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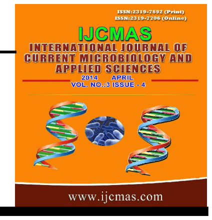

Original Research Article

Reproductive biology of eastern mosquito fish *Gambusia holbrooki* (Girard) (Poeciliiadae) in a sub-tropical Lake, Lake Nainital (India)

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A B S T R A C T

The reproductive biology of introduced Poeciliidae, *Gambusia holbrooki* (Girard, 1859), was studied over a period of two year from 2005-2007 in Lake Nainital, India. Reproductive cycle of *Gambusia holbrooki* in Lake Nainital extended from April to October during both the years. There was no marked difference in sex ratio and size distribution of female, male and juvenile fish between two years. The smallest YOY male *Gambusia* with gonopodium was 19 mm and smallest OW male was 22 mm. The smallest YOY female *Gambusia* with embryos was 17 mm and smallest OW female was 24 mm. Largest senile OW female was 56 mm. Maximum pregnancy percentage was found in August (80 %) and July (66.7 %) during first and second year respectively. The maximum adjusted fecundity was observed in month of July during both the years. Large size and small size OW females produce five broods during breeding season whereas YOY produce two to three broods. The peaks of G.S.I. of both female and male were observed in July during both years. Water temperature and photoperiod both plays important role in determining the reproductive cycle of *Gambusia holbrooki* in Lake Nainital. The population of *Gambusia holbrooki* has two generations in Lake Nainital and parent generation is replaced by its young one's but not completely therefore partly bivoltine.

Keywords

Reproductive biology; *Gambusia holbrooki*; Lake Nainital.

Introduction

Gambusia holbrooki (Girard), commonly called as mosquitofish, is a small, viviparous fish. This is a native to the eastern U.S.A. and has been introduced to various waters bodies worldwide as a mosquito control agent (Krumholz, 1948; Courtenay and Meffe, 1989). In India, the fish was brought from Italy by Dr. B. A. Rao in 1928 (Sharma, 1994). In Lake Nainital, it was introduced by Malaria Control Department in nineteen nineties (Nagdali and Gupta, 2002). Although, the fish was supposed to be useful biological agent in the past (Wilson, 1960). Recent studied have indicated the negative impacts on aquatic biodiversity (Milton and Arthington, 1983; Arthington and Lloyd, 1989; Rupp, 1996; Howe et al., 1997; Ivantsaff and Aran, 1999; Komak and Crossland, 2000, Hammer et al. 2002; Ling, 2004; Pyke, 2008; Segev et al., 2009; Stanback, 2010; Buttermore et al., 2011). In some studies it has also been found that the fish may indirectly encourage the growth of mosquito larvae by feeding on cladocerans, which were competitor of mosquito larvae (Blaustein and Karban, 1990). Although several extensive studies on the reproductive biology of *Gambusia* have been made in natural (Krumholz, 1948) and introduced areas in abroad (Brown and Fox, 1966; Sawara, 1974), the comprehensive studies on reproductive biology of this fish, has not been carried out in India. The aim of present study was thereby to provide information on life history characteristics and reproductive properties of *Gambusia* in Lake Nainital.

Study area

Lake Nainital is one of the National Lakes of India. It is situated at 1937m above sea level (29°24′ N latitude and 79°28′ E longitude) and is a subtropical water body. Based on thermal criteria, it is a warm monomictic lake undergoing circulation during winter months. The surface water temperatures range from 9° C in winter to above 24° C in summer. The surface area of the lake is 48h, mean depth 16.2m, shoreline development 1.20, and Catchment area, 3.96 km^2.

Materials and Methods

Sampling and Data collection

Fish were collected fortnightly from three selected sites (Fig.1) in Lake Nainital with the help of long handle hand net of 1mm mesh size. The captured fish were preserved in 4% formalin in wide mouth plastic jars and brought to the laboratory for analysis. The Standard length (S.L.) of all fish was determined using graph paper. All measurements referred in the text were standard lengths. Males and females were identified and separated on the basis of sexual dimorphism. Mosquitofish were categorized as males if they possessed any evidence of a gonopodium, as females if they possessed rounded anal fin. Sexes of the fish smaller than 15 mm could not be determined, therefore, they were regarded as juveniles. Females were considered gravid if they possessed developing ova and as pregnant if they possessed eyed embryos (Haynes and Cashner, 1995). Sex ratios were calculated as the number of female per male, based on above mentioned sex determination criteria. Length frequency histograms were plotted as 4 mm size classes for female, male and juvenile's fish.

Each whole fish was weighed and the gonads removed from the body cavity and weighted to give the wet weight (±10mg) using an Anamed Electronic balance. Embryos from each female were allocated to a developmental stage following Milton and Arthington (1983) and counted. To collect different developmental stages during the study period frequency of sampling was increased (weekly) in the months of May, June, July and August. The gonadosomatic index, GSI, was calculated by expressing the ratio of gonad weight to total wet weight as a percentage.

Adjusted fecundity (AF), size at maturity for both YOY and OW male and female fishes, female reproductive life spans and the length of the reproductive season of the fish was determined following Haynes and Cashner, 1995. Surface water temperature of Lake was recorded directly by using a multiparameter YSI 600 XL probe with display system and data of photoperiod was provided by the Aryabhatta Research Institute of Observational-Sciences (ARIES), Nainital.

