ISSN: 2347-5129
IJFAS 2014; 1(3): 99-104

www.fisheriesjournal.com
Received: 17-10-2013
Accepted: 28-11-2013

Sudha Devi A. R
Department of Zoology, Mary Matha Arts & Science College, Mananthavady - 670 645, Wayanad, Kerala, India.
Email: arsudhadevi@yahoo.co.in

Smija M. K
Department of Zoology, Mary Matha Arts & Science College, Mananthavady - 670 645, Wayanad, Kerala, India.
Email:smijamkkunnath@gmail.com

Correspondence:
Sudha Devi A. R
Department of Zoology, Mary Matha Arts & Science College, Mananthavady - 670 645, Wayanad, Kerala, India.
Email: arsudhadevi@yahoo.co.in
Tel: 9947163686, Fax: +91-4935-241087

Histologically Confirmed Intersex (Ovo-testis) in the Freshwater Crab *Travancoriana schirnerae* (Bott, 1969)

Sudha Devi A. R, Smija M. K

ABSTRACT
This paper provides the first report of an intersex condition in the freshwater crab *Travancoriana schirnerae*, a gonochoristic species commonly distributed in the paddy fields of Wayanad. Of the 165 males examined morphologically and histologically, one displayed intersex condition. The intersex individual investigated was a fully functional male revealed by its male-like morphology and the presence of paired testes, vasa deferentia, androgenic glands and mature spermatozoa but histological examination disclosed the presence of a previtellogenic oocyte in one of the testicular acini. The condition reported here did not present an internal bilateral division of the reproductive system into male and female components, instead the occurrence of a previtellogenic oocyte amid normal spermatogonial cell lineages. The gonadosomatic index and vas deferens factor of the intersex male were closer to normal male values indicating that the intersex condition might have occurred by feminization of a male crab.

Keywords: Freshwater crab, Intersex, Ovo-testis, *Travancoriana schirnerae*.

1. Introduction
Intersexuality is a phenomenon whereby an individual of a species with separate sexes has morphological or anatomical characteristics that are intermediary between true male and true female forms of that species. According to Nolan *et al.* [1] and Blazer *et al.* [2], intersex is an abnormal gonadal condition, characterized by the presence of oocytes in primarily testicular tissues. Intersex in naturally gonochoristic (sexes separate) species is defined as the presence of both male and female reproductive features within the same individual [3]. Ladewig *et al.* [4] and Vasquez and Lopez Greco [5] defined intersex as an individual that develops modifications in primary and/or secondary sexual characters, so that it possesses both male and female characteristics. The alterations in primary sexual characteristics include oogenesis taking place in the testes as reported in talitrid amphipods or in opposition, spermatogenesis taking place in the ovaries, which has never been observed. The variations in secondary sexual characteristics may include anomalies in chelae and cheliped dimensions or the location of gonopores which result in nonfunctional hermaphroditism [6]. According to Bateman *et al.* [7], an intersex individual possesses oocytes at various stages of development within the normal testicular tissue, which is considered as the termination of endocrine disruption. Intersexuality may also be part of a functional protandric, protogynic or synchronized hermaphroditism in the isopod *Gnorimosphaeroma oregonense*, caridian shrimp *Lysmata amboinensis* and in the crayfish *Samastacus spinifrons* [8, 9, 10].
The phenomenon of intersex has been evidenced in several animal groups, from invertebrates such as mollusks [11] and crustaceans [12, 13] to vertebrates including humans [14]. Intersexuality has been extensively studied in several species of freshwater, estuarine and marine teleost fish [15, 16, 17]. It is common among crustaceans but comparatively uncommon amongst gonochoristic crustaceans [18]. Among the non-decapod crustaceans, intersexuality has been reported as a common phenomenon in amphipods from a wide range of aquatic habitats [18]. In the amphipod *Echinogammarus marinus*, intersex individuals appear as two phenotypes - intersex male and intersex female [19]. Intersex individuals of *Gammarus minus* display protogynic hermaphroditism, typical of many amphipods [20]. Reports regarding intersexuality are also known from copepods [21, 22] and isopods [23, 24]. Among isopods, intersex condition was described in *Mesidotea sibirica* where the intersex individuals resembled mature females morphologically [24].

