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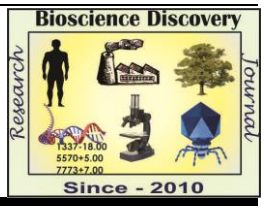

A comparative study on RNA: DNA Ratio in different tissues in two species of fresh water bivalve *Lamellidens* from lotic and lentic water

Nagawanshi M.

Department of Zoology, Vinayakrao Patil Mahavidyalay, Vaijapur, Aurangabad
meenan_2568@yahoo.com

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Abstract

In the present study an attempt has been made to understand changes in RNA: DNA ratio in different tissues in relation to reproduction in freshwater water two species of bivalve *Lamellidens marginalis* from Godavari river and *Lamellidens corrianus* from Nandrabad pond in different seasons. Seasonal variation found in the ratio in both male and female tissues. In *Lamellidens marginalis* both males and females the ratio found gill> mantle> gonad> hepatopancreas in monsoon season. High ration found in hepatopancreas and gonads in winter in both sexes and in winter male gills and hepatopancreas have shown highest RNA: DNA ratio. In *Lamellidens corrianus* males tissues like gonad gill mental an hepatopancreas have higher RNA: DNA ratio in summer season than monsoon and winter and in females the ratio found gill, mantle, gonad and hepatopancreas have higher RNA:DNA ratio in monsoon season.

INTRODUCTION

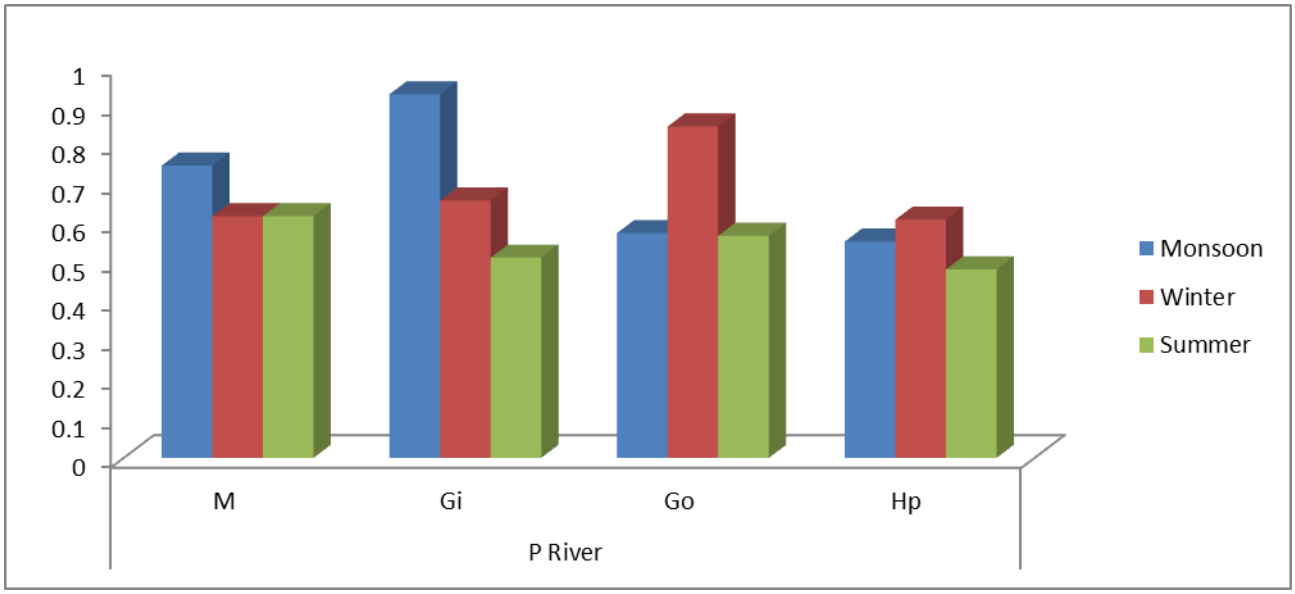
Some of the aspects of the invasive process (number of colonization events, size of the founding population, pathway of the colonization, etc.) can be revealed by molecular biology studies, and as consequence, the use of molecular biology methods in studies of evolution and population genetics of invasive species has increased dramatically in the last years (Graputto *et al.*, 2005; Timmermans *et al*., 2005). RNA: DNA ratio is now daysmost widely-used tool to determine biochemical and eco-physiological index of activity (growth, reproduction, secretion, etc.) of organisms under a given environmental condition (Lucas and Beninger, 1985). This index gives a measure of the synthetic capacity of the cell and usually correlates with nutritional status (Buckley *et al*., 1999). The RNA: DNA ratio is based on the assumption that the amount of DNA, the primary carrier of genetic information, is stable under changing environmental situations within the somatic cells of a species (Bulow, 1987), whereas the amount of RNA directly involved in protein synthesis, is known to vary with age, life-stage, organism size, disease-state and with changing environmental conditions (Bulow, 1970**)**.The basic principle of using RNA/DNA ratio as a measure of health condition in an organism is that total RNA content is primarily a function of ribosome number and is positively correlated with new protein synthesis, whereas DNA content remains constant in an individual because it is a function of chromosome number (Buckley 1984; Buckley *et al*., 1999; Dahlhoff, 2004; Chícharo & Chícharo 2008). Thus, organisms in good condition tend to have higher RNA: DNA ratios than do those in poor condition (Bulow, 1987**).** In fact RNA: DNA ratios have been used on a wide range of marine organisms, mainly phytoplankton (Dortch *et al*., 1983**)**, zooplankton (Sutcliffe, 1965; Ikeda *et al*., 2007) and larval fish

