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## INDOMYSIS ANNANDALEI W. TATTERSALL, 1914 (MYSIDACEA: MYSIDAE) FROM PAKISTAN COASTAL WATERS – EURYTHERMAL AND EURYHALINE OPOSSUM SHRIMP

*Indomysis annandalei* was collected from the Ambro Creek in Indus delta (24<sup>0</sup> 34'9 N, 67<sup>0</sup> 60'8 E), Pakistan, from November 2006 to October 2007. A total of 20647 specimens ranging in size from 1.0 to 9.7 mm were analyzed in samples: 33% were males, 53% females and 14% were indeterminate juveniles. Males ranged in size from 3.5 to 8.0 mm; females from 3.0 to 9.7 mm and juveniles from 1.0 to 4.0 mm. Females were larger than males and predominated in the majority of the samples with an overall male to female ratio of 1:1.58. Five stages of development were recognized, the number of larvae in the brood pouch ranged from 3 to 18. During the sampling period salinity ranged from 4 to 38 ppt, temperature from 13 to 39 <sup>o</sup>C, pH 8.0 to 8.5, and D.O. fluctuated from 4.9 to 9.0 mg/L. The salinity tolerance was also determined experimentally for *I. annandalei*. The upper limit of salinity tolerance to temperature and salinity, its nutritional value and its occurrence almost throughout the year in Pakistan coastal waters. *Artemia* culture is not possible below 130 ppt salinity tolerance.

Mysids belong to a highly adaptive group of Crustacea inhabiting a wide range of oceanic zones, from littoral to meso- and bathypelagic. They are an important part of coastal ecosystems, mostly as omnivores - feed on copepods, diatoms and organic detritus [3, 10, 21, 24, 33]. Domingues et al. [4] also observed strong cannibalism among this species. Different organisms, such as fish and invertebrates [9, 18, 19, 21, 25, 32 -34], birds and seals [21] feed on them. They also play an important role in the sea in transferring energy through the detrital pathways [2, 34]. Mysids are used both as live and frozen food for aquarium and for aquaculture. Mysid culture has been made using various culture system and diets [4, 5]. They are used as food for juvenile shrimp [28]: supplement of live mysids to the diet of juvenile turbot increased growth rate and FCR (food conversion rate) [16]. Wickins et a.l [35] found the combination of mysids and Artemia the best food for growth in larviculture of the European lobster, Hommarus gammarus. Seikai et al. [30] compared mysids and some granulated foods for rearing juvenile

flounder, *Paralicthys olivaceus*, and found that mysid feeding fish always had high growth rates and better FCR. Furuta *et al.* [6] also emphasized mysid potential for the use of them in aquaculture. Mysids are also considered as excellent experimental estuarine organisms for toxicity testing [15, 18, 23].

From the Northern Arabian Sea, 20 species of mysids have been recorded [11], 11 species have been recorded from Pakistan waters: *Gastrosaccus muticus* W. M. Tattersall, 1915; *Acanthomysis indica* (W. M. Tattersall, 1922), *A. pelagica* (Pillai, 1957), *A. quadrispinosa* Nouvel, 1965, *Siriella* sp., *Siriella affinis* Hansen, 1910, *Afromysis macropsis* W. M. Tattersall, 1922 and *Indomysis annandalei* W. M. Tattersall, 1914 [11, 12, 14, 22]. Among 11 reported species, *I. annandalei* is the most abundant species found from the four localities (Sandspit ( $24^0$  50' N,  $66^0$  56' E), Gizri, Hawksbay and Mauripur (Kazmi and Tirmizi, 1995). In addition to these, more new locations for *I. annandalei* were found during the recent study: Ghorabari ( $24^0$  34' 9N,  $67^0$  60' 8 E), Bhambore saltworks ( $24^0$  45' N,  $67^0$ 

55' E), Korangi Creek ( $24^0$  47'43' N, 67<sup>0</sup> 10'2 E) along Sindh Coast, and Pishukan Gwader ( $25^0$  72'8 N,  $62^0$ 49' E) along Balochistan coast [14].

