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Reproductive biology and intra marsupial development of mysids in Auckland region, New Zealand

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Abstract

Tenagomysis chiltoni and T. novaezealandiae collected from selected estuarine sites showed continuous breeding. In both species breeding is continuous. Reproductive potential of T. chiltoni is highest during winter to early summer, with major peaks during spring while T. novaezealandiae was more inconsistence in seasonal variation. Length of mysids and brood size varies with the season, being maximum during cooler months. The occurrence of the highest body size and brood size in spring may be due to favourable conditions prevailing in moderate temperatures. The larger species showed higher brood size and higher egg diameters than smaller species. Laboratory studies showed that, intra marsupial development period and each developmental stage of T. chiltoni, last longer than that of T. novaezealandiae. In both species, egg stage is shorter than other stages. All stages of larvae of T. chiltoni are larger than those of T. novaezealandiae. The total development time for T. chiltoni was, 19–20 days,T. novaezealandiae it was 17–18 days. The gravid females of T. chiltoni are negatively correlated with salinity, indicating that the higher salinity levels provide unsuitable conditions and the opposite is true for T. novaezealandiae. Except juveniles, all the life stages of T. novaezealandiae showed positive correlation with temperature, indicating that high temperatures provide better conditions for T. novaezealandiae than T. chiltoni.

Keywords: Mysid, reproduction, brood, gravid females, life stages, *Tenagomysis.*

Introduction

The intra-marsupial development time (incubation period or embryonic period) is an important aspect of population biology of mysids and is linked with the timing of the breeding season, age at maturity, frequency of broods, number of young per brood, egg size and adult body size[1].

The embryonic period of mysids within the marsupium can be separated into three stages[2-4]. These are: eggs (stage I), eyeless larvae (stage II) and eyed larvae (stage III). All larvae within a single marsupium generally are at the same developmental stage and there are occasions that stage II larva occurs amongst stage III larva, although this is rare[5]. Larvae are regularly closely packed and directing their heads posteriorly[5].

The brood size varies greatly among species, and has been positively correlated with mysid body size[5,6]. Larger *T. chiltoni* (11.75–18.6mm) carry more larvae (22–39) than the smaller *T. novaezealandiae* (5.7–9.91mm; 6–19 larvae and *T. macropsis* (6.5–11.65mm; 4–25 larvae)[7].

The size of the larvae in the marsupium can also vary seasonally and latitudinally. A seasonal variation exists in brood size in the *Neomysisinteger* population regardless of the body size, with a larger number of broods during winter and spring compared to the summer in the Wester schelde estuary South – West Netherlands[8]. Maximum brood size and egg size of *T. Macropsis* found during winter in the Avon-Heathcote Estuary[6]. However, there was no significant difference between the mean sizes of each three stages of larvae among *T. macropsis*, *T. novazealandiae* and *T. chiltoni* in the same estuary[8].

The duration of marsupial development has been reported from several studies for different species from different latitudes and may range from a few days to several months. Tropical mysid species have shorter development times, 5.5 days for *Metamysidopsis insularis* at 32.5°C than temperate mysids, at 17°C, *A. mixta australis*, *T. tasmaniae* and *Paramesopodopsis rufa* taken 15, 15 and 20 days development time respectively[1,9].

It was witnessed from *N. americana* and *P. flexuosus*, the stage III larvae within the marsupium are active and show rhythmic contractions of the body[10]. The female spreads the lamellae of the marsupium laterally and released the larvae one by one; they are unable to swim. Finally moulting take place to terminate the stage III development. Immobile larvae would be subjected to heavy predation[2,5].

Few studies have been carried out on the reproductive biology of New Zealand mysids[6,7,11,12]. A reproductive biology study of *T. macropsis* was based on monthly collection of mysids over one year[6]. He mainly studied the brood characteristics of *T. macropsis*. Although the study conducted on distribution and brood characteristics of *T. macropsis, T. chiltoni, T. novaezealandiae* and *G. australis,* data are too few to allow

detailed analysis[7]. Other studies were also not mainly focused on reproductive biology, conversely they inferred the life histories of mysids based on monthly collections[11-13]. However, all these studies are only explanatory. Therefore, the present study has been focussed mainly on life cycle in more detail in the simulated laboratory condition in order to have a clear description of the reproductive biology of the most abundant species in Auckland region.