Sant' Anna *et al.* [25] investigated intersexuality in the hermit crab *Clibanarius vittatus* using anatomical, histological and electron microscopic techniques. In majority of these cases, intersexuality has been described as a manifestation of various anomalies in the external morphology of the abdomen and its appendages [22].

In decapod crustaceans, one manifestation of intersexuality involves the presence of externally discernible male and female genital openings [25]. In the Australian red-claw crayfish *Cherax quadricarinatus*, several types of intersex animals can be found in which both male and female genital openings occur in unusual combinations [26, 27]. Achuthankutty [28] has reported the incidence of intersex individuals in the sergestid shrimp *Acetes sp*. Peer Martin and Gerhard Scholtz [29] described a fully functional intersex female, which possessed male-like first pleopods in the freshwater crayfish *Marmorkrebs sp*. Study on the American lobster *Homarus americanus* by Sangalang and Jones [30] demonstrated histological intersexes with testes containing previtellogenic oocytes inside the testicular acini packed with distinct spermatogonial cell lineages. Stentiford [31] noticed the prevalence of intersexes in the European lobster *H. gammarus*. Intersexes are relatively infrequent among brachyurans. Imposex (imposition of male characters in females on exposure to certain chemicals) phenomenon has been found to occur in males and females of the Japanese freshwater crab *Geothelphusa dehaani* [32]. In the same species, Takahashi *et al.* [33] observed dual gender intersex characterized by the existence of penis-like appendages in females and gonopore-like openings and ovo-testis in males collected from contaminated sites. There are a number of studies on intersex crustaceans dealing primarily with the morphological and physiological characteristics [27, 34]. The present study is the first report on intersexuality in the freshwater crab *Travancoriana schirnerae*.

2. Materials and methods

Adult intermoult male specimens of *T. schirnerae* (4.0-5.5 cm in carapace width (CW) and 34.910-60.910 g in body weight (BW)) were collected from the paddy fields near Mary Matha Arts & Science College campus, Mananthavady (Wayanad, Kerala) over a period of two years (June 2009-June 2011) to study the annual cyclicity in spermatogenesis. The external morphology of each specimen was analyzed prior to dissection. The morphological analyses included the size of the animal (CW and BW), chelae size (length of left and right chelipeds, length and width of propodus) and shape of the abdomen. The gonopods, external genital organs and gonopores were checked for abnormalities under a stereo zoom microscope.

For histological analysis of spermatogenesis, every month 5-10 specimens (a total of 165 males) were sacrificed. The gonads were quickly dissected out; their size, form and colour were recorded. The wet weights of gonads and vasa deferentia were recorded to calculate the gonadosomatic index (GSI) and vas deferens (VD) factor respectively. The testes were immediately fixed in Bouin's fluid, dehydrated in graded series of ethanol and embedded in paraffin. Sections 6 µm thickness were stained with hematoxylin-eosin, examined under a Leica DM 500 Research microscope and digital images were taken using a DG 330/210 camera attached to the microscope. Measurements of germ cells in various spermatogenic stages were made using an image analysis system of Biowizard software.

3. Results

The results concerning the morphological observations indicated that all the males selected were morphologically normal and possessed normal abdominal shape (T-shaped), gonopods, external genital organs and gonopores. All animals, including the intersex male had gonopores on the coxae of the 5th pereiopods, typical of male brachyuran crabs. The CW (5.4 cm) and BW (57.3 g) of the intersex male were within the range reported for normal adult males (4-5.5 cm and 34.910-60.910 g respectively) which showed that the intersex individual was a sexually mature male specimen. Data regarding chelae and cheliped dimensions of the intersex male were in accordance with the normal male values (see Table 1).

Table1: Cheliped and chelae dimensions of normal and intersex male in *Travancoriana schirnerae*.