The occurrence of I. annandalei is mainly tropical, until now it has not been reported in latitudes higher than 26<sup>0</sup> 4' N. It was first reported and described by Tattersall in 1914 from low salinity waters in the vicinity of Mumbai, India (19<sup> 0</sup> N) [31]. Its occurrence from Pakistan waters (25<sup>°</sup> N) was recorded by Kazmi and Tirmizi [13]. Murano [20] recorded it from Tarut Bay, Saudi Arabia  $(26^0 4' N)$ ; the most recent record is from Tubli Bay, Bahrain, Arabian Gulf (26<sup>0</sup> N) by Grabe et al., [7, 8]. I. annandalei remained the sole species of genus until recently when a new species, Indomysis nybini Biju & Panampunnayil, 2010, from the salt pans of Mumbai, India was described [1]. Apart from the records of its occurrence, only two biological studies have been conducted on this species. Kazmi and Tirmizi [13] presented population structure, seasonal abundance, breeding and embryonic stages of I. annandalei and Grabe et al., [7, 8] reported species composition and abundance of selected shallow water mysids of Bahrain waters including I. annandalei.

*I. annandalei* harms the growing Artemia pond industry [29]. Mysids were revealed to overwhelm Artemia in hypersaline ponds, feed on Artemia nauplii, multiply quickly and virtually replace all Artemia within a few days [29].

Despite the universally recognized multiple roles in the ecology of estuaries and aquaculture, mysids studied little in Pakistan waters. Present study was conducted to collect some new data on biology and ecology of *I. annandalei*, which are needed for environmental management and development of *Artemia* and mysid culture for their use as live food in aquaculture.

**Materials and methods.** For this study, specimens of *I. annandalei* were obtained from zooplankton samples collected from Ambro Creek, Ghorabari, Pakistan  $(24^0 \ 34' 9 \ N, 67^0 \ 60' 8 \ E)$ . The depth of water in the deepest part of Creek at high tide is approximately  $4.5 - 6.0 \ m$  whereas, at low tide  $0.9 - 2.4 \ m$ . Temperature and salinity were recorded twice a day, whereas, pH and dissolved oxygen occasionally. The air and surface water temperatures were recorded by a mercury thermometer; salinity was measured by a portable hand held salinity refractometer (ATAGO) calibrated by distilled water; pH and D.O. by pH meter and dissolved oxygen meter, respectively. The sampling was done with scoop nets (mesh size  $0.5 \ mm$ ) because

I. annandalei swarm along shallow mud banks, making operation of any trawl or zooplankton net difficult. For each sample, the net was pulled a distance of 200 m; a total of 114 samples were collected from November 2006 to October 2007. The samples were preserved initially in 10 % formalin and later transferred to 70 % ethanol after two weeks. I. annandalei was identified using the characters described by [13]; total body length was taken from the tip of the rostrum to the posterior end of the telson excluding apical spiniform setae. Specimens were categorized as juveniles (indeterminate sex), males, females with marsupia and females without marsupia. The number of larvae or eggs in marsupia was counted under a stereomicroscope. Young were categorized into one of five developmental stages [13]. For observations of feeding on Artemia, single live mysids were kept in separate beakers with different numbers (5, 10, 15, and 20) of Artemia nauplii to observe the number of larvae consumed by a single mysid in 24 hrs. An experiment was also conducted to determine the sizes of Artemia ingested by mysids. Similarly, to observe in day and nighttime the phenomenon of release of larvae, live ovigerous mysids were kept in glass beakers individually.

To determine the salinity tolerance of I. annandalei, three experiments were conducted. The first experiment was conducted in four 30 liter aquaria with 100 mysids in each aquarium. The first aquarium was kept as a control at 20 ppt and in the second, third, and fourth in aquaria, salinities were gradually increased: 5, 10, and 30 ppt /day, respectively, from an initial salinity of 20 ppt (the natural salinity of the creek at which they were collected) to 130 ppt. Freshly hatched Artemia nauplii were given as food. To determine the lethal limit of salinity for I. annandalei, a second experiment was conducted in small earthen ponds (25 m<sup>2</sup>) from 80 ppt onward. The salinity was gradually increased i.e., increase of approximately 10 ppt every day by adding sea salt to determine the lethal level of salinity. In third experiment, the intensity of salinity shock that was lethal for I. annandalei was determined in aquaria. Saturated brine of calculated quantity was added to increase the salinity by 20 ppt, 30 ppt, 40 ppt and 50 ppt simultaneously. To determine the lower lethal limit, salinity was gradually decreased 4 ppt each day by adding fresh water. The experiments were begun with 20 ppt salinity. Processing of all results was made with the use of standard statistical methods.