(Bulow, 1987; Buckley, L. 1984; Caldarone *et al*., 2003) but also juvenile and adult fish (Bulow, 1970; Thorpe *et al*., 1982), in bivalves (Chícharo *et al*., 2001; Grémare, & Vétion, 1994; Chícharo & Chícharo,1995) cephalopods (Clarke *et al*., 1989; Sykes *et al*., 2004**)** and crustaceans (Chícharo *et al*., 2007; Lemos *et al*., 2002) shown to reflect the nutritional status and may be useful in monitoring their physiological state in the field. An attempt has been made to understand seasonal changes in RNA: DNAratio of gonad of male and female *Lmarginalis* during reproduction, inhabiting at Godavari River at Paithan been carried out.

MATERIALS AND METHODS

The Godavari is the second longest river in India. it flows through Paithan of Aurangabad district. Maharashtra At Paithan the river banks and Nath Sagar dam are sites for *Lamellidens marginalis*. Ten individuals of bivalve *Lamellidens marginalis* from Godavari River and *Lamellidens corrianus* from Nandrabad pond (6.5 cm in shell length) were collected from collection sitesin different seasons summer, monsoon and winterAnimals were immediately brought to the laboratory and acclimatized for 24 hrs for defecation. Animals were dissected and gonad smears were observed under microscope in order to find male and female individuals.Gonad weighed and homogenized in 750 µl of trizol. The extraction of RNA and DNA was followed as per manufacturers'protocol. The RNAdissolved in 20 µl of DEPC water. The solution was passed through several times in order to dissolve RNA 8mM NaOH aliquot containing.DNA was mixedwith water to the final volume of 20 µl& measure the absorbance RNA and DNA concentrations were read without further dilution on Nanodrop at 260nm& at 286nm at Paul Herbert Centre for DNA barcoding and biodiversity and expressed in ng/µl.

Fig1: RNA: DNA ratio of Female *Lamellidens marginalis* in different seasons



RESULTS AND DISCUSSION

RNA: DNA ratios of male and female *L marginalis* differed significantly in different tissues. Higher RNA: DNA ratio was found in Gill tissues in monsoon season in female and in mantle in male. In summer season high RNA: DNA ratio was found in mantle tissue of male and in winter it in hepatopancreas of male. When compared with male and female tissues independently higher ratios are found in mantle of male in monsoon, in the gill tissues of female in monsoon. In winter female gonads also had shown higher values of ratio than male gonads and in male hepatopancreas than females.

