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Preliminary Notes on the Structure and Postembryonic Development of Gonads in Two Tadpole Shrimps *Triops longicaudatus* and *T. numidicus* (Crustacea: Branchiopoda, Notostraca)

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Introduction

Three sexual conditions (male, female, and hermaphroditic) have been reported in tadpole shrimps (*e. g.*, Akita, 1971; Ando and Makioka, 1999). These sexes constitute different populations: *e. g.*, populations containing only females, those containing only hermaphrodites and those containing both males and females, suggesting various modes of reproduction. Most studies on the modes of tadpole shrimp reproduction (*e. g.*, Akita, 1971), however, have contained some obscurities mainly in distinguishing hermaphrodites from females and in terminology of gonadal structures.

We describe here some structural features on testes and ovaries in *Triops numidicus* and those on ovaries and hermaphroditic ovotestes with their development in *T. longicaudatus*. We have also attempted to clarify some of the descriptive terms used by various authors, and suggest that standardized common terms be adopted in the further description.

Materials and Methods

Adult specimens of *Triops longicaudatus* were collected from rice fields in Nagano and Saitama Prefectures, and those of *T. numidicus* from rice fields in Saitama Prefecture, Japan. Specimens of both species were dissected under a stereomicroscope, and the gonads were sketched. Gonads were then fixed with Bouin's solution and prepared as usual into serial paraffin sections 4–10 μm thick. These sections were stained with Mayer's haematoxylin and eosin.

Hermaphroditic *T. longicaudatus* juveniles, obtained from the eggs laid in the laboratory and reared in separate bottles, were fixed with Bouin's solution in every instar and prepared into serial sections by the same methods as above to observe the postembryonic development of gonads.

Results

1. Adult gonads of Triops longicaudatus *and* T. numidicus

In both species, a pair of gonads were located along the gut (Fig. 1). Each gonad consisted of a longitudinal tube (gonadal trunk) with a number of relatively short branched tubules (gonadal branches) including gametogenetic zones (Fig. 2). A short straight gonoduct connected the middle portion of each gonadal trunk and a gonopore opening at the base of the 11th thoracic leg (Fig. 1).

Three types of gonads were distinguished: testes with only spermatogenetic zones (Figs. 2A, 3A) seen only in male *T. numidicus*, ovaries with only oogenetic zones (Figs. 2B, 3B) seen in females of *T. longicaudatus* and *T. numidicus*, and ovotestes with spermatogenetic and oogenetic zones (Figs. 2C, 3C) seen only in hermaphroditic *T. longicaudatus*.

2. Postembryonic development of hermaphroditic Triops longicaudatus *gonad*

In the fourth instars, gonadal primordia first appeared as a pair of indifferentiated cellular masses at both sides of

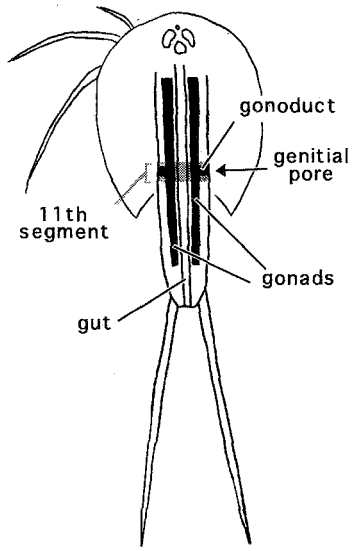

Fig. 1 Diagrammatic representation of location of tadpole shrimp reproductive system.

the gut (Fig. 4A). In the eighth instars, these cellular masses were elongated along the longitudinal body axis. In this stage, several larger and spherical germ cells were found in smaller cell masses. No differentiation as to whether they were to become male or female germ cells could be recognized (Fig. 4B). In the 13th instars, surface epithelia and inner lumens were distinguished in the cellular masses, which were developed into a pair of the ovotesticular trunks. From the middle lateral portion of each ovotesticular trunk, a sac-like structure, a proximal part of the ovotesticular duct, emerged. On the surface of each ovotesticular trunk, several primordia of ovotesticular branches with young oogenetic zones including oocytes at their tips, were also found (Fig. 4C). In the 15th instars, the ovotesticular branches were extended and oogenetic zones enlarged. In the epithelia of some ovotesticular branches near the oogenetic zones, the spermatogenetic zones were found (Fig. 4D). In the 18th instars, oviposition through the