The Ambro Creek is an inshore creek in Indus delta and receives fresh water through drain canal discharged from agriculture lands. The fresh water is drained through regulatory gates during low tide, whereas, at high tide the regulatory gates are kept closed to prevent entry of the sea water to upland agricultural areas (Fig. 1). The Ambro Creek has a typical semi-diurnal pattern of tides, characteristic of the Pakistan Coast, and is replenished by seawater twice daily during high and low tides. The tidal amplitude is over 3 m. The creek is devoid of any aquatic vegetation. The sediment is compact, fine grained, and range between the fine sand and clay size fractions. The turbidity was apparently high resulting in low water clarity. Air temperatures were very close to water ones, differences was not more than  $\pm 1 - 2^{\circ}$ C. Salinity ranged from 4 to 38 ppt; water temperature from 13 to 39 <sup>o</sup>C; pH range was 8.0 to 8.5, dissolved oxygen (D.O.) in daytime fluctuated from 4.9 to 9 mg/L.

Results. Ecology. I. annandalei was in abundance in shallow inland coastal waters and creeks in a salinity range from 4 to 38 ppt. It was also found from coastal salt works up to 100 ppt salinity. Individuals I. annandalei are very active swimmers and move in swarms, mostly along the shallow banks in sandy muddy/muddy areas. They were found almost throughout the year (Table 1). except in August and the first week of September when salinity was less than 4 ppt due to the excessive freshwater discharge in the creek from land due to heavy monsoon rains. The population seems to be appeared in late September and progressively increased each month to reach the peak abundance during Jan-Feb, it declined from March onward with minimum numbers in July. The highest percentage of juveniles was found during September - October, and they were absent only in July - August (Fig. 2).

Sex was determined in specimens as from 3 - 4 mm in length. In the absence of a brood pouch, sex can be differentiated on the basis of fourth pleopod of males, which is long in male and much shorter and hook-like in females. The brood pouch is developed in females of >5.0 mm total length. The size of the smallest juveniles in the sample was 1.0 mm, which also verifies the size at which larvae released.

20692 specimens of I. annandalei, having a size range 1.0 - 9.7 mm were collected and analyzed according to the above-mentioned procedure (Table 1). On average there were 33 % (6867) of males, 53 % (10865) of females, and 14 % (2960) of indeterminate juveniles (Fig. 2). Females predominated in the majority of the samples. The average male to female ratio was 1:1.58 (limits -1.14 - 2.55). Males ranged in size from 3.5 to 8.0 mm; females 3.0 - 9.7 mm and juveniles from 1.0 - 4.0 mm. Females had a larger length than the males in all seasons, on average on 10 %. The number of ovigerous females was higher than the non-ovigerous females during September to April (Fig. 3), what shows the peak breeding activity during the period. The overall size range was 1.0 - 9.7 mm. Fig. 4 shows length frequency distribution of I. annandalei from November 2006 through March 2007. The size of individuals in the spring population was significantly larger than those of the autumn population (p < 0.5).

During November and December 2006, the population was dominated by 1 mm size class, whereas, during January 4 – 5 mm size class dominated in the population and from February through March, 5 – 6 mm size classes were in abundance. There were fewer individuals in size classes 7 – 8 mm, whereas, individuals of  $\geq$ 9 mm were found only in May 2007.

Larvae. The entire larval development of *I. annandalei* is completed within the marsupium; the next development stages were recognized in the present samples: the embryonic development (stage I), early nauplioid stage (stage II), early postnauplioid stage (stage III), late postnauplioid stage (stage IV).

At the first stage the embryo is more or less egg like, oval in shape, creamy white in color and is composed of yolk globules which are roughly polygonal in shape packed in a thin egg membrane; the size of embryo is 0.35 - 0.45 mm. Early nauplioid larva is bent ventrally to form a perfect comma shape body (Fig. 5).