Cheliped size	Normal range (cm)	Intersex (cm)
Major cheliped length	7.1-12.2	11.5
Major propodus length	1.7-3.1	3.0
Major propodus width	1.6-3.3	3.2
Minor cheliped length	6.4-10.0	7.2
Minor propodus length	1.3-2.1	1.7
Minor propodus width	1.2-2.4	1.4

3.1 Reproductive system of normal and intersex male

Morphological observations of the male reproductive system from normal and intersex individuals presented an identical pattern. Both normal and intersex males had paired testes with vasa deferentia divided into anterior, middle and posterior regions. Morphologically, the testis of intersex specimen showed no structural abnormalities. The testes were creamy white, elongated and lobulated structures positioned in the cephalothoracic region. The size (length and width 23.0 and 2.3 mm respectively) of the intersex gonad conformed to that of normal adult males (length 20-26 mm and width 2.0-2.5 mm). The paired vasa deferentia, creamy white in colour, arose from the posterior ends of the testes and extended posteriorly in the form of thick coiled tubules. The posterior vas deferens narrowed to form a thin ejaculatory duct which pierced the muscles of the 5th pereiopod to open at the base of the coxa through external penis. The wet weight of testis (110 mg) and vas deferens (330 mg) of the intersex specimen lie within the range reported for normal adult males (80-160 mg and 220-400 mg respectively). The GSI (0.191) and VD factor (209) of the intersex male were very close to normal male values (GSI 0.155-0.316; VD factor 101-254).

3.2 Histology of testis of normal and intersex male

Our observations revealed that the gonads from normal and intersex males had identical histoarchitecture. Histological evaluation of the testes bestowed the normal lobular structure of seminiferous tubules and germ cells in all stages of spermatogenesis (primary and secondary gonia, primary and secondary spermatocytes, spermatids and spermatozoa) were present in all the samples assessed (Figs. 1A-F and 2A-F). The germ cells in various developmental stages could be identified by their nuclei size. Each acinus was lined by a single layer of germinal epithelium. The gonia cells were seen arranged either towards the periphery of the acini or fully packed in individual acinus. The primary (9.1-12 µm in

diameter) and secondary gonia (6.1-9 µm) possessed large nuclei surrounded by thin rim of cytoplasm. Spermatocytes (primary 5.1-6.0 µm and secondary 3.1-4.0 µm in diameter) are polygonal cells with oval nuclei and have more amount of cytoplasm than the gonia cells. Spermatids were spherical cells measuring 2-3.5 µm in diameter.

Mature sperms (4-5 µm in diameter) were noted fully packed in acini of the intersex gonad as in normal males. Highly condensed residual sperms were seen arranged either towards the periphery or towards the centre of the acini. The presence of mature spermatozoa in the seminiferous tubules indicates that normal spermatogenesis is taking place in the intersex male. The only difference noticed in histology was the presence of a previtellogenic oocyte in one of the testicular acini of the intersex male (Fig. 2D). The previtellogenic oocyte was closely associated with secondary spermatocytes within the acinus in which it was present. The oocyte measured 71 µm in diameter, possessed oval, multinucleolated basophilic nucleus (26 µm in diameter). The nucleoli positioned towards the periphery of the nucleus and the chromatin was granular in nature. This oocyte exhibited the characteristic features of the chromatin nucleolus stage II of previtellogenic phase in oogenesis. There were no signs of yolk accumulation in the ooplasm.

