenport J., Healy A., Casey N. and Jeffron J. (2004) Diet-dependent UVAR and UVBR resistance in the high shore harpacticoid copepod *Tigriopus brevicornis*. *Marine Ecology Progress Series* 276, 299–303.
- Detto T., Zeil J., Magrath R.D. and Hunt S. (2004) Sex, size and colour in a semi-terrestrial crab, *Heloecius cordiformis* (H. Milne-Edwards, 1837). *Journal of Experimental Marine Biology and Ecology* 302, 1–15.
- Detto T., Hemmi J.M. and Backwell P.R. (2008) Coloration and colour changes of the fiddler crab, *Uca capricornis*: a descriptive study. *PLoS ONE* 3, e1629.
- Edge R., McGarvey D.J. and Truscott T.G. (1997) The carotenoids as anti-oxidants: a review. *Journal of Photochemistry and Photobiology B: Biology* 41, 189–200.
- Etherington L., Eggleston D.B. and Stockhausen W.T. (2003) Partitioning loss rates of early juvenile blue crabs from seagrass habitats into mortality and emigration. *Bulletin of Marine Science* 72, 371–391.
- Halperin J., Ansaldo M., Pellerano G.N. and Luquet C.M. (2000) Bimodal breathing in the estuarine crab *Chasmagnathus granulatus* Dana 1851—physiological and morphological studies. *Comparative Biochemistry and Physiology Part A* 126, 341–349.
- Hartnoll R. (2001) Growth in Crustacea—twenty years on. *Hydrobiologia* 449, 111–122.

- 379 Heck Jr K.L., Hays G. and Orth R.J. (2003) Critical evaluation of the
380 nursery role hypothesis for seagrass meadows. *Marine Ecology*
381 *Progress Series* 253, 123–136.
- 382 Hines A.H., Lipcius R.N. and Haddon A.M. (1987) Population dynamics
383 and habitat partitioning by size, sex and molt stage of blue crabs
384 *Callinectes sapidus* in a subestuary of central Chesapeake Bay.
385 *Marine Ecology Progress Series* 36, 55–64.
- 386 Hunt H. and Scheibling R. (1997) Role of post settlement mortality in
387 recruitment of benthic marine invertebrates. *Marine Ecology Progress*
388 *Series* 155, 269–301.
- 389 Huntingford F.A., Taylor A., Smith I.P. and Thorpe K.E. (1995)
390 Behavioural and physiological studies of swimming crabs. *Journal of*
391 *Experimental Marine Biology and Ecology* 193, 21–39.
- 392 Hultgren K.M. and Stachowicz J. (2008) Alternative camouflage strat-
393 egies mediate predation risk among closely related co-occurring kelp
394 crabs. *Oecologia* 155, 519–528.
- 395 Lipcius R., Rochelle T., Seitz D., Seebo M. and Colon-Carrion D. (2005)
396 Density, abundance and survival of the blue crab in seagrass and
397 unstructured salt marsh nurseries of Chesapeake Bay. *Journal of*
398 *Experimental Marine Biology and Ecology* 319, 69–80.
- 399 Iribarne O., Bortolus A. and Botto F. (1997) Between-habitats differ-
400 ences in burrow characteristics and trophic modes in the south-
401 western Atlantic burrowing crab *Chasmagnathus granulata*. *Marine*
402 *Ecology Progress Series* 155, 137–145.
- 403 Isacch J.P., Costa C., Rodríguez-Gallego L., Conde D., Escapa M.,
404 Gagliardini D.A. and Iribarne O. (2006) Distribution of salt marsh
405 plant communities associated with environmental factors along a lati-
406 tudinal gradient on the south-west Atlantic coast. *Journal of*
407 *Biogeography* 33, 888–900.
- 408 Luppi T., Spivak E.D., Bas C.C. and Anger K. (2004) Molt and growth of
409 an estuarine crab, *Chasmagnathus granulatus* (Brachyura: Varunidae),
410 in Mar Chiquita coastal lagoon, Argentina. *Journal of Applied*
411 *Ichthyology* 20, 333–344.
- 412 Luquet C.M., Cervino C.O., Ansaldo M., Carrera Pereyra V., Kocmur S.
413 and Dezi R.E. (1998) Physiological response to emersion in the
414 amphibious crab *Chasmagnathus granulata* Dana (Decapoda
415 Grapsidae): biochemical and ventilatory adaptations. *Comparative*
416 *Biochemistry and Physiology Part A* 121, 385–393.
- 417 McGaw I.J. and Naylor E. (1992) Salinity preference of the shore crab
418 *Carcinus maenas* in relation to coloration during inter-moult and to
419 prior acclimation. *Journal of Experimental Marine Biology and*
420 *Ecology* 155, 145–159.
- 421 Melville-Smith R., Wing Cheng Y. and Thomson A. (2003) Factors
422 affecting colour change in 'white' western rock lobsters, *Panulirus*
423 *cygnus*. *Journal of Experimental Marine Biology and Ecology* 291,
424 111–129.