Fig. 1 The studied area: satellite image (A); Ambro Creek at low tide (B) and at high tide (C); drain canal (D) Рис. 1 Район исследований: спутниковый снимок (A); Ambro Creek в отлив (B) и прилив (C); дренажный канал (D)



Fig. 2 Sex-age structure of annandalei Ι. in Ambro population Ghorabari, Creek, Pakistan during study Рис. 2 Половозрастная структура популяции Ι. annandalei в Ambro Creek, Ghorabari, Пакистан в период исследований

Month	Total number	Size range	Male number	Male Size range	Female number	Female Size range	Juven. number	Juven. Size range	Sex Ratio, F/M
Nov-06	1555	1.5 - 7.0	487	3.5-7.2	820	3.1-7.2	248	1.3-4.0	1.68
Dec- 06	1929	1.0 - 8.2	441	3.9-8.2	1125	4.0-8.2	360	1.2-3.5	2.55
Jan- 07	5976	1.2 - 8.5	2305	4.0-7.5	3016	4.0-8.5	655	1.2-4.0	1.31
Feb-07	6148	1.2 - 8.5	1923	4.5-8.0	3165	4.1-8.5	1060	1.2-3.0	1.65
Mar-07	987	1.2 - 8.0	356	4.9-7.0	474	4.6-8.0	157	1.2-2.0	1.33
Apr-07	683	1.2 - 7.5	278	4.9-7.0	316	4.1-7.5	89	1.2-2.0	1.14
May-07	1355	2.5 - 9.7	410	3.7-6.9	910	4.2-9.7	35	1.3-3.5	2.22
Jun-07	520	3.2 - 6.9	210	3.5-4.0	300	4.4-6.9	10	1.2-3.0	1.43
Jul-07	101	3.5 - 7.1	45	3.5-6.5	59	4.0-7.1	0	0	1.31
Aug-07	4	4.0 - 6.4	3	4.0-5.9	1	4.5-6.4	0	0	1.31
Sep-07	679	1.0 - 6.7	202	4.0-6.0	332	4.5-6.7	145	1.0-2.0	1.64
Oct-07	755	1.0 - 6.8	207	4.0-6.0	347	4.5-6.8	201	1.0-3.0	1.68

Table 1 Population structure of *Indomysis annandalei* in Ambro Creek during November 2006 to June 2007 Табл. 1 Популяционная структура *Indomysis annandalei* в Ambro Creek (ноябрь 2006 – июнь 2007)



Fig. 3 The proportion of ovigerous and nonovigerous *I*. annandalei females (Ambro Creek, Ghorabari, Pakistan) Рис. Соотношение 3 самок І. annandalei c личинками и без них (Ambro Creek, Ghorabari, Пакистан)

The late nauplioid stage is characterized by almost complete segmentation of thorax and abdomen; the abdomen is quite distinct and eyes clearly pigmented at this stage. The larval cuticle missing. The early postnauplioid stage is recognized by the presence of eyestalks and free thoracic appendages, uropods and telson. At the late postnauplioid stage the appendages are quite developed and larvae look like miniature adults. Larvigerous females are found during almost every month. The number of larvae in the marsupium ranged from 5 to 20, whereas 14 - 17 larvae were found most frequently, very few females (2.43 %) had 20 larvae in their brood pouch (Fig. 6). The smallest brood (N = 5 - 10) found in size class 4.0-5.0 mm and largest brood (N = 15 - 20) were Морський екологічний журнал, № 3, Т. Х. 2011

those in size classes 6.5 mm to 8.5 mm. The number of produced larvae has a positive correlation with the total length of female ( $R^2$ =0.90). This relation can be approximated by linear regression:

$$N = 2.39 + 0.30 L,$$

where N - number of larvae in marsupium, L - female size, mm.

Release of larvae from marsupium takes place at night. The female spreads the lamellae laterally to release the larvae one by one; 4 - 7larvae released at a single time from the brood pouch under the laboratory conditions. The larvae were not active until they moult the early postnauplioid stage. The moulted mysids are miniature adults, 1.2 - 1.5 mm in body length.