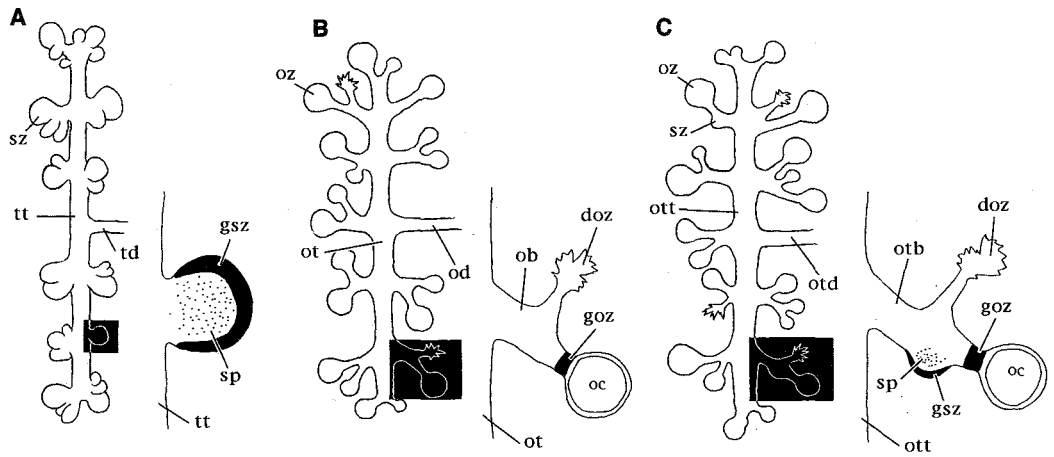

Fig. 2 Diagrammatic representation of three types of gonads of tadpole shrimps. A. Testis of *Triops numidicus*, B. Ovary of *T. longicaudatus* and *T. numidicus*, C. Ovotestis of *T. longicaudatus*. doz: degenerated oogenetic zone after ovulation, goz: germarium of oogenetic zone, gsz: germarium of spermatogenetic zone, ob: ovarian branch, oc: oocyte, od: oviduct, ot: ovarian trunk, otb: ovotesticular branch, otd: ovotesiticular duct: ott: ovotesiticular trunk, oz: oogenetic zone, sp: sperm, sz: spermatogenetic zone, td: testicular duct, tt: testicular trunk.

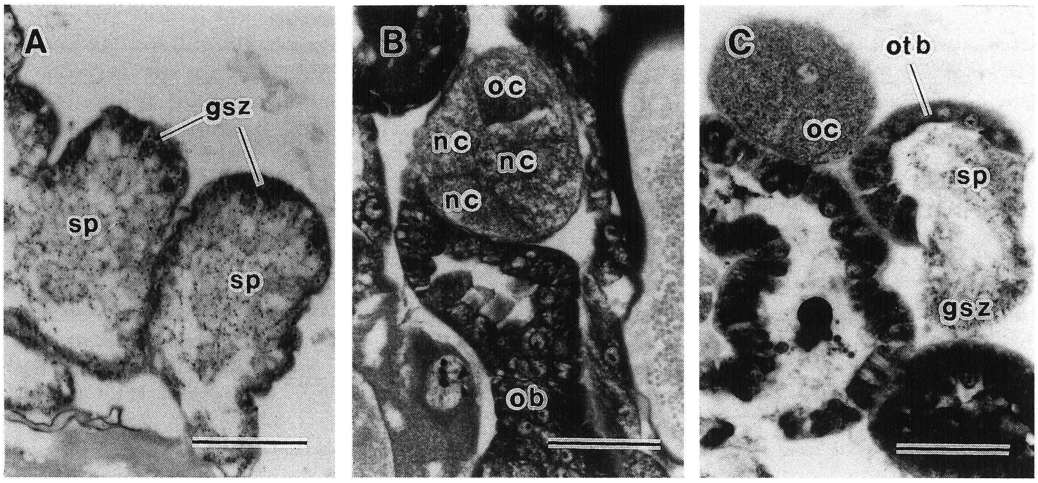

Fig. 3 Gametogenetic zones in three types of gonads of tadpole shrimps. A. Spermatogenetic zones of male *Triops numidicus* containing sperms. B. Oogenetic zone of female *T. longicaudatus* containing oocyte and three nurse cells. C. Spermatogenetic zone and Oogenetic zone of hermaphroditic *T. longicaudatus*. gsz: germarium of spermatogenetic zone, nc: nurse cell, ob: ovarian branch, oc: oocyte, otb: ovotesticular branch, sp: sperm. Bars =50 μm.

ovotesticular ducts were first observed, showing this stage to be the 1st adult stage, in which the hermaphroditic gonads became matured (Fig. 4E).

Discussion

Both the ovotesticular trunks and the ovotesticular ducts in hermaphroditic *Triops longicaudatus*, as well as the ovarian trunks and the oviducts in females of *T. longicaudatus* and *T. numidicus*, have often been called "oviducts" (Table 1) because of their common nature as passages for ovulated eggs. However, the ovotesticular trunks bearing the ovotesticular branches with both the oogenetic zones and the spermatogenetic zones are doubtless parts of gonads, not of the gonoducts, because they originated from the gonadal primordia and they include germ cell regions as seen in the present results, and because they correspond to the gonads in many other crustaceans in their structure, locality, and

