

Inter-population variability in the reproductive morphology of the shore crab (*Carcinus maenas*): evidence of endocrine disruption in a marine crustacean?

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Abstract

Environmental contaminants that are capable of causing endocrine disrupting effects are currently a major cause for concern. These chemicals are known to influence the reproductive development of vertebrates by mimicking or antagonising the actions of endogenous hormones. However, little is known regarding their potential effects on invertebrates. Here we examine variations in the reproductive morphology of the shore crab (*Carcinus maenas*) for evidence of endocrine disruption. Crabs were collected from a number of sites comprising a putative gradient of exposure to endocrine disrupting chemicals. Patterns of inter-population variability in the expression of sexually dimorphic traits were then examined for evidence of hormone disruption. Extensive variability was detected and patterns of chelal morphology were consistent with the gradient of endocrine disruption. However, overall, the patterns of morphological variability were not consistent with hormonally-mediated effects. This suggests that shore crabs are not susceptible to the same type of endocrine disrupting effects that have been detected in vertebrates, which are most commonly mediated via the oestrogen receptor. However, the potential for androgenic effects on crustacean morphology are discussed.

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Keywords: Shore crab; *Carcinus maenas*; Endocrine disruption; Oestrogen; Reproductive development; Morphology

1. Introduction

There is now unequivocal evidence that a wide variety of chemicals that enter the aquatic environment are capable of disrupting endocrine function in wildlife and humans (IEH, 1999). Endocrine disrupting chemicals (EDCs) that interfere with the actions of the sex hormones are of particular concern, having been associated with reproductive dysfunction in all classes of vertebrate (Ashby et al., 1997; Tyler et al., 1998). The mechanisms responsible for these effects are very similar across this group of organisms as the vertebrate hormone-receptor system is highly conserved. Conse-

quently, this phenomenon is relatively well understood. In contrast, little is known regarding the potential implications of these chemicals for invertebrates, largely due to our rudimentary understanding of invertebrate hormone-receptor systems. This paucity of knowledge is dangerous, given that invertebrates comprise 95% of all animal species and play a pivotal role in ecosystem dynamics (Defur et al., 1999; Depledge and Billingham, 1999).

The phenomenon known as “imposex” remains one of the few clear examples of endocrine disruption in invertebrates in the field. This morphological abnormality occurs in gastropod molluscs exposed to organotin compounds and it is characterised by the superimposition of male reproductive characteristics, including a penis and vas deferens, on the female genitalia (Bryan et al., 1986). The mechanism by which this occurs is still under investigation. However, interference with the

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aromatase enzymes, which are normally responsible for the conversion of testosterone to 17β estradiol, is the most widely accepted explanation. The resulting accumulation of testosterone is thought to be responsible for these masculinising effects (Matthiessen and Gibbs, 1998).

There is putative evidence that the reproductive morphology of crustaceans may also be affected by EDCs. For example, increased rates of intersex and female biased sex ratios have been reported in harpacticoid copepods from sewage polluted locations along the East coast of Scotland, although a direct correlation between the frequency of intersex and distance from the discharge locations was not observed (Moore and Stevenson, 1991, 1994). Ovotestes formation has also been reported in lobsters (*Homarus americanus*) around the coast of Nova Scotia (Sangalang and Jones, 1997) and dual-gender intersex, characterised by the presence of penis-like appendages on females and gonopore-like openings and ovotestes in males, has been observed in Japanese freshwater crabs (*Geothelphus dehaani*) from contaminated sites (Takahashi et al., 2000). Recent data have revealed increased rates of intersex and female biased sex ratios in the marine amphipod, *Echinogammarus marinus*, from the Scottish coast (Ford et al., 2004). Furthermore, discriminant analysis of sexual dimorphisms, such as gnathopod length, revealed that “normal” males from polluted sites closely resembled intersex specimens. Similar abnormalities have been reported in amphipods (*Hyalella azteca*) exposed to ethinylestradiol (Vandenberg et al., 2003), which provides further evidence that these effects may be endocrine-mediated. These findings are consistent with the hypothesis that, like vertebrates, crustaceans are susceptible to the effects of EDCs.