Data presentation and statistics

Monthly data were presented in all the analyses (mean of three selected sites + fortnight mean). Student's test (t-test) was used to determine the significance of difference in G.S.I. values between months. A Chi-square goodness of fit test showed deviations of sex ratio from 1:1. All analyses were performed using Microsoft Excel Programme (Window's XP Professional).

Results and Discussion

Sex ratio

The monthly variation in the sex ratio of *Gambusia* is shown in Table 1. The sex ratio varied from 1:1 (January) to 1:5.1 (July) during first year while it ranged from 1:1.1(March, May, August) to 1:2.7 (October) during the next year (Table 1). The sex ratio for the pooled monthly observations was highly significant ($p<0.01$) during first year with 1.5 female per male and but not significant during second year with 1.09 female per male

Size distribution

Length frequency distribution histograms of females showed almost similar pattern during both years therefore only one year data is shown here (Fig. 2).

Large sized females dominated from September to December while small sized dominated in January and February. The small size fish of January and February gradually increased in size from March onwards and gained maturity in April. Again small females dominated the samples in August. These were borne in June.

The length frequency distribution histograms of males (Fig. 3) and juvenile (Fig. 4) also showed similar pattern during both years.

Large sized males dominated most of the time during the two-years study. Small sized males dominated in the month of August during first year and in the month of February and August during second year (Fig. 3). Juveniles were present during June to October in both years, however, they were also collected in January of the first year and January and February of the second year (Fig. 4).

The smallest size of mature YOY female was found to be 17 mm (June, 2006-07) and smallest mature OW female was found to be 24 mm (April, 2006-07). However, in our winter catch most of the large OW females were found with degenerated ovary and could be regarded as senile females (Fig. 2). The largest senile OW female was of 56 mm (December, 2005-06) during the period of two years study.

The smallest size mature YOY male was 19 mm and was found in August 2006. Smallest and largest OW males were found to be 22 mm (January of both years) and 30 mm (November and December of both year respectively). However, some OW males were found in February and March during both years which were still immature at the length of 24 mm (gonopodium still developing).

Pregnancy percentage

It gradually declined from September to April during both years (Fig. 5). A sharp increase in pregnancy percentage was noticed thereafter till August. The maximum percentage of pregnancy was found in the month of August (80 %) during first year and in July (66.7 %) during the second year (Fig. 5).

Fecundity

Adjusted fecundity showed a decline from September to February and thereafter it started increasing gradually till August during both the years (Fig. 5). The maximum fecundity was found in July during both the years (Fig. 5).

Gonadosomatic index (G.S.I.)

The gonadosomatic index of female fish started to increase from March onwards till July (Fig. 6). The values of G.S.I. remained high till September. Thereafter, it started to decrease and attained the minimum level in February. The values were markedly high from May to September and low from October to April (Fig. 6). This trend was noticed during both years. The G.S.I. of male fish showed trend similar to that of female (Fig. 6).

Stage of development of embryos

Seasonal variations in the proportions of each embryonic development stages in *G. holbrooki* females was shown in Figures 7. It followed almost similar trend during both years i.e. why only one year data were presented here. Seasonal shifts in the developmental stages in *G.holbrooki* suggested that 5 broods were produced each year. Reproductive cycle extended from April to October during both the years. Gestation period varied from 24 to 28 days as suggested by observations of embryonic developmental stages.

Length/Weight relationship with fecundity

There was a significant co-relation ($r = 0.50$; $p<0.01$) between the length and fecundity of the fish (Fig. 8). Also, the weight-fecundity data were significantly co-related ($r =.0.57$; $p<0.01$.) (Fig. 8).

Sex ratio of any population may differ from situation to situation. At the time of birth the ratio may remain 1:1 but may change thereafter due to selective mortality or different habitat preference of males or females (Fernandez-Delgado, 1989; Fernandez-Delgado and Rossomanno, 1997). In some studies males were found to dominate over females due to heavy mortality of females. The female's mortality was attributed to their larger size, slower movement and better perceivability (Fernandez-Delgado and Rossomanno, 1997). However in some investigations females have been found to dominate over males because of short life span of males (Krumholz, 1948; Fernandez-Delgado, 1989; Vargas and de Sostoa, 1996). In our observation in Lake Nainital, females dominated over males. The males mortality could be attributed to their shorter life span and (or) increased competition with juveniles for food. During gut analysis it was found that food of males of *G. holbrooki* appeared to be more sensitive to environmental stress than females. Extreme temperature, overcrowding, starvation (Krumholz, 1948) and hypoxia (Cech et al. 1985) have been found to be responsible for heavy mortality of males. Besides these, some workers believe that males die to reduce the competition with juveniles for food, as females store sperms for a long time after they were inseminated (Krumholz, 1948) thus males were not required after the females were inseminated. In our observation also, female were harder than male and dominated them most of the part of the study period (Table 1).

Discrepancies exist in the literature for minimum standard length at first reproduction and reproductive season for female mosquito fish (Haynes and Cashner, 1995). These results were exactly similar to Barney and Anson (1921) (natural population) as minimum S.L. at first reproduction of OW female was 24 mm and that of YOY was 17 mm. The length of reproductive season, i.e. from April to October, were consistent with Haynes and Cashner (1995) (natural population) and Krumholz (1948) (introduced population) (Haynes and Cashner, 1995).