<u>Feeding.</u> Under the laboratory conditions, an adult *I. annandalei* can ingest *Artemia* nauplii and small metanauplii of 500 - 700 micron size, i.e. newly hatched *Artemia* nauplii to three-day old larvae only. The size of *Artemia* larvae on the fourth day becomes too large for ingestion. The average number of *Artemia* nauplii consumed by a single mysid was 8 - 10 /day (24 hrs).



Fig. 4 Length frequency distribution of *I. annandalei* from November 2006 through March 2007

Рис. 4 Частотное распределение особей по размерным группам в популяции *I. annandalei* с ноября 2006 по март 2007 гг.

Fig. 5 Early nauplioid (A) and late postnauplioid larvae stage (B) of *I. annandalei* Рис. 5 Ранняя науплиальная стадия (A) и поздняя постнауплиальная личиночная стадия *I. annandalei* 

Salinity tolerance. According to the first salinity tolerance experiment in glass aquaria, it is observed that the gradual increase of salinity by 5 – 10 ppt/day is well tolerated by *I. annandalei* and no negative effect was observed until 130 ppt salinity reached. The movement of individuals slowed down considerably and feeding stopped at <130 ppt, however, they survived up to 96 hours.



Fig. 6 Frequency distribution of larvae number in the marsupium of L annandalei Рис. 6 Частотное распределение количества личинок в марсупиуме самок *I*. annandalei

According to the second experiment in ponds, increase of salinity was 10 ppt/day from 80 ppt onward; the lethal limit of salinity was 130 ppt. In the third experiment the salinity shock of 50 ppt was most effective in killing the mysids, but even after increasing the salinity abruptly from 80 to 130 ppt, it took three hours to kill the mysids. The lower limit of salinity, determined by gradually lowering the salinity by addition of fresh water, was 4 ppt. The lower limit of 4 ppt salinity was also verified by regular sampling from creek. These results are in good accordance with abovementioned field data.

Discussion. I. annandalei inhabits shallow coastal waters and inland creeks, mostly in small channels, where water action is not much strong. Kazmi and Tirmizi [13] discussed their presence in protected areas and complete absence in the plankton samples from Manora channel, most probably due to the strong water movement prevailing in the area. They also reported the occurrence of I. annandalei in hypersaline waters of 45 - 50 ppt salinity. The salinity recorded at the three new locations i.e., Bhambore, Korangi Creek, and Pishukan from Pakistan coast is ranged from 55 to 100 ppt [14]. I. annandalei were found from the seawater supply canals to salt works at Bhambore and Korangi Creek (90 - 100 ppt) whereas at Pishukan they were found in shallow inshore waters (45 ppt) and experimental ponds for penaeid shrimps (55 ppt).

Taking into account these data as well as

our experimental ones we conclude that *I. annandalei* is one of the most extremely euryhaline and eurythermal species among mysids – total salinity tolerance from 4 to 130 ppt and temperature – from 13 to  $39^{\circ}$ C.

The overall dominance of females over males is also an interesting feature not reported previously by Grabe et al [8] possibly due to the low density of species. Kazmi and Tirmizi [13] reported the dominance of females over males in January-February 1987 and vice versa in November-December 1986, whereas juveniles dominated adults in March 1987. They also observed the population during 1993/94 when in four samples from five males dominated during December 1993 to February 1994. During the present study, the considerable number of I. annandalei were found almost throughout the year except in August and the first week of September when salinity was below 4 ppt, though the number reached its peak in January-February in inshore creeks, shallow coastal waters and salt works. Tirmizi [13] documented Kazmi and the appearance of population in November that reached its maximum in December; January-February being the breeding months, whereas the species started disappearing in early March and completely disappeared by the end of the month. Grabe et al [8] presented a report on shallow water Mysids of Bahrain, from Tubli Bay, Arabian Gulf presented the occurrence and relative and abundance of I. annandalei, the occurrence of larvigerous females. They reported an overall low abundance and maximum number during May 1992 associated with maximum number of larvigerous females and an overall male to female ratio of 1:1. There are no principal differences in population dynamics of *I. annandalei* in different localities. Taking into account all these data we conclude that sex ratio in *I. annandalei* as well as its population dynamics are under environmental control.

Gut content revealed only the digested organic material and detritus in preliminary studies. Feeding is accomplished by grabbing the food with the gnathopods. Our data show that small crustaceans, such as *Artemia* nauplii, also can play important role in *I. annandalei* feeding.