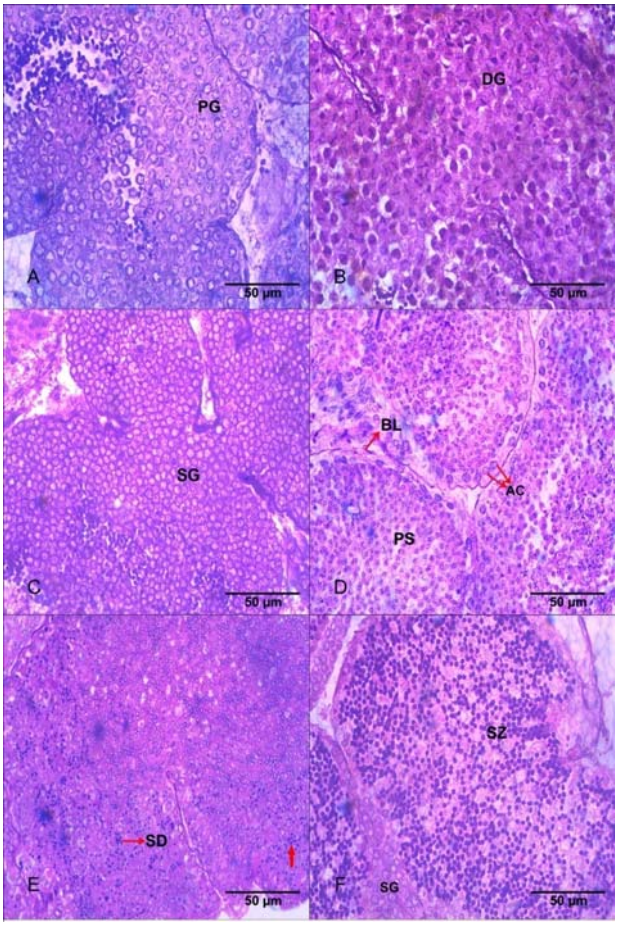

Fig 1: Testis of normal adult male *Travancoriana schirnerae* with acini containing various spermatogenic stages.
(A) Primary gonia, (B) Division of primary gonia (C) Secondary gonia (D) Primary spermatocytes, (E) Spermatids undergoing spermiogenesis, (F) Mature spermatozoa. PG: Primary gonia; DG: Dividing gonia; SG: Secondary gonia; BL: Basal lamina; AC: Accessory cells; PS: Primary spermatocyte; SD: Spermatid; SZ: Spermatozoa; Arrow indicates spermiogenesis (H & E)

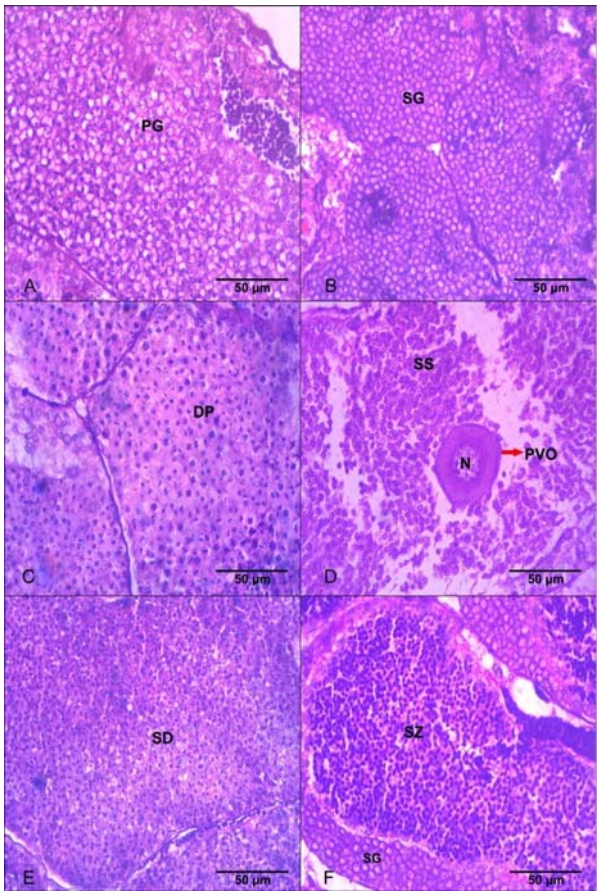

Fig 2: Testis of intersex male *Travancoriana schirnerae* with acini containing various spermatogenic stages.
(A) Testicular acini containing primary gonia, (B) Secondary gonia, (C) Primary spermatocytes undergoing meiosis, (D) Previtellogenic oocyte interspersed among secondary spermatocytes, (E) Spermatids under spermiogenesis, (F) Mature spermatozoa tightly packed inside the acini. PG: Primary gonia; SG: Secondary gonia; DP: Dividing primary spermatocyte; SS: Secondary spermatocyte; PVO: Previtellogenic oocyte; N: Nucleus; SD: Spermatid; SZ: Spermatozoa (H & E)

4. Discussion

The present investigation showed that the intersex individual investigated was a functional male indicated by its male like morphology and the presence of paired testes, sperm ducts, androgenic glands and viable spermatozoa. No evidence was observed for the presence of an atrophied male reproductive system, neither testicular tissue nor vasa deferentia. The condition reported here did not present an internal bilateral division of the reproductive system into male and female constituents, alternately by the presence of a previtellogenic oocyte among normal spermatogonial cell lineages. The GSI and VD factor of the intersex male were within the range reported for normal male values indicating that the sampled intersex was a sexually mature male specimen. In crustaceans, intersex individuals are most often reported to be males based on the presence of testicular tissue and male reproductive tract with rudimentary female characteristics [13]. In the American lobster *H. americanus*, Sangalang and Jones [30] demonstrated histologically confirmed intersex males with testicular lobules containing previtellogenic oocytes intermingled among spermatogenic stages. In *C. quadricarinatus*, the morphological, biochemical and endocrinological studies of intersexes have demonstrated that the intersex individuals are functional males with previtellogenic oocytes [27, 35]. In several