In this study, we examine patterns of variability in the reproductive morphology of the shore crab, *Carcinus maenas* L., for evidence of endocrine disruption. The shore crab provides an ideal focus for this type of study as it has a particular affinity to estuarine habitats, which are particularly susceptible to pollution from anthropogenic sources. Recent evidence indicates that this species is sensitive to contaminant-induced effects (Galloway et al., 2004). The shore crab also fulfills many of the criteria for the selection of sentinel species outlined at the Institute for Environmental Health workshop on “The Ecological Significance of Endocrine Disruption” (Leicester, 1997) in that it is common and widespread in Northern Europe, it reproduces sexually and is sexually dimorphic. Unlike a number of decapods that are naturally hermaphroditic, the shore crab is single sexed throughout life and adult males and females are readily identifiable on the basis of their morphology.

However, male shore crabs are known to be capable of exhibiting an intersex condition, which is induced by parasitic castration by the thoracican barnacle, *Saccu-*

lina carcini (Charnioux Cotton, 1960). This leads to the development of more feminine features, such as a broadened abdomen and a reduction in dominant claw size, through changes in their endogenous endocrine regime. Preliminary data indicate that these feminised features are also exhibited by male shore crabs from polluted environments, with males from the Tyne and Tees estuaries, which are impacted by EDCs (Allen et al., 1999a,b; Lye et al., 1999; Matthiessen et al., 1998), appearing to be less male than those from a reference population (unpublished data). This indicates that the reproductive development of this species may be susceptible to endocrine disruption by exogenous agents such as EDCs. Here we present the findings of an extensive field survey that aimed to investigate these patterns of inter-population variability in shore crab morphology and assess their potential use as a biomarker of endocrine disrupting effects on crustaceans in the field.

2. Materials and methods

Approximately one hundred shore crabs were collected from each of eight sites around northern Britain. Sampling site locations are shown in Fig. 1. These locations were selected on the basis of previous reports of endocrine disruption, including the induction of vitellogenin and intersexuality in wild flounder (Allen et al., 1999a,b). In increasing order of impact, these were located in the estuaries of the rivers Dee, Clyde, Tyne, Mersey and Tees. Three reference sites were also identified, two of which were located on the west coast of Scotland at Arisaig and Appin, and one of which was located on the east coast at Belhaven Bay. No evidence of endocrine disrupting inputs was available for these sites, but given their remoteness from centres of population and industry, the risk of endocrine disrupting effects is likely to be low.

Samples were collected during a six-week period in the summer of 2001. Adult crabs (>30 mm carapace width) were collected by hand from the intertidal zone. Individuals that exhibited signs of infection by *S. carcini* were discarded. The remaining crabs were returned to the laboratory for morphological examination (see Fig. 2). This focused on the analysis of sexually dimorphic traits. Carapace length and width and cephalothorax depth were measured at the widest, longest and deepest dimensions, respectively. The depth of the chelae was also measured between the maximum points. The degree of heterochely was taken to be the difference in depth between the left and right claws. Individuals that had lost chelae or that were suspected to have regenerated one or more cheliped were omitted from the analysis of claw morphology. Periopod and propodus lengths were measured by taking an average from the fourth and fifth pairs of limbs, respectively. Again, data from crabs with