Fig2: RNA: DNA ratio of Male *Lamellidens marginalis* in different seasons

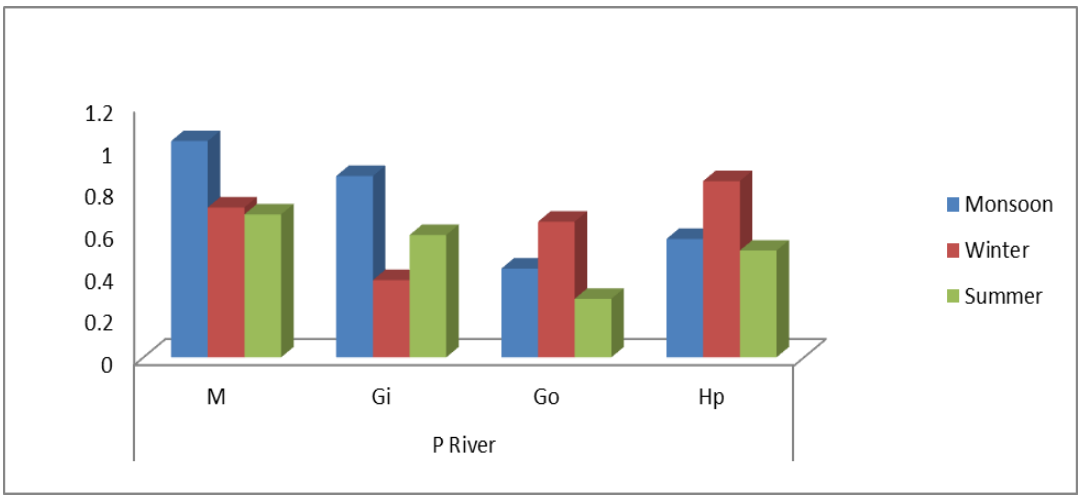


Fig3: RNA: DNA ratio of Male *Lamellidens corrianus* in different seasons

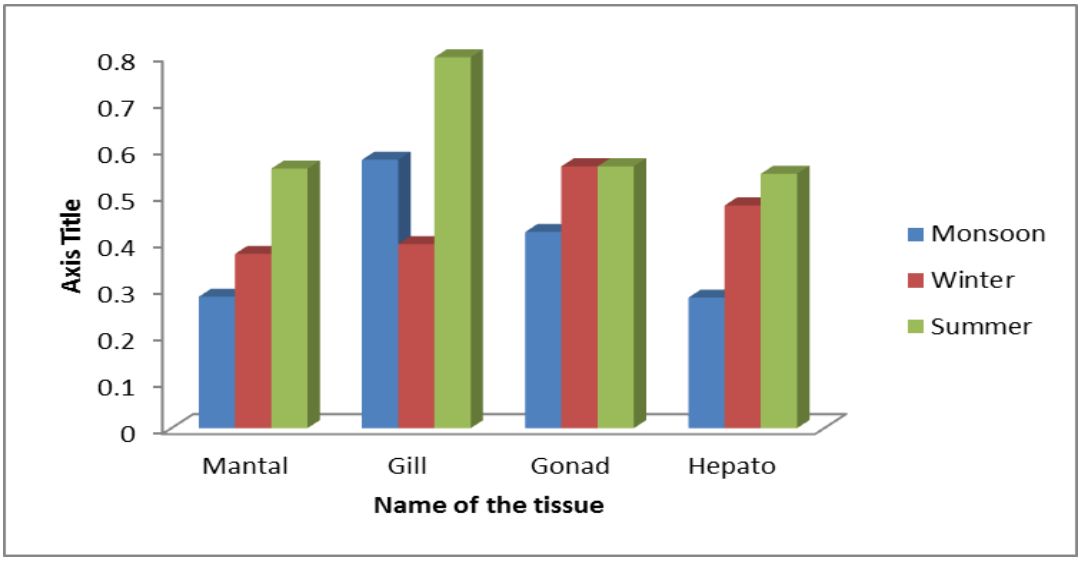
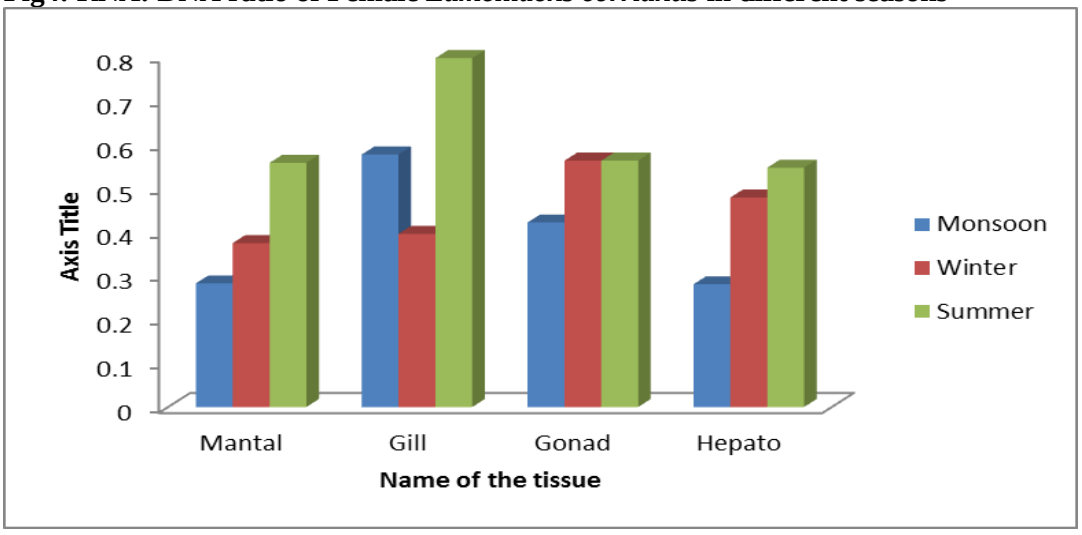


Fig4: RNA: DNA ratio of Female *Lamellidens corrianus* in different seasons



Much difference was observed in ratio in different tissues in the present study. This may be due to physiological changes occurring in the tissue according to the reproductive status of the animals. Changes in protein contentis also seen in different tissues in different seasons maximum protein content was found in gonads in all seasons than in any other tissue. Mantle and hepatopancreas have shown nearly constant values, high protein content in mantle in winter (Jadhav and Jadhav, 2012).

RNA: DNA ratios of male and female *Lamellidens corrianus* differed significantly in different tissues. Higher RNA: DNA ratio is found in Gill and gonad tissues in monsoon season in female and in mantle if male. In summer season high RNA: DNA ratio is found in gill tissue of male and in winter it in hepatopancreas of male. When compared with male and female tissues independently higher ratios are found in gill of male in summer, in the all tissues of female in summer. In winter female gonads also had shown higher values of ratio than male gonads and in male hepatopancreas than females. Much difference is observed in ratio in different tissues in the present study. This may be due to physiological changes occurring in the tissue according to the reproductive status of the animals.