species of teleost fish, intersex condition manifests as the presence of ovo-testis, a combination of male testicular tubules containing different spermatogenic stages and oocytes in various stages of vitellogenesis [16, 17]. On the other hand, among gammaridean amphipods, intersexes usually function as females [18]. Female intersexes possessing male genital apophyses and male intersexes with oostegites have been described in amphipods [36] and isopods [37]. In the hermit crab *C. vittatus*, intersex individuals carried both male and female reproductive systems with functional male and female gonads in the same individual and ducts opened to the gonopores [25]. In *T. schirnerae*, only 0.6% of the males sampled exhibited intersex condition. The pattern of intersexuality is considered abnormal in most decapod species with a typical background incidence of <1%, excluding hermaphroditic crayfish in which a higher incidence is normal [13].

Different causes have been attributed to intersexuality. Previous researches suggest that contact with anthropogenic chemicals and mimics of estrogen and testosterone may modify sexual development in many vertebrate groups including fishes [38], amphibians [39], reptiles [40] and birds [41]. Among invertebrates, molluscs show the imposex condition which is linked to tributyltin contamination [42]. Water pollution may also persuade intersexuality in crustaceans [22]. Olmstead and Le Blanc [43] demonstrated that intersex condition can be induced chemically in crustaceans by juvenile hormone (JH) mimics.

Though there are scattered references to intersexes in the literature, very little work has been carried out on their cause or function in crustaceans. Parasitism has been linked to intersexuality in several crustaceans [44]. The parasites that cause intersexuality in crustaceans include microsporidians and digeneans in amphipods and rhizocephalans in brachyurans. Rhizocephalan and epicaridean parasites were found to feminize some male brachyurans and completely modify their secondary sexual characteristics [45, 46]. A direct link between microsporidean infection and decline in androgenic gland hormone (AGH) production has been noticed in the amphipod *G. duebeni* [47]. Parasitism may be a plausible elucidation for the intersex condition in the isopod *M. sibirica* [48]. Rigaud and Juchault [49] had stated a direct link between intersexuality and the presence of bacterial endoparasites. Male shore crabs of *Carcinus maenas* are known to exhibit an intersex condition caused by parasitic castration by the thoracian barnacle *Sacculina carcini* [50].

Environmental factors such as photoperiod and temperature are also accountable for the induction of intersexuality in crustaceans [45, 51]. Furthermore, crustacean intersexuality has been correlated to viral infections [52] and genetic abnormalities [53].

Ford *et al.* [54] related crustacean intersexuality to an irregular production of AGH secreted by the AG. The AGH in crustaceans plays a major role in male sex differentiation and development and the expression of primary and secondary sexual characteristics [55]. It has been reported that intersexes could be created by the manipulation of the AG in the crayfish *C. quadricarinatus* [55]. Intersexuality can be stimulated in amphipods by the extirpation or implantation of AGs from/into males and females respectively [56]. The intersex condition described in the present investigation may possibly be elemented to a malfunction of the sex determining mechanism controlled by the AG.

In gonochoristic crustaceans, the AG is also linked to intersexuality where the co-occurrence of various male and female characteristics has been established. Previous studies on AG ablation in amphipods led to the initiation of oogenesis, development of oviducts and female gonopores [57]. In isopods, it has been suggested that intersex individuals that are functional males are the products of a belated expression of AGH in genetic males [58]. Sagi *et al.* [27, 59] revealed that the AG which controls the primary and secondary sexual characteristics in males is also an imperative factor in the formation of intersex individuals and sexual plasticity in crustaceans.

The occurrence of intersexes has major inference for studies on the evolution and ecology of sex ratios and the use of crustaceans as indicators of environmental quality [19]. Intersexes might also manipulate the size of crustacean populations by causing population increases in cases where they function as members of the limiting sex [60] or population decline in cases where costly intersex forms occur at the expense of normal animals [61]. Ford *et al.* [62] detailed a number of costs associated with intersexuality at the individual level, which include reduced fecundity and fertility and belated maturation. Intersexes offer a unique opportunity to study various endocrine irregularities and development.