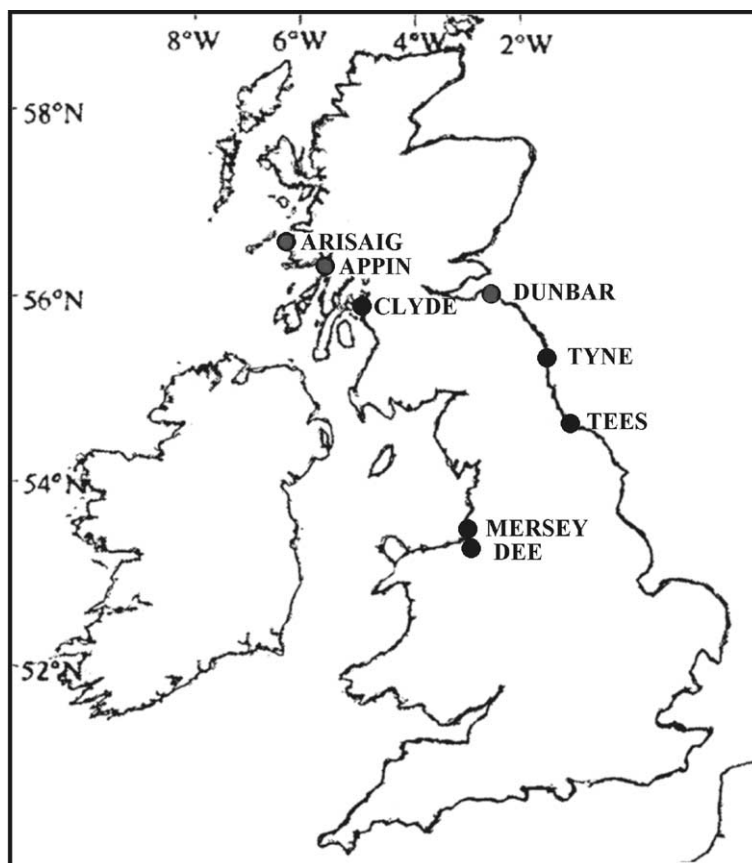


Fig. 1. Locations of the sampling sites.

missing or regenerating limbs were ignored. These dimensions were measured using digital callipers (Browne and Sharpe). The length of the first pair of pleopods was measured under a dissecting microscope. These structures exhibit clear sexual dimorphism, with the pleopods of males being modified for copulation and those of females being used to hold eggs. Pleopod structure was therefore used to confirm the sex of each crab. The size and shape of the abdomen also exhibits sexual dimorphism. Hence, the area of the abdomen was recorded using the image analysis package, Image Tool (UTHSCSA).

Each of the characters measured was plotted against carapace width in order to investigate their relationship with body size. All characters increased with body size, although the nature of these allometric relationships varied between traits. It was necessary to remove the effects of size dependence to allow the morphological comparison of crabs of varying size. This was achieved by calculating the residuals of the line of best fit between each trait versus carapace width, which was used as a reference dimension. These residuals were then used as adjusted trait values (Reist, 1985; Debuse et al., 2001). For some traits, the residuals required log transformation in order to fulfill the assumptions of normality. In

other cases, the adjusted trait values increased with body size. This required that they were divided by carapace width in order to achieve homogeneity of variance. These transformations enabled the statistical analysis of inter-population variations in morphology, which was carried out using ANOVA and Tukey's pairwise comparisons.

The correlations between each trait and the gradient of exposure to EDCs was explored by ranking the data and calculating Pearson's product moment correlation coefficient. Traits were ranked from 1 to 8 according to their mean adjusted values. Sites were ranked according to their pollution status: the three reference sites were given a mean rank of 2 and the remaining five contaminated sites were ranked 4–8 according to the extent of the effects reported by Allen et al. (1999a,b).