In the present study, breeding period of *G. holbrooki* varied from April to October during both the years (Fig. 5 and Fig.6). It released its first brood in the lake in the month of June (Fig.7). The YOY female born early in the breeding season (2005-06), grew at a very fast rate and attained maturity within two months at smaller sizes to take part in reproduction. YOY females after spawning two or three times died in November and December (2005-06). Those, which survived, lived as large overwintered females (OW) with degenerated ovaries (senile) during winter and prepared themselves for participating in next breeding season in April (2006-07). The YOY female born late in the breeding season did not take part in reproduction and grew very slowly and survived as small overwintered females. They attained maturity by April and constituted the major portion of parent stock of the next breeding season (2006-07). Females of parent stock (2005-06), which gave birth to juveniles from June to October, died after breeding season in winter. Haynes and Cashner (1995) also found similar results in their study.

Hubbs (1971) and Daniels and Felley (1992) reported that adult males born early in the breeding season also matured rapidly at smaller sizes, while those born later in the summer exhibited slower growth, achieving much larger sizes. Early in the reproductive season, males matured quickly so as to begin reproduction as soon as possible; at the end of the season, larger sizes were advantageous to increase the probability of overwintered survival. Males that grow faster and mature at smaller sizes also make a trade-off: they were capable of reproducing sooner but do not live as long as larger, slower growing males (Haynes and Cashner, 1995). In the present study, YOY males matured quickly to participate in reproduction and died within the same season after fertilizing the females. YOY males born late in the breeding season did not take part in reproduction. They attained larger size (Figs. 3) to overcome winter and reproduce in next breeding season.

Gambusia females may have multiple broods over a single breeding season, with older and larger females having more broods during the breeding season than younger females (Pyke, 2005). According to Minckley (1973), a single female may produce upto about five broods in her lifetime. Six consecutive broods during one breeding season have been observed on a number of occasions (Milton and Arthington, 1983), and the maximum observed number of broods per female per season has been nine (Milton and Arthington, 1983). Krumholz (1948) and

Fig.1 Location of Lake Nainital (After Valdiya, 1988). Dots showing the three sampling sites

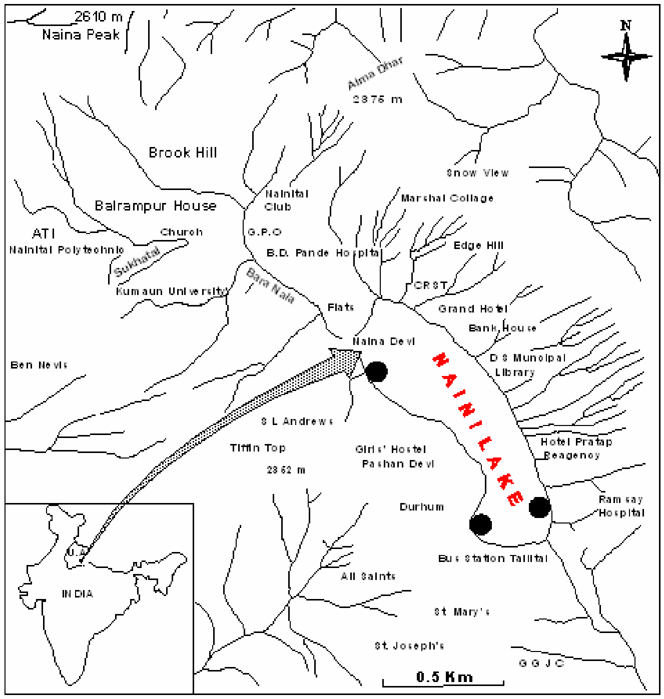


Table.1 Monthly variation in sex ratio of *G. holbrooki* during 2005- 06 and 2006-07 in Lake Nainital

Months	Male/Female	Chi-square calculated value
Sep.,05	1 : 2.0	2.34
Oct.	1 : 1.6	0.68
Nov.	1.2 : 1	0.08
Dec.	1.6 : 1	0.08
Jan.,06	1 : 1	0.32
Feb.	1 : 1	0.12
Mar.	1 : 1.4	0.86
Apr.	1 : 1.8	2.12
May	1.1 : 1	0.32
Jun	1 : 2.1	2.90
Jul	1 : 5.1	8.00**
Aug.,06	1 : 4.5	4.44*
Yearly	**1 : 1.5**	8.92**
Sep.,06	1 : 2.2	1.80
Oct.	1 : 2.7	3.26
Nov.	1 : 2.0	1.07
Dec.	1.7 : 1	0.43
Jan.,07	1.3 : 1	0.20
Feb.	1.4 : 1	0.66
Mar.	1 : 1.1	0.40
Apr.	1.6 : 1	1.09
May	1 : 1.1	0.40
Jun	1 : 1.4	0.38
Jul	1 : 1.7	1.8
Aug.,07	1 : 1.1	0.40
Yearly	**1 :1.09**	0.43

*p<0.05; **p<0.01

Fig.2 Seasonal pattern of length-frequency distribution of female *Gambusia holbrooki* during 2005-06