Methodology

Mysid samples were collected monthly from May 2006 to September 2008 from six estuarine sites (Kakamatua, Cornwllis, Mill bay, Big Manly Bay, Okoromai Bay, and Orewa). Mysid samples were collected using a hand-held dip net of 500 µm. Total body length and, sex of each mysid were determined. The reproductive state of each mysid (juveniles, immature males and females, adult males and females, gravid females and post-spawned females) and brood sizes of each gravid female were determined. Each brood were examined to determine the number of larvae, and their development (egg, stage II, stage III) within the brood.

For both species different life stages were identified and categorized as juveniles (without secondary sexual characteristics), immature males (elongated fourth pleopods), mature adult males (fourth pleopods reach the posterior edge of the last abdominal segment or/and the lobus masculinus appeared with setose and the prominent penis) immature females (rudimentary pleopods and brood pouches are formed but not noticeable from lateral side), mature adult females (laterally visible marsupia but small and empty), gravid females (marsupium filled with eggs or larva), and post-spawned females (larvae released from the marsupium)[14]. In this study, for the purpose of distinguishing the population structure, immature males and females were considered as sub adults.

Mysid samples were collected for culture experiment to observe the development stages, during October 2007 from two main sites Kakamatua (*T. chiltoni*) and Manly Bay (*T. novaezealandiae*). Collected mysids were safely transferred to plastic buckets containing water from the collection site. They were taken to the laboratory and kept in aerated containers at ± 15°C (based on the water temperature of the site, at the time of collection).Subsequently selected gravid females were examined using light microscope to observe the development stage of larvae.

Three replicate glass beakers were used for each species (2000 ml beakers for *T. chiltoni* and 1000 ml beakers for *T. novaezealandiae*). In each beaker, four males and six mature females (gravid females/ post-spawned females), were kept for six weeks. The number of gravid females and post-spawned females per replicate beaker was determined by the availability of each life stage in wild-caught samples. Males were kept in the beaker to allow for reproduction to obtain fresh broods with egg stage.

Total body length was taken in order to identify the individual female mysids over a period of six weeks and different lengths were kept in each replicate beaker. Daily, every individual was examined to determine the stage of larval development within the marsupium. For observation purposes mysids individually taken into a small vial and kept carefully in a Sedgwick rafter cell with water and observed under the light microscope. The numbers of days taken to complete each developmental stage was recorded.

Water collected from the sampling site was used throughout the experiment in aerated containers. Water parameters: temperature, pH, dissolved oxygen and salinity were monitored using WTW 3400i Multi-Parameter Water Quality Field Meter (Geotech Environmental Equipment, USA). Each day ammonia levels were monitored using an Ammonia Test Kit. Replicated experimental setups were placed in a temperature and light controlled incubator at 15°C under 12 hour of light and 12 hour of dark.

Each day faeces, possible moults and dead mysids, were removed. Dead mysids were replaced with new female or male (as needed) of known size; nearly one third of the water was renewed; and mysids were fed with newly hatched *Artemia*. Every fourth day, the water in each beaker was completely renewed.

Before starting the experiment, *Artemia* culture was maintained using *Artemia* cysts in a glass beaker with seawater kept at 23 ± 1°C. Series of new cultures were subsequently started on every third day.

A pilot study was carried out to find the appropriate non-lethal stocking density for each mysid species at temperatures 13±1°C and 23±1°C. Mysid densities selected as 15, 10 and 7, in each beaker volume. Three replicate glass beakers were used for each stocking density and each temperature. Survivors were observed daily up to two weeks. Water renewal, monitoring of environmental variables and food supply, were undertaken as in the main experimental set up. In order to avoid higher mortality rates, it was decided to introduce 10 individuals into a beaker as the most suitable density when compared to other two densities. Similarly detailed life cycle stages were analysed for *Gastrosacus australis*.

Preliminary investigation was carried out to decide the proper food for mysids, using fish flakes and newly hatched *Artemia*, in these experimental conditions. Three replicate glass beakers containing 10 mysids were used for each food item at 13±1°C temperature. Survivors were observed for two weeks with supply of each food item daily. Water exchange and monitoring of environmental variables were undertaken as in the above experiments. Due to high mortality of mysids in beakers which were provided with fish flakes as food, it was established that newly hatched *Artemia* was the most suitable food item for mysids.