3. Results

The mean body size of crabs collected varied extensively between sites. In general, crabs were smallest at the Dee estuary, where the majority of individuals were of between 35 and 40 mm carapace width, and were largest at Arisaig, where the majority of crabs fell within the

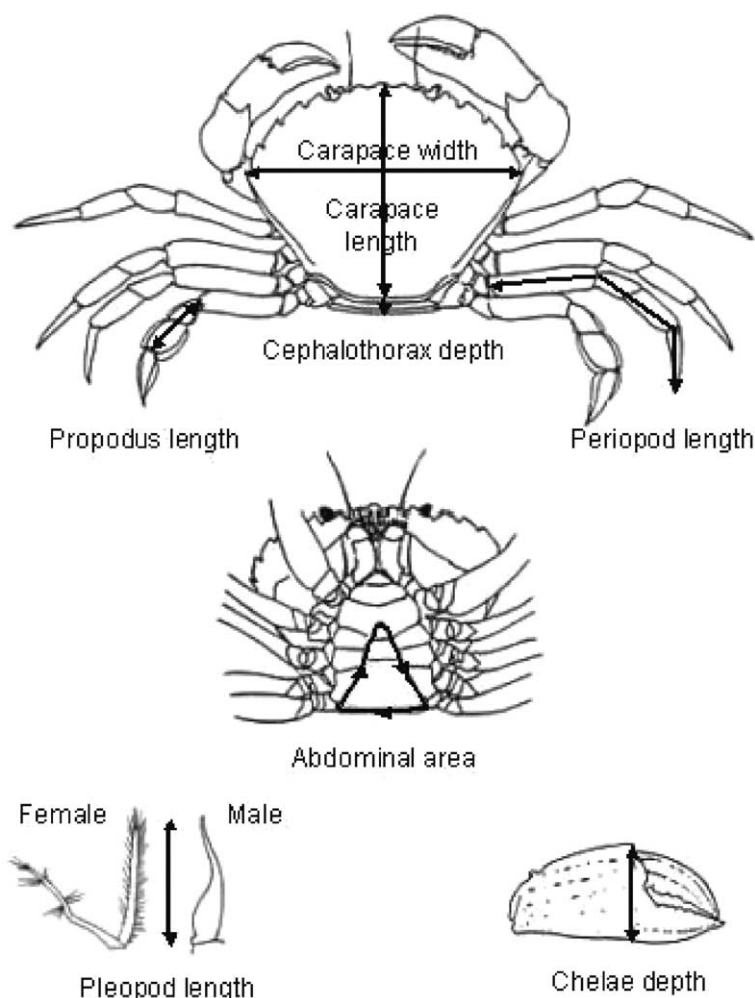


Fig. 2. Illustration of morphological dimensions measured. (Reproduced and modified from Crothers (1967) with the kind permission of Crothers).

45 and 50 mm carapace width range. For this reason, the mean trait values presented in Table 1(a and b) have been calculated for a crab of average size.

Each of the traits analysed exhibited some degree of sexual dimorphism. This required that male and female crabs were considered independently for the analysis of inter-population variability. These analyses revealed that males exhibited significant variability in nine out of the ten traits measured and that females exhibited significant inter-site variability in all ten traits (see Table 2). However, no consistent pattern in the distribution of this variability among populations from reference sites and those that had evidence of endocrine disruption was apparent from the results of the post-hoc tests.

The pattern of variability expressed by each trait was then considered in terms of exposure to EDCs. In male crabs, a correlation between depth of the right chelae and the putative gradient of endocrine disrupting effects was observed ($r = -0.81$, $p < 0.05$). This pattern was not evident from the analysis of the left chelae ($r = 0.27$, $p = 0.52$). This meant that the degree of heterochely also

correlated with the pollution gradient, with male crabs from sites with deeper right chelae exhibiting greater differences between the size of the left and right claw ($r = -0.88$, $p < 0.01$). Although this characteristic is generally more pronounced in male crabs, the degree of heterochely expressed by female crabs also correlated with the pollution gradient ($r = -0.78$, $p < 0.05$). These patterns are highlighted on Table 1. Tukey's tests revealed that there was a significant difference between the degree of heterochely expressed by male and female crabs at the reference sites compared with those at the Mersey and Tees, and to a lesser extent, the Clyde and Dee. The only anomaly to this pattern was at the Tyne, where the chelal morphology of male and female crabs differed little from that at the reference sites.