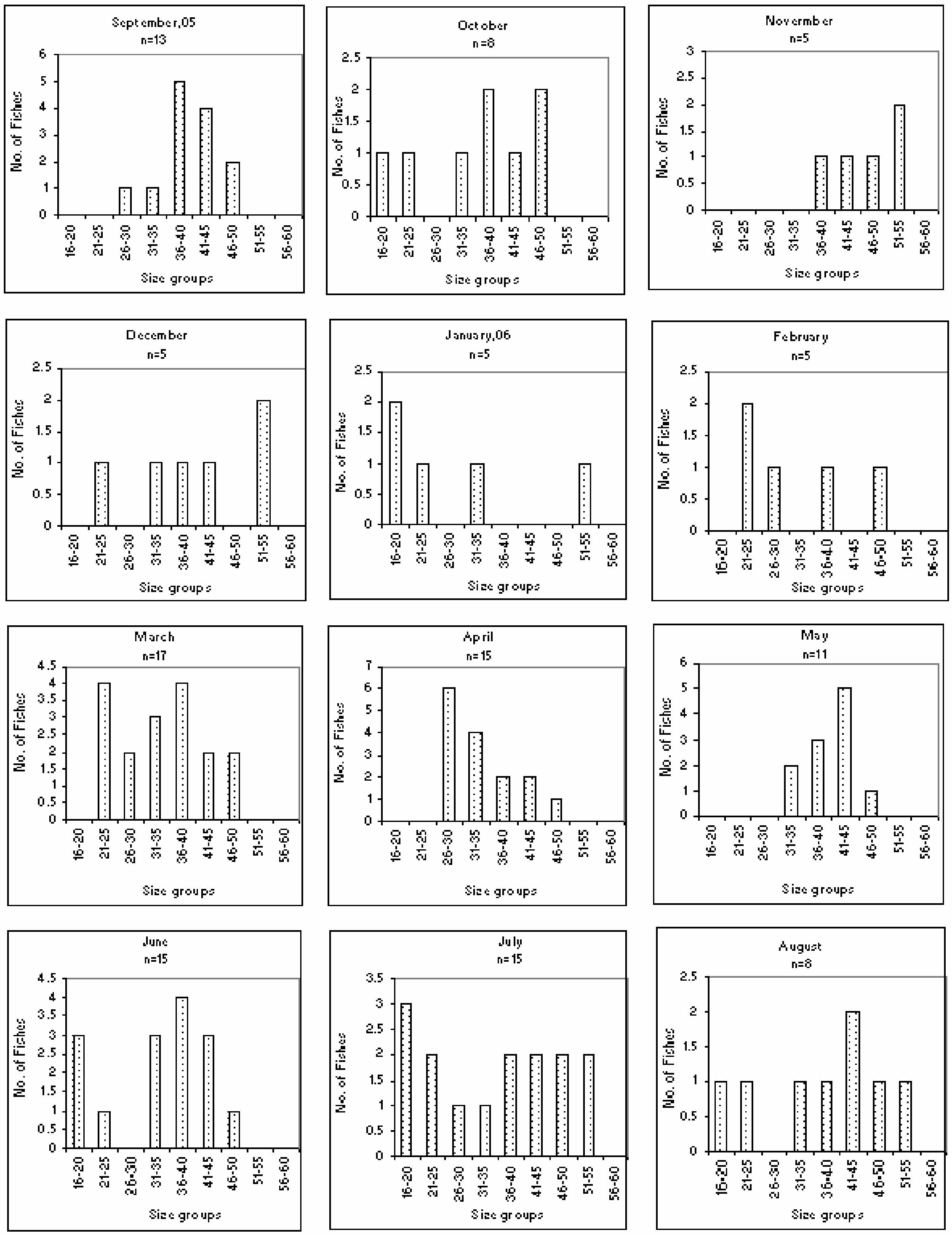


Fig.3 Seasonal pattern of length-frequency distribution of male *Gambusia holbrooki* during 2005-06 and 2006-07

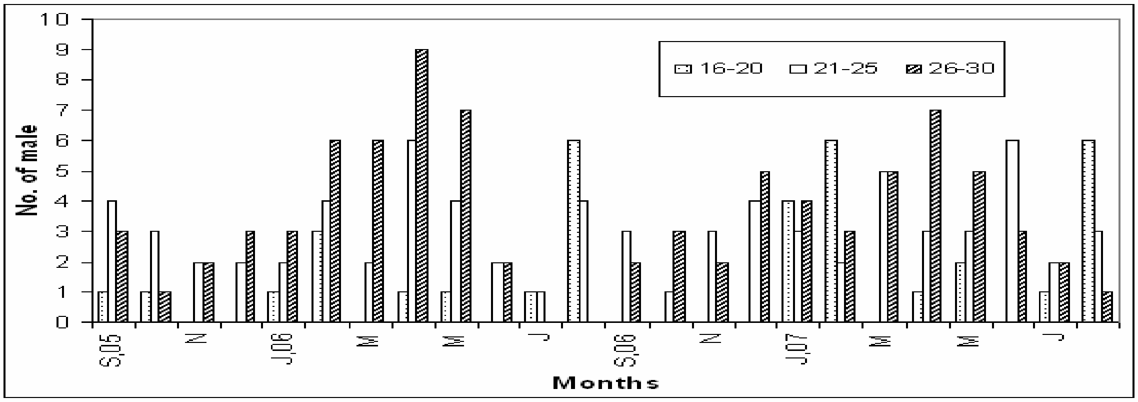


Fig.4 Seasonal pattern of length-frequency distribution of juvenile *Gambusia holbrooki* during 2005-06 and 2006-07