- 425 Minello T.J., Able K.W., Weinstein M.P. and Hays C.G. (2003) Salt
426 marshes as nurseries for nekton: testing hypotheses on density,
427 growth and survival through meta-analysis. *Marine Ecology Progress*
428 *Series* 246, 39–59.
- 429 Moeller R.E., Gilroy S., Williamson C.E. and Grad G. (2005) Dietary
430 acquisition of photoprotective compounds (mycosporine-like amino
431 acids, carotenoids) and acclimation to ultraviolet radiation in a fresh-
432 water copepod. *Limnology and Oceanography* 50, 427–439.
- 433 Rao K.R. (1985) Pigmentary effectors. In Bliss D.E. and Mantel L.H. (eds)
434 *The biology of Crustacea, integuments, pigments, and hormonal pro-*
435 *cesses, Volume 9*. New York: Academic Press, pp. 395–462.
- 436 Reid D.G., Abelló P., Kaiser M.J. and Warman C.G. (1997) Carapace
437 colour, inter-moult duration and the behavioural and physiological
438 ecology of the shore crab *Carcinus maenas*. *Estuarine, Coastal and*
439 *Shelf Science* 44, 211–302.
- 440 Reta R., Martos P., Perillo G., Piccolo M.C. and Ferrante A. (2001)
441 Características hidrográficas del estuario de la laguna Mar Chiquita.
In Iribarne O. (ed.) *Reserva de biosfera Mar Chiquita: características,*
físicas, biológicas y ecológicas. Mar del Plata, Argentina: Editorial
Martin, pp. 31–52.
- Sagardes G., Castillo R. and Segonzac M. (2000) Carotenoid pigments
and trophic behaviour of deep-sea shrimps (Crustacea, Decapoda,
Alvinocarididae) from a hydrothermal area of the Mid-Atlantic
Ridge. *Comparative Biochemistry and Physiology Part A* 127, 293–300.
- Silbiger N. and Munguia P. (2008) Carapace color change in *Uca pugila-*
tor as a response to temperature. *Journal of Experimental Marine*
Biology and Ecology 355, 41–46.
- Silva P., Luppi T., Spivak E. and Anger K. (2009) Reproductive traits of
an estuarine crab, *Neohelice* (= *Chasmagnathus*) *granulata* (Brachyura:
Grapsoidae: Varunidae), in two contrasting habitats. *Scientia Marina*
73, 117–127.
- Sneddon L., Huntingford F. and Taylor A. (1997) Weapon size vs. body
size as predictor of winning fights between shore crabs, *Carcinus*
maenas (L.). *Behavioral Ecology and Sociobiology* 41, 237–242.
- Spivak E., Anger K., Luppi T., Bas C. and Ismael D. (1994) Distribution
and habitat preferences of two grapsid crab species in Mar Chiquita
Lagoon (Province of Buenos Aires, Argentina). *Helgoländer*
Meeresuntersuchungen 48, 59–78.
- Stuart-Fox D. and Moussalli A. (2009) Camouflage, communication
and thermoregulation: lessons from colour changing organisms.
Philosophical Transactions of the Royal Society of London B 364,
463–470.
- Styrishave B., Rewitz K. and Andersen O. (2004) Frequency of moulting
by shore crabs *Carcinus maenas* (L.) changes their colour and their
success in mating and physiological performance. *Journal of*
Experimental Marine Biology and Ecology 313, 317–336.
- Thurman C.L. (1988) Rhythmic physiological color change in crustacea: a
review. *Comparative Biochemistry Physiology Part C* 91, 171–185.
- Thurman C.L. (1990) Adaptive coloration in Texas fiddler crab (*Uca*).
In Wicksten M. (ed.) *Adaptive coloration in invertebrates:*
Proceedings of a Symposium of the American Society of Zoologists.
College Station, TX: Texas A & M University Press, pp. 109–125.
- Thlusty M. and Hyland C. (2005) Astaxanthin deposition in the cuticle of
juvenile American lobster (*Homarus americanus*): implications for
phenotypic and genotypic coloration. *Marine Biology* 147, 113–119.
- Wade N., Goulter K., Wilson K., Hall M. and Degnan B. (2005)
Esterified astaxanthin levels in lobster epithelia correlate with shell
colour intensity: Potential role in crustacean shell colour formation.
Comparative Biochemistry Physiology Part B 141, 307–313.
- and
- Zar J.H. (1999) *Bioestatistical analysis*. Englewood Cliffs, NJ:
Prentice-Hall, Inc.
- Correspondence should be addressed to:**
A.M. Casariego
Departamento de Biología (FCEyN)
Universidad Nacional de Mar del Plata CC 573
Correo Central, B7600WAG, Mar del Plata, Argentina
email: mendezc@mdp.edu.ar