4. Discussion

This study has revealed that there is extensive inter-population variability in the morphology of male and

Table 1
Relative mean trait values for male (a) and female (b) shore crabs at each site

Trait value (mm)	Reference sites			Increasing degree of contamination				
	Arisaig	Appin	Dunbar	Dee	Clyde	Tyne	Mersey	Tees
<i>Panel (A)</i>								
Carapace width	50.4 ± 8.66	47.7 ± 8.35	48.1 ± 10.46	41.5 ± 6.34	44.1 ± 8.85	45.3 ± 9.78	43.5 ± 7.56	43.2 ± 9.65
Carapace length	34.5 ± 0.47	34.5 ± 0.09	34.7 ± 0.12	34.9 ± 0.09	34.8 ± 0.09	34.6 ± 0.76	34.5 ± 0.84	34.5 ± 0.41
Cephalothorax depth	18.9 ± 0.45	19.0 ± 0.64	19.1 ± 0.10	18.9 ± 0.58	18.6 ± 0.59	18.7 ± 0.60	18.8 ± 0.06	19.0 ± 0.39
Left chelae depth	9.59 ± 0.55	9.62 ± 0.39	10.3 ± 0.57	9.81 ± 0.48	9.54 ± 0.47	9.92 ± 0.29	9.89 ± 0.33	9.92 ± 0.46
Right chelae depth	13.4 ± 1.12	13.1 ± 0.93	13.6 ± 1.02	12.4 ± 0.06	11.8 ± 0.68	12.9 ± 0.96	11.6 ± 0.94	12.1 ± 0.77
Heterochely	3.84 ± 0.90	3.45 ± 0.81	3.37 ± 0.59	2.61 ± 0.62	2.39 ± 0.46	3.17 ± 0.65	1.83 ± 0.82	2.23 ± 0.63
Pleopod length	11.5 ± 0.48	12.8 ± 0.49	13.3 ± 0.43	13.2 ± 0.38	12.6 ± 0.61	13.1 ± 0.44	13.1 ± 0.41	12.9 ± 0.41
Abdominal area	145 ± 8.20	149 ± 9.16	155 ± 11.15	145 ± 8.46	145 ± 7.81	149 ± 8.03	148 ± 6.49	153 ± 8.98
Periopod length	48.8 ± 1.74	50.0 ± 1.88	48.9 ± 1.46	48.9 ± 1.73	48.4 ± 1.79	49.6 ± 2.02	48.7 ± 1.42	49.8 ± 1.7
Propodus length	16.7 ± 0.47	16.8 ± 0.47	16.7 ± 0.42	16.5 ± 0.54	16.5 ± 0.43	16.7 ± 0.46	16.5 ± 0.30	16.8 ± 0.46
<i>Panel (B)</i>								
Carapace width	46.5 ± 6.72	46.5 ± 6.71	40.3 ± 6.93	33.9 ± 4.09	49.4 ± 5.76	43.2 ± 10.7	38.1 ± 5.72	37.4 ± 5.48
Carapace length	34.7 ± 0.88	34.7 ± 0.46	34.8 ± 0.59	35.2 ± 0.07	34.8 ± 0.07	35.2 ± 0.57	34.9 ± 0.12	34.5 ± 0.05
Cephalothorax depth	19.3 ± 0.74	19.4 ± 0.46	19.5 ± 0.66	19.4 ± 0.36	19.2 ± 0.52	19.1 ± 0.63	19.0 ± 0.37	19.4 ± 0.31
Left chelae depth	9.02 ± 0.31	9.62 ± 0.28	9.15 ± 0.26	8.66 ± 0.25	8.98 ± 0.26	9.26 ± 0.36	8.66 ± 0.13	8.79 ± 0.26
Right chelae depth	11.6 ± 1.22	10.6 ± 0.82	11.1 ± 0.72	10.2 ± 0.36	10.0 ± 0.51	11.2 ± 0.95	9.10 ± 0.22	9.64 ± 0.43
Heterochely	2.66 ± 1.04	1.74 ± 0.85	2.11 ± 0.65	1.73 ± 0.40	1.06 ± 0.46	2.04 ± 0.85	0.43 ± 0.23	0.84 ± 0.33
Pleopod length	20.2 ± 1.06	19.1 ± 0.87	20.1 ± 1.14	20.7 ± 1.29	19.0 ± 1.38	19.8 ± 1.05	19.2 ± 0.75	20.1 ± 0.80
Abdominal area	255 ± 22.7	262 ± 10.7	277 ± 19.4	257 ± 11.9	294 ± 19.9	277 ± 26.3	234 ± 10.3	258 ± 13.9
Periopod length	46.2 ± 1.90	47.8 ± 1.34	44.4 ± 1.63	44.0 ± 0.74	46.5 ± 1.02	47.8 ± 1.83	45.9 ± 0.77	46.5 ± 1.19
Propodus length	16.3 ± 0.65	16.2 ± 0.48	15.7 ± 0.46	14.8 ± 0.31	16.0 ± 0.38	15.8 ± 0.52	15.7 ± 0.37	15.9 ± 0.36