Results and discussion

Sex ratio: The sex ratio of both *T. Chiltoni* and *T. Novaezealandiae* which were determined on the basis of examination of all adult mysids collected during the two year survey (Figures-1a–b). During each month, the sex ratios expressed as percentage of male to female of *T. chiltoni* and *T. Novaezealandiae* varied more or less higher percentages of females than males.

Population structure: Proportional population structures (juvenile/sub adult, adult female and male, gravid female) of *T. chiltoni* and *T. novaezealandiae* from all six sites during January to December are expressed in percentages (Figures-2a–2b). During January to December, the major proportion of the *T. chiltoni* population was represented by the juvenile and sub adults (36%–67%). However, during the period of July to December, a high proportion of the reproductive females (gravid and adult females) was observed (33%–36%) (Figure-2a). High proportion of males was observed during May, August and November (29%, 30%, 28% respectively) (Figure-2a).

The population of *T. novaezealandiae* was predominantly represented by adults of both sexes and gravid females, and they were most frequent during most of the sampling period (Figure-2b). The population of *T. novaezealandiae* was indicated by a higher number of gravid females throughout the year, with the highest proportions during March, November, October, January, July and September (71%, 61%, 59%, 58%, 57% and 55% respectively). The highest proportions of juvenile and sub adults occurred in August (46%) and April to June (35%–39%) (Figure-2b).

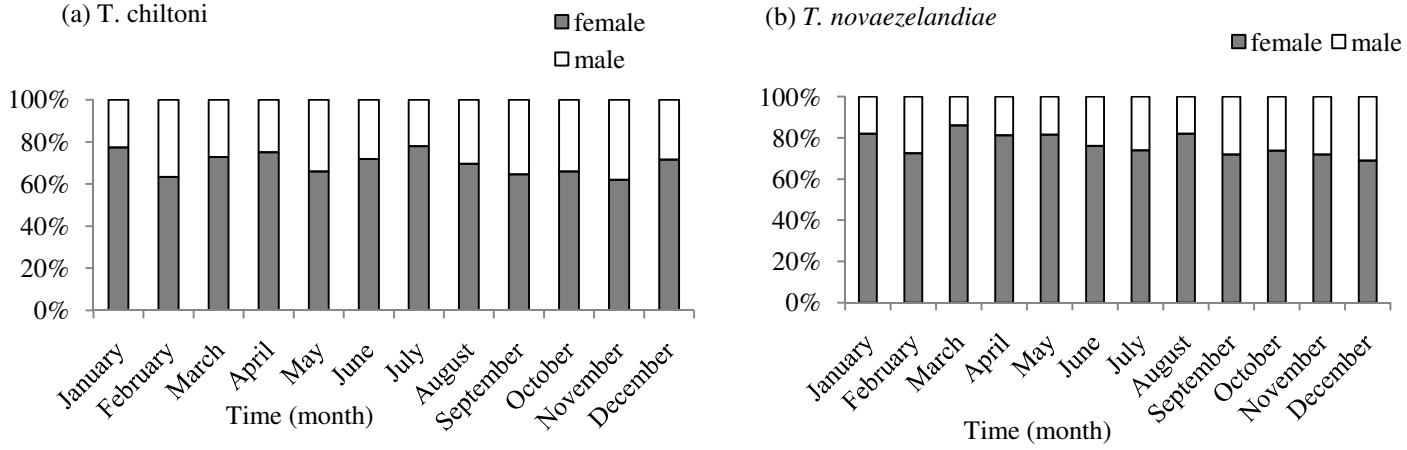

Figure-1: Sex ratio of mysids collected over two years.