5. Conclusions

We conclude that the intersexuality in *T. schirnerae* does not indicate a case of parasitism or viral infection or endocrine disruption. The case of intersexuality described here presents a sole model for the study of the role of the AG in the regulation of sex differentiation in crustaceans. Our investigation is in the preliminary stage and further research is needed before the effects can be attributed with any degree of certainty.

6. Acknowledgements

The authors wish to thank the Kerala State Council for Science, Technology & Environment for the financial support in carrying out the research.

7. Reference

1. Nolan M, Jobling S, Brighty G, Sumpter JP, Tyler CR. A histological description of intersexuality in the roach. J Fish Biol 2001; 58:160-176.
2. Blazer VS, Iwanowicz LR, Iwanowicz DD. Intersex (Testicular oocytes) in smallmouth bass from the Potomac river and selected nearby drainages. J Aquat Anim Health 2007; 19:242-253.
3. Jobling S, Tyler CR. Endocrine disruption in wild freshwater fish. Pure Appl Chem 2003; 75:2219-2234.
4. Ladewig V, Jungmann D, Koehler A, Schirling M, Triebskorn R, Nagel R. Intersexuality in *Gammarus fossarum* Koch, 1835 (Amphipoda). Crustaceana 2002; 75:1289-1299.
5. Vasquez FJ, López Greco LS. Intersex females in the red claw crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae). Rev Biol Trop 55 (Supp 1) 2007; 25-32.
6. Charniaux-Cotton H. Hermaphroditism and gynandromorphism in malacostracan Crustacea. In: Intersexuality in the animal kingdom (ed. by R. Reinboth), Springer-Verlag, New York, 1975; 91-105.
7. Bateman KS, Stentiford SD, Feist SW. A ranking system for the evaluation of intersex condition in