With the exception of carapace width, the mean trait values have been calculated for a crab of average size (45.6mm carapace width) to enable the comparison of populations of varying body size. This was achieved by regressing the relationship between each trait and carapace width. Error bars represent the mean of the residuals of this relationship for each population. Traits that correlated with the gradient of endocrine disrupting effects are highlighted.

Table 2
Inter-population differences in the morphological characteristics of male and female crabs

Trait	Males			Females		
	df	F	p	df	F	p
Carapace width	340	5.31	<0.01	305	17.56	<0.01
Carapace length	338	4.26	<0.01	299	3.99	<0.01
Cephalothorax depth	253	1.83	0.08	223	2.76	<0.05
Left chelae depth	185	6.60	<0.01	153	3.98	<0.01
Right chelae depth	187	18.14	<0.01	157	17.33	<0.01
Heterochely	176	18.65	<0.01	151	19.40	<0.01
Pleopod length	216	6.22	<0.01	171	4.16	<0.01
Abdominal area	211	5.48	<0.01	179	6.83	<0.01
Periopod length	209	5.09	<0.01	165	15.68	<0.01
Propodus length	215	5.49	<0.01	192	13.98	<0.01

female shore crabs around the coast of Northern Britain. However, examination of the patterns of spatial variability expressed by each trait revealed that only the size of the dominant claw, and hence the degree of heterochely, exhibited any correlation with previous reports of endocrine disrupting effects in fish (Allen et al., 1999a,b). In male shore crabs, the dominant claw is a secondary sexual characteristic that is used in aggressive and sexual interactions. As its development is determined by sex hormones (Charnioux Cotton, 1960), variations in the size or shape of this structure could be indicative of endocrine disruption. However, if this were

the case, we would also expect there to be effects on the expression of other sexually dimorphic traits, such as abdominal area, that are known to be affected by endogenous endocrine disruption. We might also expect to see an increase in the degree of sexual dimorphism expressed by crabs at the affected sites. The lack of further evidence of feminisation, combined with the fact that similar patterns of variability in claw morphology were evident in female crabs, indicates that endocrine disruption is an unlikely explanation for the patterns observed.

This finding was not consistent with that of a previous, smaller scale survey of crab morphology, although

the same patterns of variability were expressed by each of the traits analysed at each of the sites in question (Brian, unpublished data). This should serve to caution against claims of endocrine disruption when small datasets, containing fewer sites and/or lower sample sizes, are used to investigate such effects. In contrast, the results of this study were in close agreement with those of a similar survey of shore crab morphology, which was carried out under the EDMAR programme (Allen et al., 2002). This revealed patterns of inter-population variability in the morphology of the right chelae that were analogous to those presented in this study, but overall, it was concluded that the data was equivocal and the mechanism responsible for variations in claw size was unknown.