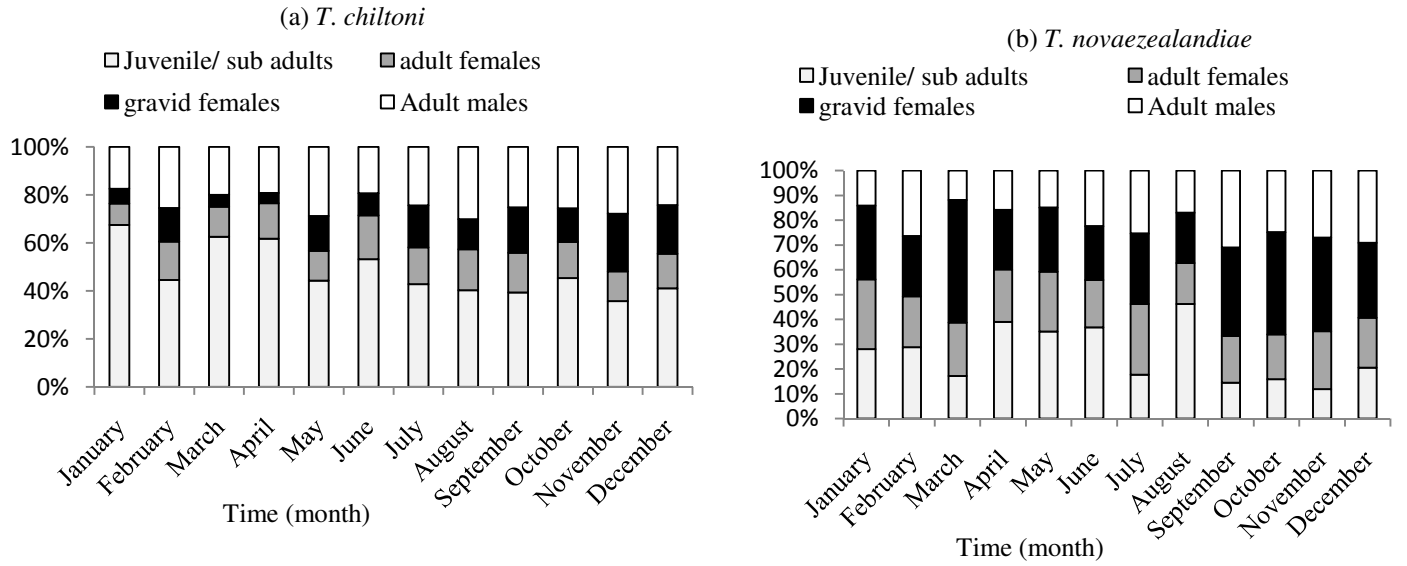

Figure-2: Proportional population structure of mysids.

Comparison of life stages: The monthly mean number of juvenile/sub adults of *T. chiltoni* and *T. novaezealandiae* represented by three peaks: January (*T. chiltoni*), June (both species), October (*T. chiltoni*), August and December (*T. novaezealandiae*) (Figure-3a) that shows the seasonality of the mean abundance of both species.

Higher abundance values for juveniles/sub adults were observed for *T. chiltoni* during winter, spring and early summer and for *T. novaezealandiae* during June, August and early summer. There was a reduction in the abundance for *T. chiltoni* during late summer and autumn and for *T. novaezealandiae* it was during March, July, September and November.

The abundance of gravid females of *T. novaezealandiae* was higher in December, January, May–June, and September–November (Figure-3b). The abundance of *T. chiltoni* varies throughout the year, and three peaks appeared during June, July, September to November and December (Figure-3b). The mean monthly abundance of reproductive population of both species showed that the abundance values were higher during June, September to November and December (Figure-3c). For both species, three abundance peaks were apparent and there were lower abundance values during March and April (both gravid females and the reproductive population).

Aspects on brood size: Number of gravid females (marsupium consist of larval stages) of *T. Chiltoni* and *T. Novaezealandiae* recorded from all sites over a two year period is given in Table-1. Gravid females of *T. chiltoni* were found throughout the year at Cornwallis. Mill Bay they were found almost year around except in March and April. Kakamatua they were found from June to January but none for the late summer and autumn.