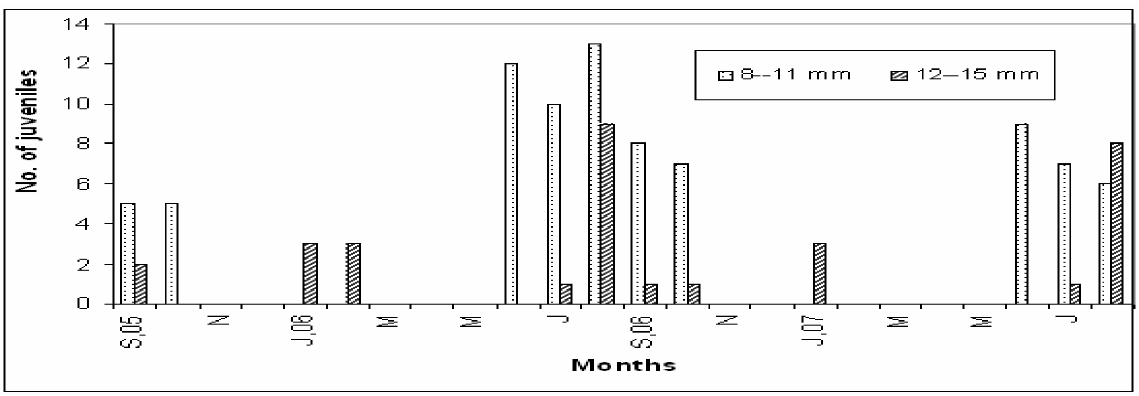


Fig.5 Seasonal variation in pregnancy percentage and adjusted fecundity of female *G. holbrooki*during 2005-06 and 2006-07.Vertical bars represent mean ± standard deviation

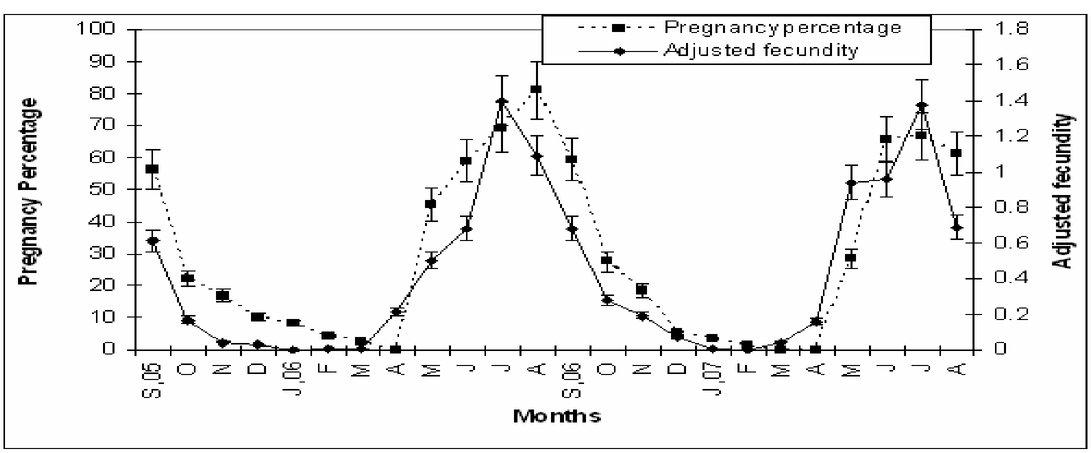


Fig.6 Seasonal variation in gonadosomatic index (GSI) of female and male *G. holbrooki* during 2005-06 and 2006-07. Vertical bars represent mean ± standard deviation

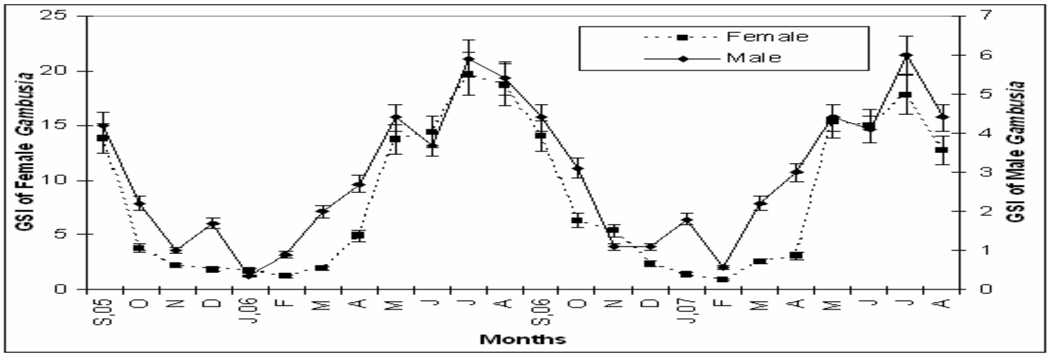


Fig.7 Seasonal changes in numbers of female fish bearing embryos in each embryonic development stage during 2005-06 N. A. (Not available)