Potential explanations for these patterns of morphological variability are wide ranging. For example, it is possible that differences in the chelal morphology of crabs from reference and contaminated sites reflect a more general effect of pollution on crustacean health. Alternatively it may be that the patterns observed have arisen as an indirect consequence of contamination on ecological parameters. For example, changes in community composition may alter the availability of different prey types. Diet has been found to play an important role in the chelal development of the blue crab, *Callinectes sapidus* (Smith and Palmer, 1994). Variations in morphology may have also arisen in response to natural environmental differences between the sites, such as the structure of the substrate. Further research is required to establish the potential influence of the habitat characteristics on morphological variability.

However, the lack of evidence of a relationship between variations in shore crab morphology and the putative gradient of endocrine disruption does not rule out the possibility that shore crabs may be susceptible to the same type of effects reported in crustaceans in the literature (e.g. Ford et al., 2004; Moore and Stevenson, 1991; Sangalang and Jones, 1997; Takahashi et al., 2000). It is possible that a correlation was not detected because the levels of EDCs encountered by the crabs from these populations did not exceed the threshold required to elicit an affect. Alternatively, it may be that the methods employed in this study were not sufficiently sensitive to reflect the effects of exposure to environmentally relevant concentrations of EDCs or that the high levels of background variability have obscured any contaminant-induced patterns in morphology. However, it is also possible that these results reflect the fact that, unlike vertebrates, crustaceans such as the shore crab are not susceptible to developmental effects exerted by EDCs.

The reproductive development of crustacea is similar to that of vertebrates in that sex is genetically determined, but the expression of secondary sexual characteristics is largely under hormonal control (Highnam and

Hill, 1976). However, the structures and functions of crustacean hormones are very different to those found in vertebrates. Although vertebrate-like oestrogens and androgens have been identified in some invertebrate phyla, such as cephalopods, bivalves and gastropods (DeLoof and DeClerk, 1986; Joosse, 1982), the principal sex steroids in the crustacea are the ecdysteroids. Non-steroidal compounds such as methyl-farnesoate have been associated with the reproductive control of some species (Baldwin et al., 1995). This indicates that crustaceans are unlikely to be affected by the same type of chemicals that are capable of interfering with the reproductive development of vertebrates.

Previous research has revealed that changes in the reproductive morphology of the shore crab can be induced by the manipulation of the androgenic gland, which is the site of male sex hormone synthesis in crustacea (Barki et al., 2003). The removal of this gland from juvenile male shore crabs has been found to inhibit the growth of male secondary sexual characteristics and result in the development of the female form. Conversely, the implantation of an androgenic gland into immature females stimulates the development of male characteristics and inhibits female reproductive processes, such as vitellogenesis (Charnioux Cotton and Payen, 1988). This indicates that the expression of male and female morphological characteristics is largely determined by the presence or absence of androgenic hormones. This would explain the absence of a correlation between variations in shore crab morphology and the gradient of endocrine disrupting effects in flounder, which reflect the levels of estrogenic, as opposed to androgenic, activity.

The significance of androgenic hormones in determining the reproductive development of the shore crab highlights the potential for androgenically-mediated effects on crustacean morphology. It is unlikely that androgenic endocrine disruptors are responsible for the patterns of inter-population variability detected in this study, which were not consistent with the effects of hormone disruption. However, the de-masculinising actions of anti-androgenic chemicals provide a plausible explanation for previous reports of feminisation and intersexuality in copepods, decapods and amphipods *in situ*. Further research is clearly required to elucidate the potential for androgenically-mediated effects on the reproductive development of crustacea and to establish the implications for invertebrate populations in the field.

Acknowledgment

This work was funded by a Napier University studentship. The author would like to thank Trevor Blackall, Richard Ladle and Jennifer Batty for assistance with

the sampling and analysis of the crabs and John Sumpter for comments on the original manuscript.

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