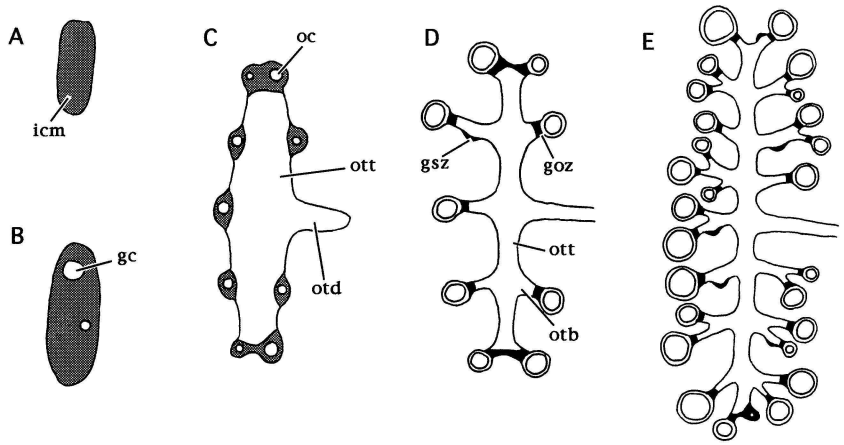

Fig. 4 Diagrammatic representation of postembryonic development of ovotestis in hermaphroditic *Triops longicaudatus*. A. Fourth instar. B. Eighth instar. C. Thirteenth instar. D. Fifteenth instar. E. First adult stage. gc: early germ cell, goz: germarium of oogenetic zone, gsz: germarium of spermatogenetic zone, icm: indifferentiated cell mass, oc: oocyte, otb: ovotesticular branch, otd: ovotesiticular duct, ott: ovotesticular trunk.

Table 1 Terms used for gonadal structures of tadpole shrimps.

Gonads	Terms used in the present study	Terms having been used in various papers
Testis	spermatogenetic zone (sz)	lobe[1], branched tuble[2], testis lobe+seminiferous duct[3]
	testicular trunk (tt)	longitudinally proceeding duct[2], vas deferens[3]
	testicular duct (td)	efferent duct[1,3]
	germarium of spermatogenetic zone (gsz)	
Ovary	oogenetic zone (oz)	follicle[2,4,5], ovum[3,6], ovarian follicle[7], oogenetic pouch[8]
	ovarian branch (ob)	follicular duct[2], follicle duct[3,6], tubular folliclar duct[7], ovarian branch[4,8,9]
	ovarian trunk (ot)	longitudinal oviduct[2,3], oviduct[3,6], central longitudinal oviduct[7], ovarian trunk[4,8,9]
	oviduct (od)	efferent oviduct[3,6], oviduct[3,4,8,9]
	degenerated oognetic zone after ovulation (doz)	empty follicle[9], empty oogenetic pouch[8]
	germarium of oogenetic zone (goz)	germinal zone[7], germarium[4,8,9]
Ovotestis	spermatogenetic zone (sz)	testis zone[1], testicular zone[5], testicular lobe[3,5,10,11], testicular follicle[12], testis[3]
	oogenetic zone (oz)	follicle[1,10], oocyte follicle[5], egg follicle[12], ovarian follicle[12], ovarian lobe[10]
	ovotesticular branch (otb)	follicle duct[1,3,5,10,11], uterus[13]
	ovotesticular trunk (ott)	longitudinal oviduct[1,5], oviduct[10], ovary[13], uterus[13]
	ovotesticular duct (otd)	longitudinal oviduct[5], efferent oviduct[1,10], ovary[13]
	degenerated oogenetic zone after ovulation (doz)	empty follicle[1,5]
	germarium of spermatogenetic zone (gsz)	
	germarium of oogenetic zone (goz)	germinal region[1], germarium[5]

1: Longhurst (1955), 2: Engelmann *et al.* (1997), 3: Akita (1973), 4: Ando (1992), 5: Trentini and Scanabissi (1982), 6: Akita (1971), 7: Trentini and Scanabissi (1978), 8: Ando (1998), 9: Ando and Makioka (1992), 10: Ogata (1981), 11: Zaffagnini and Trentini (1980), 12: Wingstrand (1978), 13: Tomassini *et al.* (1989).

function (Ando, 1998; Ando and Makioka, 1999). In contrast, the ovotesticular ducts are true gonoducts, because they are directly connected with the gonopores and they do not include germ cell regions as seen in the present results, and they correspond to the gonoducts in many other crustaceans in structure, locality, and function (Ando, 1998; Ando and Makioka, 1999).

The oogenetic zones in the females and hermaphrodites have long been called "follicle" (Table 1). However, it was found in female *T. longicaudatus* that the epithelium surrounding oocyte in each oogenetic zone was not closed, but was connected with the epithelium of the ovarian branch (Ando, 1998).

In the present study, we also have found in hermaphroditic *T. longicaudatus* and female of *T. numidicus* that the epithelium surrounding each growing oocyte is a part of the epithelium of each ovotesticular or ovarian branch. The space containing each growing oocyte is, therefore, not the "follicle", but the terminal space of the lumen of each ovotesticular or ovarian branch.

The spermatogenetic zones in male *T. numidicus* and hermaphroditic *T. longicaudatus* have variously been called (Table 1). In the present study, we have used the term as a common term corresponding with the homologous structure in females and hermaphrodites, the oogenetic zones.

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