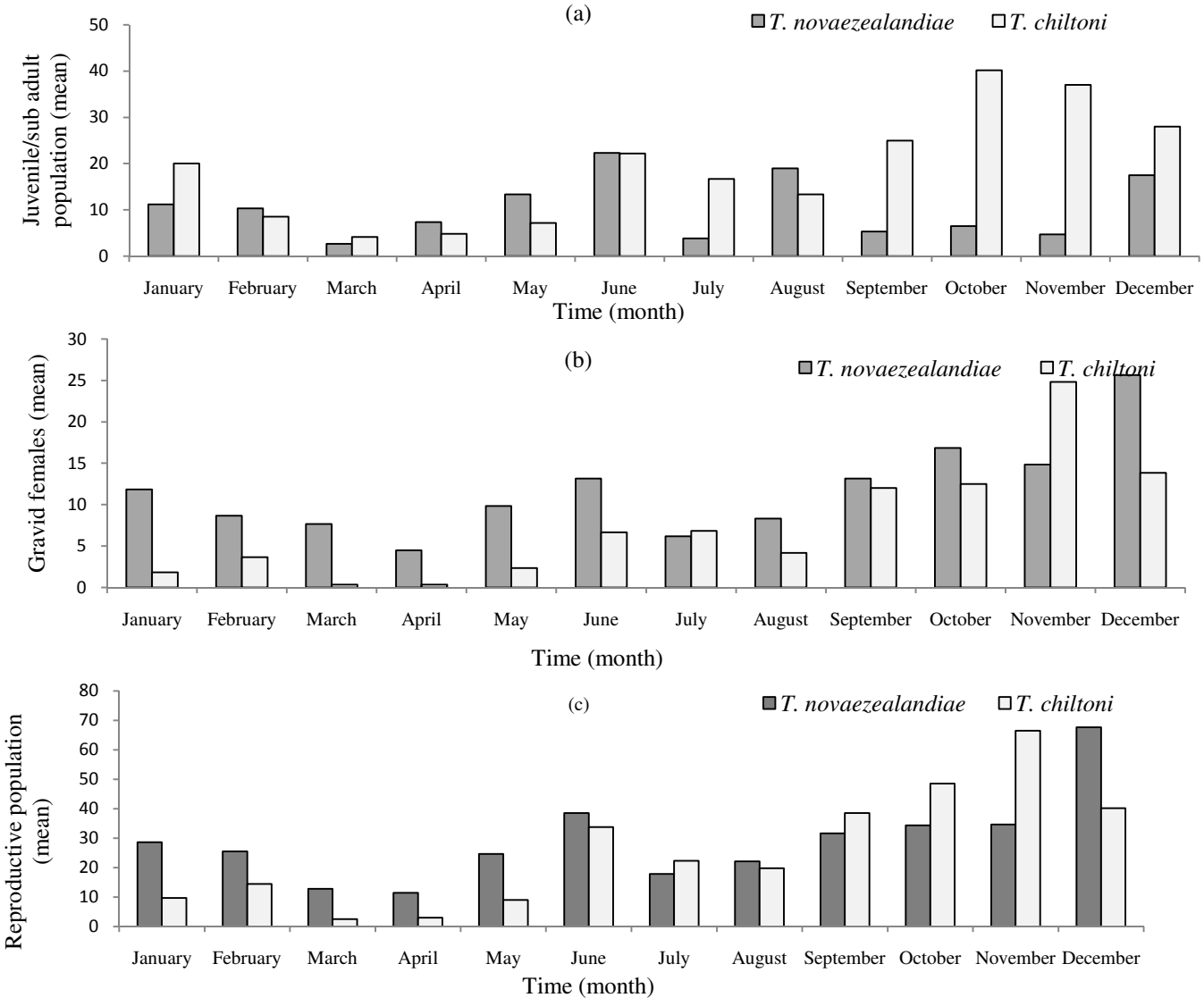

Figure-3: The monthly mean number of (a) juveniles/subadults (b) gravid females (c) reproductive population.

Gravid females of *T. novaezealandiae* were collected from the sites of Manly Bay and Orewa Stream throughout the year but from Okoromai Bay they were not found from March to July (Table-1). However, according to pooled data gravid females of both *T. chiltoni* and *T. novaezealandiae* were found throughout the year (Table-1).

According to monthly collected broods, *T. chiltoni* brood sizes were found ranging from 4–31 and for *T. novaezealandiae*, it ranging from 2–21 (Table-1). The maximum brood sizes for both species were detected during spring and winter (Table-1) showed that the number of eggs per brood varied seasonally. The highest mean brood sizes were shown during spring (*T. chiltoni* -14.95 eggs and *T. novaezealandiae* - 9.72 eggs). The one-way ANOVA showed that a significant difference is exist on the average number of eggs per female ($P \leq 0.05$) throughout the seasons of the year for *T. chiltoni* and *T. novaezealandiae* (Table-1 and 2). Tukey test confirmed that spring brood size was significantly different to that of summer (11.5 eggs) and winter (10.96 eggs). However, summer brood sizes were not significantly different from that of winter (Table-2). The mean brood sizes (eggs per female) in winter and autumn are 9.29 and 8.58 respectively and it was lowest in summer (7.82 eggs per female) (Table-2). The brood sizes in spring are significantly different from summer. And also summer, brood sizes are significantly different from winter in Manly Bay (Table-2).