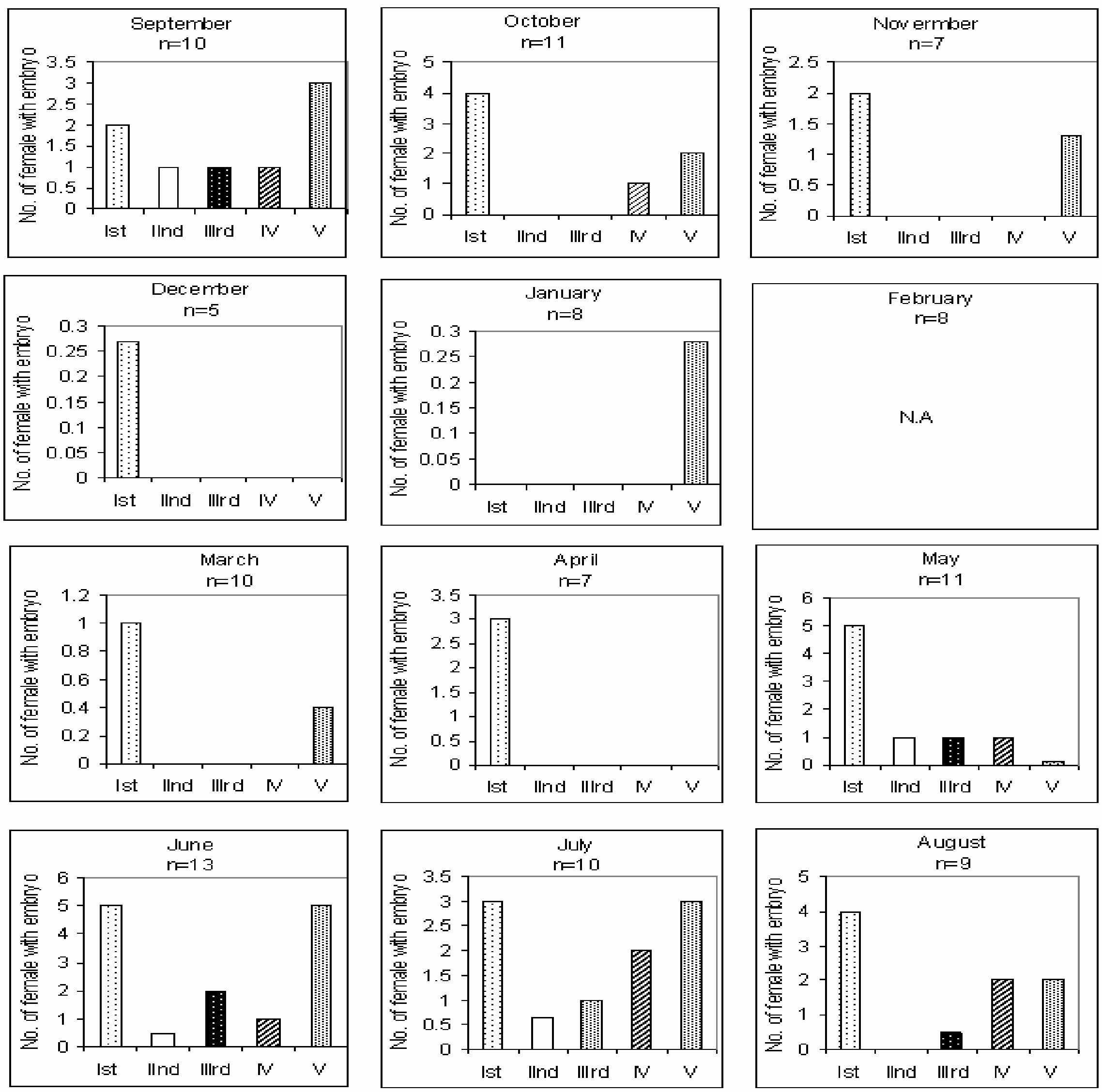


Fig.8 Relationship between fecundity and length and fecundity and weight of *G. holbrooki* in Lake Nainital

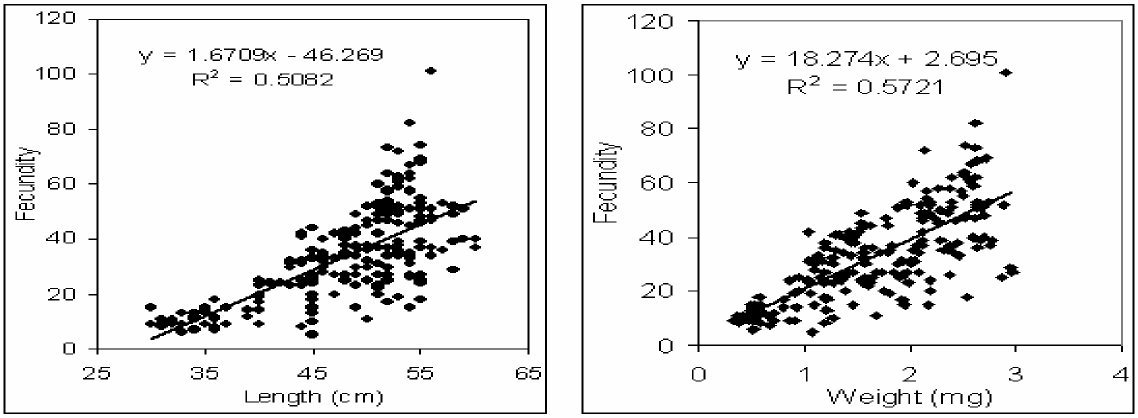
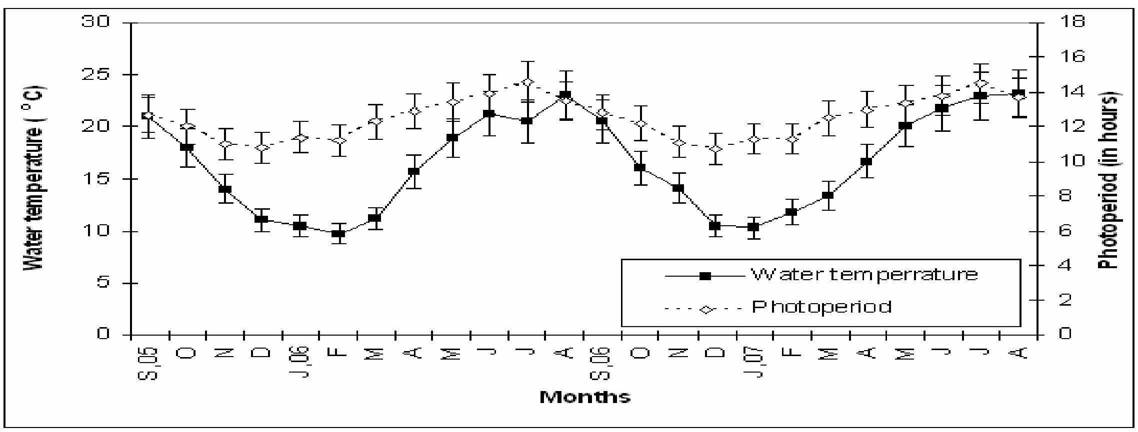