Maturation and reproduction, which differ between males and females, often require different amounts of energy, as discussed by Paon and Kenchington (1995) and Pérez *et al.* (2003). According to authors, reproductive costs are much greater for females than for a male, which implies that protein synthesis and therefore RNA content is greater in females than in males. The clam *R. decussates* (Pérez *et al.*, 2003) exhibits clear sexual differentiation in biochemical composition. Protein synthesis for females, especially during spawning activity, which could explain the higher RNA content and RNA: DNA ratios of female bivalves observed in the present study. However, this is not only restricted to spawning period: *C. crangon*was analyzed after spawning and in the resting period, and greater RNA content and RNA: DNA ratios were still evident in females. Behavior could also lead to differences in RNA: DNA ratios between sexes. According to Salgado *et al.,* (2004) higher RNA: DNA ratios of female *P.s microps* could be a consequence of differences in the activity patterns of sexes, because parental care is supported by males. Males are probably limited to foraging for food available around the nest during the breeding season, whereas females behave as food maximizers. Feeding *R. decussate* sexhibited the lowest RNA: DNA ratios, indicating very poor condition (Chicharo *et al.,* 2001), but the general pattern of low values for males and high values for females in terms of RNA concentrations and RNA: DNA ratios were still observed. In conclusion, caution is needed when interpreting RNA: DNA ratio data from adult organisms, because sex differences in nucleic acid concentrations bias RNA indices if the frequencies of sexes in samples are not representative of those in the population. Samples in which males are over-represented result in an underestimate of the condition of the population, and those in which females are over-represented result in an over estimate. Mitochondria and protein content are also greater in female rats than in males. Mean RNA: DNA ratios of females tended to be greater than those of males in all 3 species studied, largely owing to greater RNA concentrations Justo *et al.,* (2005). Robbins *et al.,* (1990), studied RNA: DNA ratio in male and female *P. maximus*, the authors reported that the RNA: DNA ratio of the male gonad was consistently ten times lower than that of the female gonad. In somatic tissues this could suggest a reduced protein synthetic activity. RNA: DNA ratios in spring and summer suggested that mussel physiology is h~ghlyplastic in response to site and area-related variations in environmental factors (Dahlhoffand Menge, 1996). Patterns of the RNA/DNA ratio are not always consistent across tissue types and food availability in different bivalve species (Mayrand *et al.*,1994; Norkko& Thrush, 2006). Variation of RNA/DNA ratios of HP size effect was statistically significant in mussels only in which the RNA/DNA ratio decreased with increasing size. Small and medium mussels consistently showed the lowest RNA/DNA ratio when compared for two different sites**(**Yeung and Leung, 2013).

The seasonal variation in biochemical components is not related only to the reproductive cycle but is also influenced by the food availability and temperature as emphasized by Ansell (1974A, 1974B, 1974C,Darribaet. al., 2005). Seasonal changes in condition indices and biochemical components of the digestive gland, anterior adductor muscle, foot, and gonad of *Ensisarcuatus* (Jeffreys, 1865) were in relation to environmental conditions and reproductive events to meet the energy requirement. Gametes production occurs at the expense of other body reserves, from tissues like mantle and hepatopancreas when the gametes are

not ripe in *M. edulis* (Bayne and Thompson, 1970) and *P. viridis* (Nagabhushanam and Mane, 1978). Variations in organic constituents in different soft body parts of *L. marginalis* were observed by Sheikh (2011) in Pravara River in different seasons. In the present study the *L. marginalis* both the male and the female have shown beginning of gametogenic activity in the gonads during the summer season. The gonads showed accumulation of lipid globules and nutritive cells in the tissue. Proliferation of cells from germinal layer is also seen. Maturation of the gametes was observed during the monsoon season in both male and female gonads.

In the present study an attempt has been made to correlate the reproductive status of the bivalve molluscs and role of the other tissues in relation to RNA: DNA ratio. Since tissues of bivalves are contributing their role in the reproductive activities by accumulating and diverting biochemical substances as per need of gonad in different seasons. Some reports indicate mantle also plays important role in reproduction in movement of metabolites (Nagawanshi, 2013; 2014; Jadhav and Jadhav, 2012). Gill tissue plays an important role in housing the developing glochidia after fertilization by modifying it into marsupial (Nagawanshi, 1998). Thus the changes in RNA: DNA ratios in these tissues observed in different seasons can be correlated with the reproductive cycle of *L. marginalis*

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