Intra-marsupial development: The larval development within the marsupium of both *T. chiltoni* and *T. novaezealandiae* was observed at 15°C under laboratory conditions, with three stages being identified: Stage I: Egg, spherical shape, filled with oil globules. Stage I ended with hatching from the egg membrane. In some occasions, extraordinary grey-coloured eggs were observed, (both field and laboratory samples) those seem to be unfertilized eggs that disappeared from the marsupium; Stage II: eyeless larvae, with comma-shaped body (eyeless larva); the antennae and thoracic appendages develop during this stage and eyes become pigmented; this stage terminates in a moult; Stage III:eyed larvae (moulted larva), has eyes on stalks with well-developed appendages; this stage also terminates in a moult and juveniles are released from the marsupium. The free living young juveniles are morphologically similar to the adults, except lacking of obvious sexual characteristics.

The recorded total body lengths of larvae (stage I–III) of field collected broods for *T. chiltoni* and *T. novaezealandiae*, are given in Table-3. All three stages of larvae of *T. chiltoni* are larger thanthose of *T. novaezealandiae*.

Table-1: Number of gravid females and the description of brood size of mysids (pooled data).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Number of gravid females												
T. chiltoni	11	15	02	02	14	40	41	25	72	75	149	83
T. novaezealandiae	64	35	22	12	45	34	13	46	101	87	81	131
Min-Max brood size												
T. chiltoni	7-10	4-13	4-13	8-16	8-23	4-18	4-25	8-25	7-31	4-25	4-21	4-15
T. novaezealandiae	2-9	5-11	6-10	4-11	2-14	8-14	4-15	5-17	6-15	2-21	3-16	6-14

Table-2: Description of variation of brood size of *T. novaezealandiae* and *T. chiltoni* among seasons.

Site	Species	F value	Df	Significance	Season	Mean±SE	Min. (no of eggs)	Max (no of eggs)
Kakamatua	*T. chiltoni*	24.70	3	s	Autumn	0.00	0	0
					Spring	14.96±0.33	31	4
					Summer	11.57±0.40	15	3
					Winter	10.96±0.45	25	4
Big Manly Bay	*T. novaezealandiae*	12.01	3	s	Autumn	8.58±0.39	13	4
					Spring	9.72±0.28	21	4
					Summer	7.82±0.19	14	4
					Winter	9.29±0.30	17	5

SE = standard error, s = significant at $P \leq 0.05$, Df =degree of freedom.

The recorded intra-marsupial development time for each larval stage at 15°C for *T. chiltoni*, and *T. novaezealandiae* are given in Table-3, Figures-4a and 4b. The total development time within the marsupium for *T. chiltoni* was, 19–20 days and for *T. novaezealandiae* it was 17–18 days. It was apparent that each larval stage showed that they have a different time duration for completion of development. Further, the development time for *T. chiltoni* for each stage is longer than that of *T. novaezealandiae*. Stage I of *T. chiltoni* took five days to develop into stage II and for *T. novaezealandiae*, it was four days. Development time for stage II and stage III of *T. chiltoni* was 6–7 days, 7–8 days and for *T. novaezealandiae* it was 6 and 7 days respectively.

Table-3: Total body lengths of stage I–III larvae, *T. chiltoni* and *T. novaezealandiae*.

Species	Stage I length (mm)	N	Stage II length (mm)	N	Stage III length (mm)	N
T. chiltoni	0.40–0.64	156	0.64–1.40	216	1.50–2.20	128
T. novaezealandiae	0.36–0.48	136	0.52–1.16	182	1.20–1.50	132

n = number of individuals.