Fig.9 Seasonal variation in water temperature (surface water) and photoperiod of Lake Nainital during 2005-06 and 2006-07.Vertical bars represent mean ± standard deviation



Maglio and Rosen (1969) found that females that were breeding in their second season had 4-5 broods, whereas smaller young of the year females generally produced no more than two broods. In the present study, gestation period ranged from 24 to 28 days. Considering the gestation period of 24-28 days and examining the Figure 6, the pattern of change in the developmental stages suggested that in Lake Nainital a maximum of 6-7 broods were produced by parent female. The YOY female produced 2-3 broods while the large OW females (which had bred already in the previous breeding season) produced 2-3 additional broods and then died early in the breeding season. Thus, the large OW females produced 8-10 (6 to 7 + 2 to 3) broods in their life span. The decrease in brood size of overwintered females was observed in the present study. This could be due to physiological changes in the mother with aging (Krumholz, 1948). The fecundity and embryo size were functions of mother's size. A common pattern was seen in most studies that YOY born early in the season mature quickly (at smaller sizes) but their initial brood sizes (number of young ones produced) were small, while those born late in the season grow more slowly and mature at larger sizes and thus can carry much larger broods (Krumholz, 1948; Haynes and Cashner, 1995; Fernandez-Delgado and Rossomanno, 1997). In the present investigation, the number of broods produced by OW and YOY females was perhaps associated with reproductive life span (Haynes and Cashner, 1995).

Clutch size in *Gambusia* varies considerably from habitat to habitat. In some studies average clutch sizes have ranged from 5 to over 100 (Krumholz, 1948, Brown-Peterson and Peterson, 1990). The minimum clutch sizes have ranged from 1-84 (Smith, 1912; and Gall et al, 1980) and the maximum from 10 to 375 (Krumholz, 1948; Trendall, 1982) in other studies. In the present investigation, clutch size ranged from 3-120 with an average of 40 and 35 young, being produced during breeding season in the first and the second year, respectively.

A search in literature reveals that temperature and photoperiod period play important role in breeding of *Gambusia.* For example, Medlen (1951) found that reproduction could be stimulated in *Gambusia* at temperatures above 15.5 ^{0}C. In the present investigation, temperature remained above 15.5 ^{0}C during the breeding season (April to October) (Fig. 9). The fish ceased reproduction in winter when water temperature fell below 15.5°C. Some authors were of the view that the timing of the reproductive cycle in mosquito fishes was governed by photoperiod (Sawara, 1974; Milton and Arthington, 1983; Haynes and Cashner 1995). Similar to these observations the reproductive cycle in *G. holbrooki* in Lake Nainital occurred when day length exceeded 11-13 hours (Fig. 9). In the light of these data, 2 different generations of mosquito fish propagate in one reproduction period. However, because not all of the new generations matures and takes part in reproduction, the mosquito fish was considered to be a partly bivoltine species (Fernandez-Delgado, 1989; Fernandez-Delgado and Rossomanno 1997;).

These results were consistent with other studies (Krumholz, 1948; Trendall, 1982; Fernandez-Delgado, 1989) who reported that mosquitofish populations contain two age groups; after first reproduction, and the parental generations disappear and are replaced by their young ones.

References

Arthington, A.H. and Lloyd, L. N., 1989. Introduced Poeciliidae in Australia and New Zealand. In Meffe, G. K. and Snelson, F. F. (eds.), Evolution and Ecology of Livebearing Fishes (Poeciliidae). Prentice-Hall, New York: 333-348.

Blaustein, L. and Karban, R., 1990. Indirect effects of the mosquitofish *Gambusia affinis* on the mosquito *culex tarsalis*. Limnology and Oceanography 35: 767-771.

Brown, C. J. D. and Fox, A. C., 1966. Mosquitofish (*Gambusia affinis*) in a Montana pond. Copeia 3: 614-616.

Brown-Peterson, N. and Peterson, M. S., 1990. Comparative life history of female mosquitofish, *Gambusia affinis*, in tidal freshwater and oligohaline habitats. Environmental Biology of Fishes 27 : 33-41.

Buttermore, K. F., Litkenhaus, P. N., Torpey, D.C., Smith, G. R. Retting, J. E. 2011. Effects of Mosquitofish (*Gambusia affinis*) cues on wood frog (*Lithobates sylvaticus*) tadpole activity. Acta Herpetologica 6 (1) 81-85.

Cech, J. J., Jr, Massingill, M. J., Vondracek, B. and Linden, A. L. 1985. Respiratory metabolism of mosquitofish, *Gambusia affinis* effects of temperature, dissolved oxygen, and sex difference. Environmental Biology of Fishes 13: 297-307.

Courtenay, W.R and Meffe, G. K. 1989. Small fish in strange places: A review of introduced poeciliids. In Meffe, G. K. and Snelson F.F. (eds.), Ecology and Evolution of Livebearing Fishes. Prentice-Hall, New York, pp. 319-332.