Mysids have been recognized for their important role as live food organisms in aquaculture, aquarium, and in toxicity testing as it is mentioned above. *I. annandalei* appears to be a

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- 7. *Grabe S. A., Price W., Abdulqader E.A A.* Shallow water Mysida of Bahrain (Arabian Gulf): species composition, abundance and life history parameters

suitable organism for culture due to its high tolerance to temperature and salinity, its nutritional value (protein 18.2 % on wet weight basis [6]) and its occurrence almost throughout the year. On the other hand, *Artemia* growing is not possible at salinity below 130 ppt due to *I. annandalei* presence. As a matter of fact both *Artemia* and *I. annandalei* are important as live food organisms in the aquaculture and aquarium trades, but commercial cultivation for these species must be developed separately.

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Поступила 22 ноября 2010 г. После доработки 24 февраля 2011 г. Еврітермальна і евригалинна креветка-опосум *Indomysis annandalei* W. Tattersall, 1914 (Mysidacea: Mysidae) з прибережних вод Пакистану. Р. Султана, К. В. Казмі, М. Насір, Ф. Амір, В. Алі, М. В. Шадрін. *Indomysis annandalei* збирався в Ambro Creek дельти Інду, Пакистан, з листопада 2006 по жовтень 2007. Всього піймано 20647 рачків розміром от 1 до 9.7 мм. Серед них: 33 % – самці, 53 % – самки, 14 % - невизначена за статтю молодь. Розмірний діапазон самців – 3.5 - 8.0 мм, самки – 3.0 - 9.7 мм і молоді – 1.0 - 4.0 мм. Самки переважали у всіх пробах – 1 : 1.58, їх розмір в середньому на 10% більше, ніж у самців. Личинковий розвиток повністю завершується в марсипіуме. Всього виділяється п'ять стадій розвитку *I. Аппаndalei*. Кількість личинок в марсипіуме – від 3 до 18, позитивно корелює з розміром самок. В період вивчення физико-хімічні параметри змінювалися в межах: солоність – 4 - 38 ppt, температура – від 13 до 39 °C, ph – 8.0 - 8.5, розчинений кисень – від 4.9 до 9.0 мг/л. Експериментальне визначення соленосної толерантності дало наступні межі – від 4 до 130 ppt. Із-за хижацтва *I. annandalei* культуру артемій можна успішно розвивати в Пакистані лише при сольностях вище 130 ppt.

Ключові слова: мізиди, *Indomysis annandalei*, розмірно-возрастна структура, полова структура, соленосна толерантність

Эвритермальная и эвригалинная креветка-опоссум *Indomysis annandalei* W. Tattersall, 1914 (Mysidacea: Mysidae) из прибрежных вод Пакистана. Р. Султана, К.В. Казми, М. Насир, Ф. Амир, В. Али, Н.В. Шадрин. *Indomysis annandalei* собирали в Ambro Creek дельты Инда, Пакистан, з ноября 2006 по октябрь 2007. Всего отловлено 20647 рачков размером от 1 до 9.7 мм. Среди них – 33% – самцы, 53% – самки и 14% – неопределенная по полу молодь. Размерный диапазон самцов – 3.5 - 8.0 мм, самок – 3.0 - 9.7 мм и молоди – 1.0 - 4.0 мм. Самки преобладали во всех пробах – 1 : 1.58, их размер в среднем на 10% больше, чем у самцов. Личиночное развитие полностью завершается в марсипиуме. Всего выделяется пять стадий развития особей. Количество личинок в марсипиуме колебалось от 3 до 18, положительно коррелируя с размером самок. В период изучения физико-химические параметры изменялись в пределах: соленость – 4 - 38 ppt, температура - от 13 до 39 °C, pH – 8.0 - 8.5, растворённый кислород – от 4.9 до 9.0 мг/л. Экспериментальное определение солёностной толерантности дало следующие пределы – от 4 до 130 ppt. Из-за хищничества *I. annandalei* культуру артемий можно успешно развивать в Пакистане только при солёностях выше 130 ppt.

Ключевые слова: мизиды, *Indomysis annandalei*, размерно-возрастная структура, половая структура, соленостная толерантность