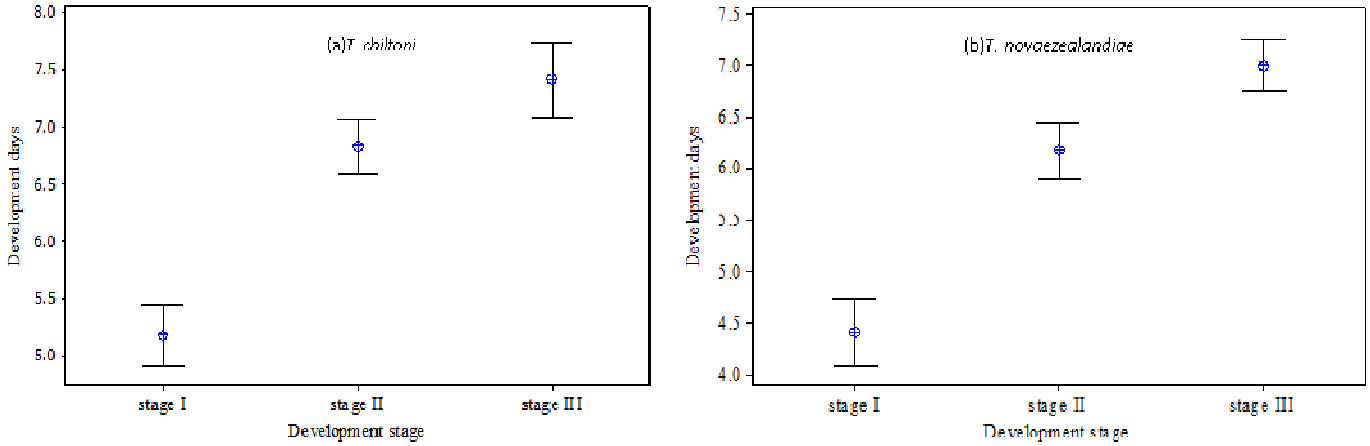

Figure-4: Intra marsupial development time of mysids, at 15°C.

Table-4: Correlation coefficients (r) of bivariate analysis of pooled environmental parameters on different life stages of mysids.

Species		pH	DO ($mg^{-1}L$)	Salinity (‰)	Tempe (°C)	Rainfall (mm)
T. chiltoni	Juvenile/sub adults	-0.417**	0.223	0.02	0.219	-0.129
	Gravid females	-0.395**	-0.305*	-0.368*	0.294*	-0.208
	Adult females	-0.372*	0.223	0.018	0.128	-0.167
	Male	-0.395**	-0.119	-0.2	0.267	-0.205
T. novaezealandiae	Juvenile/sub adults	0.296*	0.227	0.232	0.077	-0.028
	Gravid females	0.378**	-0.249*	0.062	0.276*	-0.216
	Adult females	0.328**	0.019	0.219	0.246*	-0.247*
	Male	0.334**	-0.037	0.241*	0.346**	-0.173

* Significant at $P \leq 0.05$ level,** Significant at $P \leq 0.01$ level, Do=Dissolved oxygen Tempe=temperature

The correlation among the environmental parameters and abundances of different life stages of mysids is shown in Table-4. The correlations indicated that the all life stages of *T. chiltoni* are correlated negatively with pH, whereas *T. novaezealandiae* correlated positively with pH ($p \leq 0.05$). In addition, gravid females of both species correlated negatively with dissolved oxygen and correlated positively with temperature. However, gravid females of *T. chiltoni* correlated negatively with salinity ($p \leq 0.05$). Further, *T. novaezealandiae* males are highly correlated with pH, salinity and temperature whereas *T. chiltoni* male correlated negatively with pH. The closer examination of correlation coefficients indicated that *T. novaezealandiae* males possessed a negative correlation with monthly rainfall ($p \leq 0.05$) (Table-4).

Ten gravid females of *Gastrosacus australis*, 10.00-13.6 mm in length had 8-20 eggs or larvae in their marsupium. Development time observed for egg stage, stage II and stage III of *G. australis* was 4, 6/7 and 6/7 respectively at 15°C. The total development time within the marsupium was, 17-18 days.

Discussion: During the sampling period, the number of *T. chiltoni* and *T. novaezealandiae* females exceed the number of males. *Neomysis americana* and *A. thailandica* also showed similar differences in the sex ratio[15,16].