European Flounder (*Platichthys flesus*). Environ Toxicol Chem 2004; 23:2831-2836.
8. Brook HJ, Rawlings TA, Davies, RW. Protogynous sex change in the intertidal isopod *Gnorimosphaeroma oregonense* (Crustacea: Isopoda). Biol Bull 1994; 187:99-111.
9. Fiedler GC. Functional, simultaneous hermaphroditism in female-phase *Lysmata amboinensis* (Decapoda: Caridea: Hippolytidae). Pac Sci 1998; 52:161-169.
10. Rudolph E. New records of intersexuality in the freshwater crayfish *Samastacus spinifrons* (Decapoda, Parastacidae). J Crust Biol 2002; 22:377-389.
11. Gibbs PE, Bryan GW, Pascoe PL. TBT-induced imposex in the dogwhelk, *Nucella lapillus*: geographical uniformity of the response and effects. Mar Environ Res 1991; 32:79-87.
12. Bauer RT. Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. J Crust Biol 2000; 20:116-128.
13. LeBlanc GA. Crustacean endocrine toxicology: a review. Ecotoxicology 2007; 16:61-81.
14. Walsh PC, Madden JD, Harrod MJ, Goldstein JL, MacDonal PL, Wilson JD. Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. N Engl J Med 1974; 291:944-949.
15. Jobling S, Nolan M, Tyler CR, Brightly G, Sumpter JP. Widespread sexual disruption in wild fish. Environ Sci Technol 1998; 32:2498-2506.
16. Allen Y, Scott AP, Matthiessen P, Haworth S, Thain JE, Feist, SW. Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effect on gonadal development of the flounder *Platichthys flesus*. Environ Toxicol Chem 1999; 18:1791-1800.
17. Stentiford GD, Feist SW. First reported cases of intersex (ovotestis) in the flatfish species dab *Limanda limanda*: Dogger Bank, North Sea. Mar Ecol Prog Ser 2005; 301:307-310.
18. Ford AT, Fernandes TF. Notes on the occurrence of intersex in amphipods. Hydrobiologia 2005; 548:313-318.
19. Ford AT, Fernandes TF, Read PA, Robinson CD, Davies IM. The cost of intersexuality: a crustacean perspective. Mar Biol 2004a; 145:951-957.
20. Buikema AL, Chester AL, Steeves, HR. Intersexuality in *Gammarus minus* Say. Crustaceana Supplement 1980; 6:112-117.
21. Markevitch GI. Intersexuality in *Eudiaptomus gracilis* (Crustacea, Copepoda). Zool Zh 1982; 61:140-143.
22. Moore CG, Stevenson JM. Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland. J Nat Hist 1994; 28:1213-1230.
23. Smith DG. An occurrence of a female intersex of *Asellus communis* Say (Isopoda, Asellidae). Crustaceana 1977; 32:89-90.
24. Korczynski RE. Intersexuality in the arctic isopod *Mesidotea* (*Saduria) sibirica*. Arctic 1985; 38:68-69.
25. Sant'Anna BS, Turra A, Zara FJ. Simultaneous activity of male and female gonads in intersex hermit crabs. Aquat Biol 2010; 10:201-209.
26. Parnes S, Khalaila I, Hulata G, Sagi A. Sex determination in crayfish: are intersex *Cherax quadricarinatus* (Decapoda, Crustacea) genetically females? Genet Res 2003; 82:107-116.
27. Sagi A, Khalaila I, Barki A, Hulata G, Karplus I. Intersex red claw crayfish, *Cherax quadricarinatus* (von Martens): functional males with previtellogenic ovaries. Biol Bull 1996; 190:16-23.
28. Achuthankutty CT. Sexual abnormalities in the genus *Acetes* (Sergestidae, Decapoda). Curr Sci 1973; 42:827-828.
29. Peer Martin, Gerhard Scholtz. A case of intersexuality in the parthenogenetic *Marmorkrebs* (Decapoda; Astacida: Cambaridae). J Crust Biol 2012; 32:345-350.
30. Sangalang G, Jones G. Oocytes in the testis and intersex in lobsters (*Homarus americanus*) from Nova Scotian sites: natural or site-related phenomenon? Can Tech Rep Fish Aquat Sci 1997; 2163:46.
31. Stentiford GD. Histological intersex (Ovotestis) in European lobster *Homarus gammarus* and a commentary on its potential mechanistic basis. Dis Aquat Org 2012; 100:185-190.
32. Araki A, Matsura S. Gynandromorph of a freshwater crab, *Geothelphusa dehaani*. Sci Bull Fac Agr Kyushu Univ 1995; 49:133-137.
33. Takahashi T, Araki A, Nomura Y, Koga M, Arizono K. The occurrence of dual gender imposex in Japanese freshwater crab. J Health Sci 2000; 406:376-379.
34. Zou E, Fingerman M. External features of an intersex fiddler crab, *Uca pugilator* (Bosc, 1802) (Decapoda, Brachyura). Crustaceana 2000; 73:417-423.
35. Khalaila I, Sagi A. Intersexuality and its control by the androgenic gland in the crayfish *Cherax quadricarinatus*. Invert Reprod Dev 1997; 43:69-70.
36. Ginsburger-Vogel T. Temperature-sensitive intersexuality and its determinism in *Orchestia gammarella* Pallas. In: Intersexuality in the animal kingdom (ed. by R. Reinboth), Springer-Verlag, Berlin, Heidelberg, New York. 1975; 106-120.
37. Juchault P, Legrand JJ. Contribution à l'étude qualitative et quantitative des facteurs contrôlant le sexe dans les populations du crustacé isopode terrestre, *Armadillidium vulgare* Latreille, II Populations hébergeant le facteur féminisant F (Bactéroïde intracytoplasmique). Arch Zool Exp Gén 1981; 122:65-74.
38. Williams RJ, Keller VDJ, Johnson AC, Young AR. A national risk assessment for intersex in fish arising from steroid estrogens. Environ Toxicol Chem 2009; 28:220-230.
39. Reeder AL, Foley GL, Nichols DK, Hansen LG. Forms and prevalence of intersexuality and effects of environmental contaminants on sexuality in cricket frogs (*Acris crepitans*). Environ Health Perspect 1998; 106:261-266.
40. Guillette LJ, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 1994; 102:680-688.
41. Fry DM, Toone CK. DDT-induced feminisation of gull embryos. Science 1981; 213:922-924.