Daniels, G. L. and Felley, J. D. 1992. Life history and foods of *Gambusia affinis* in two waterways of south-western Louisiana. The Southwestern Naturalist 37: 157-165.

Fernandez-Delgado, C., 1989. Life-history patterns of the mosquitofish, *Gambusia affinis*, in the estuary of the Guadalquivir river of south-west Spain. Freshwater Biology 22: 395-404.

Fernandez-Delgado, C. and S. Rossomanno, 1997. Reproductive biology of the maosuitofish in a permanent natural lagoon in south-west Spain: two tactics for one species. Journal of Fish Biology 51: 80-92.

Gall, G. A. E., Cech, J. J., Garcia, R., Resh, V. H. and Washino, R. K. 1980. Mosquitofish an established predator. Calif. Agricul., 34: 21-22.

Girard, C. 1859. Ichthyological notices. Proceedings of the academy of Natural Science Philadelphia, 11: 56-68.

Hamer, A.J., Lane, S.J. & Mahony, M.J. (2002). The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. Oecologia 132, 445–452.Haynes, J. L., 1993. Annual reestablishment of mosquitofish populations in Nebraska. Copeia 11(1): 232-235.

Haynes, J. N. and R.C. Cashner, 1995. Life history and population dynamics of the Western mosquitofish: a comparison of natural and introduced populations. Journal of Fish Biology 46: 1026-1041.

Howe, E., C. Howe, R. Lim and M. Burchett, 1997. Impact of the introduced Poeciliid *Gambusia holbrooki* (Giarad, 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner, 1865) in Australia. Marine and Freshwater Research 48: 425-434.

Hubbs, C. 1971. Competition and isolating mechanisms in the *Gambusia affinis* X *G. heterochir* hybrid swarm. Bulletin of the Texas Memorial Museum 19, 1-46.

Ivantsoff, W and Aran, 1999. Deection of predation on Australian native fishes by *Gambusia holbrooki*. Marine and Freshwater Research 50: 467-468.

Komak, S. and M. R. Crossland, 2000. An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. Wildlife Research 27: 185-189.

Krumholz, L. A., 1948. Reproduction in the western mosquitofish *Gambusia affinis* and its use in mosquito control. Ecological Monographs 18: 1-43.

Ling, N. 2004. Gambusia in Newzealand: really bad or just misunderstood? Newzealand Journal of Marine and Freshwater Research, 38: 473-480.

Maglio, V. J and Rosen, D. E. 1969. Changing preferences for substrate colour by reproductively active mosquitofish, *Gambusia affinis* (Baird and Girard) Poeciliidae:

Anthriniformes). Amer. Museum Novitates, 2397: 1-39.

Medlen, A. B. 1951. Preliminary observations on the effects of temperature and light upon reproduction in *Gambusia affinis*. Copeia, 148-152.

Milton, D. A. and A. H. Arthington, 1983. Reproductive biology of *Gambusia affinis holbrooki* Baird and Giarard, *Xiphophorus helleri* (Gunther) and *X. maculates* (Heckel) Pisces; Poeciliidae) in Queensland, Australia. Journal of Fish Biology 23: 23-41.

Minckley, W. L. 1973. Fishes of Arizona. Arizona Game and Fish Department, Phoenix.

Nagdali, S. S. and Gupta, P. K. 2002. Impact of mass mortality of mosquitofish, *Gambusia affinis* on the Ecology of a Freshwater Eutrophic Lake, (Lake Nainital, India). Hydrobilogia, 468: 45-52.

Pyke, G. H. 2005. A review of the biology of *Gambusia affinis* and *Gambusia holbrooki*. Reviews in Fish Biology and Fisheries, 15: 339-365.

Pyke, G. H. 2008. Plague minnow or Mosquitofish? A review of the biology and impacts of introduced *Gambusia* species. Ann Rev. Ecol. Syst. 39: 171-191.

Rupp, H. R., 1996. Adverse assessments of *Gambusia affinis*: an alternative view for mosquito control practitioners. Journal of the American Mosquito control Association 12: 155-166.

Sawara, Y. 1974. Reproduction of the mosquitofish (*Gambusia affinis affinis*), a freshwater fish introduced into Japan. Japanese Journal of Ecology, 24: 140-146.

Segev, O., Mangel, M. and Blaustein, L. 2009. Deleterious effects by Mosquitofish *Gambusia affinis*) on the endangered fire salamander (*Salamandra infraimmaculata*) 12: 29-37.

Sharma, V.P. 1994. Role of fishes in vector control in India. In: Larvivorus Fishes of Inland Ecosystems, p. 1-19, V.P Sharma and A. Ghosh (Eds.), Malaria Research Centre, Delhi.

Smith, H. M. 1912. The prolificness of *Gambusia*. Science 36, 224.

Stanback, M. 2010. *Gambusia holbrooki* predation on *Pseudacris feriarum* tadpoles. Herpetol. Conserv. Biol. 5: 486-489.

Trendall, J. T. 1982. Covariation of life history traits in the mosquitofish, *Gambusia affinis*. The American Naturalist 119, 774-783.

Vargas, M. J. and A. de Soatoa, 1996. Life history of *Gambusia holbrooki* (Pisces, Poeciliidae) in the Ebro Delta (NE Iberian Peninsula). Hydrobiologia 341: 215-224.

Wilson, F., 1960, “A review of the biological control of insects and weeds in Australia and Australian New Guinea”. Common Wealth Agricultural Bureaux, Bucks, England, pp. 102.