The reproductive population and the gravid females of both *Tenagomysis* species were found throughout the year and follow a pattern reflecting peak abundance values during a particular period in the year. The wider distribution of *T. novaezealandiae* in New Zealand indicated its ability to exist under diverse environmental conditions[17]. Therefore, in *T. novaezealandiae* breeding occurs throughout the year is not a surprising event.

These findings indicate that breeding is continuous in *T. chiltoni* and its agree with the findings on reproductive period of *T. chiltoni* in Lake Waahi in North Island[12]. However, the breeding season of *T. chiltoni* populations recorded in South Island is different, and their distinct reproductive periods were identified as only in spring and summer[11,13]. This could be associated with environmental parameters (mainly temperature) that vary with the latitude[1,5,18]. Cessation of breeding in winter is caused by the winter temperatures falling below 10°C[1]. Further, Breeding is continuous for estuarine mysids at the latitudes lower than 40°S, and Kakamatua is situated at 37°S also indicate the similar agreement[19].

Comparatively, *T. chiltoni* produces more juveniles than *T. novaezealandiae* and a juvenile/sub adult population of *T. chiltoni* is more or less higher throughout the year. There are site specific and seasonal differences in the brood size of each species. Maximum brood sizes of both species during spring, and winter indicated that the colder seasons (in Auckland) are highly favourable for mysid breeding and it reflects a higher abundances in these periods. Similarly a seasonal variation exists in brood size of the *N. integer* population regardless of the body size, with a larger number of broods during winter and spring compared to the summerin Westerschelde estuary of South - West Netherlands[8]. It's apparent that brood size is highest in larger species than smaller species, *T. chiltoni* (maximum length,18.52mm)had larger brood size (31) than smaller *T. novaezealandiae* (maximum length,10.32mm) (21).

The mean body lengths of both sexes of *T. chiltoni* and *T. novaezealandiae* were highest in winter and spring and the largest individuals of *T. chiltoni* and *T. novaezealandiae* of both sexes were also recorded in spring. Similar findings have been reported for *T. macropsis* in a New Zealand Estuary (South Island) and for three Australian mysids, *A. mixta australis*, *T. tasmaniae* and *P. rufa*[6,20]. Therefore, body length as well as number of eggs per female *T. chiltoni* and *T. novaezealandiae* vary with the season. The occurrence of the highest body size and brood size in spring may be due to favourable conditions prevailing in colder months (in moderate temperatures).

Theintra marsupial development period of *T. chiltoni* (19-20 days) is longer than that of *T. novaezealandiae* (17-18 days). Similarly, each developmental stage (stage I–III) of *T. chiltoni* lasts longer than *T. novaezealandiae*. Total development time for *Gastrosacus australis* also similar to *T. novaezealandiae*.

Furthermore, in all three species, the egg stage (stage I) is shorter than stages II and III at each temperature. For example, at 13°C the development periods of these species are as follows: *T. chiltoni* stage I, 6 days, stage II and III, each ≈9 days; *T. novaezealandiae* stage I, 5–6 days, stage II and III, each 7–8 days; *G. Australis* stage I, 4 days, stage II and III 6–7 days. All three stages of larvae of *T. chiltoni* are larger than those of *T. novaezealandiae*.

The gravid females of *T. chiltoni* are negatively correlated with salinity, indicating that the higher salinity levels provide unsuitable conditions for *T. chiltoni* and the opposite is true for *T. novaezealandiae*. Except juveniles, all the life stages of *T. novaezealandiae* showed positive correlation with temperature. This indicating that, high salinities and high temperatures provide better habitat suitability for *T. novaezealandiae* than *T. chiltoni*.

Conclusion

The present finding proved that both *Tenagomysis* sp. have year around breeding. Further *T. chiltoni* showed seasonal variation in their reproductive output while *T. novaezealandiae* was more variable in seasonal expression. The highest body size and brood size achieved in moderate temperatures (spring). Each developmental stage of larvae of *T. chiltoni* are larger and last longer than those of *T. novaezealandiae*. Higher salinity levels provide unsuitable conditions for gravid females of *T. chiltoni* and suitable conditions for *T. novaezealandiae*. Higher temperatures provide better conditions for all the life stages, except juveniles of *T. novaezealandiae* than *T. chiltoni*.

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