42. Bettin C, Oehlmann J, Stroben E. TBT-induced imposex in marine neogastropods is mediated by an increasing androgen level. Helgol Meeresunters 1996; 50:299-317.
43. Olmstead AW, LeBlanc GA. The environmental endocrine basis of gynandromorphism (intersex) in a crustacean. Int J Biol Sci 2007; 3:77-84.
44. Charniaux-Cotton H, Payen GG, Ginsburger-Vogel T. Arthropoda-Crustacea: sexual differentiation. In: Reproductive biology of invertebrates: sexual differentiation and behavior (ed. by K.G. Adiyodi and R.G. Adiyodi), John Wiley and Sons, Chichester. 1992; 281-323.
45. Bulnheim HP. Interaction between genetics, external and parasitic factors in sex determination of the crustacean amphipod *Gammarus duebeni*. Helgol Meeresunters 1978; 31:1-33.
46. Hastings, MH. Intersex specimens of the amphipod *Ampelisca brevicornis* (Costa). Crustaceana 1981; 41:119-205.
47. Rodgers-Gray TP, Smith JE, Ashcroft AE, Isaac RE, Dunn AM. Mechanisms of parasite-induced sex reversal in *Gammarus duebeni*. Int J Parasitol 2004; 34:747-753.
48. Korczynski RE. Comparison of freshwater and saltwater populations of the isopod *Mesidotea entomon* from Dolomite Lake, Northwest Territories and Pauline Cove, Yukon Territory. Ph D Thesis, Institute of Oceanography, McGill University, Montreal, Quebec, Canada, 1983.
49. Rigaud T, Juchault P. Sterile intersexuality in an isopod induced by the interaction between a bacterium (*Wolbachia*) and the environment. Can J Zool 1998; 76:493-499.
50. Charniaux-Cotton H. Sex determination. In: The physiology of Crustacea (ed. by T.H. Waterman), Academic Press, New York. 1960; 411-447.
51. Ginsburger-Vogel T, Magniette-Mergault F. The effects of temperature on sexual differentiation in the temperature sensitive thelygenic-intersexual offspring of *Orchestia gammarella* (Pallas) (Amphipoda: Crustacea). Effects of temperature on pubescent females. Int J Invert Reprod 1981; 4:39-50.
52. Juchault P, Louis C, Martin G, Nouling G. Masculinization of female isopods (Crustacea) correlated with non-Mendelian inheritance of cytoplasmic viruses. Proc Nat Acad Sci USA 1991; 88:10460-10464.
53. Hough AR, Bannister NJ, Naylor E. Intersex in the mysid *Neomysis integer*. J Zool 1992; 226:585-588.
54. Ford AT, Rodgers-Gray TP, Davies IM. Abnormal gonadal morphology in intersex, *Echinogammarus marinus* (Amphipoda). Mar Biol 2005; 147:913-918.
55. Sagi A, Khalaila I. The crustacean androgen: a hormone in an isopod and androgenic activity in decapods. Am Zool 2001; 41:77-484.
56. Charniaux-Cotton H. The androgenic gland of some decapod crustaceans and particularly of *Lysmata seticaudata*, a species with functional protandrous hermaphroditism. C R Hebd Seances Acad Sci 1958; 246:2814-2817.
57. Nagamine C, Knight AW, Maggenti A, Paxman G. Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn *Macrobrachium rosenbergii* (de Man) (Decapoda, Palemonidae), with first evidence of induced feminization in a non-hermaphrodite decapod. Gen Comp Endocrinol 1980; 41:423-441.
58. Azzauna A, Greve P, Martin G. Sexual differentiation traits in functional males with female genital apertures (♂fga) in the woodlice *Armadillidium vulgare* Latr. (Isopoda, Crustacea). Gen Comp Endocrinol 2004; 138:42-49.
59. Sagi A, Manor R, Segall C, Davis C, Khalaila I. On intersexuality in the crayfish *Cherax quadricarinatus*: an inducible sexual plasticity model. Invert Reprod Dev 2002; 41:27-33.
60. Forbes MR, McCurdy DG, Lui K, Mautner SI, Boates JS. Evidence for seasonal mate limitation in populations of an intertidal amphipod, *Corophium volutator*. Behav Ecol Sociobiol 2006; 60:87-95.
61. Ford AT, Martins I, Fernandes TF. Population level effects of intersexuality in the marine environment. Sci Total Environ 2007; 374:102-111.
62. Ford AT, Fernandes TF, Rider SA, Read PA, Robinson CD, Davies IM. Endocrine disruption in a marine amphipod? Field observations of intersexuality and de-masculinisation. Mar Environ Res 2004b; 58:169-173.