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# Study of the reproduction of the Karamote shrimp *Peneaus* (*Melicertus*) *kerathurus* in Amvrakikos Gulf, western Greece

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The reproduction of the karamote prawn, Penaeus (Melicertus) kerathurus (Forskål 1775), was studied for the native population in Amvrakikos Gulf (Ionian Sea; western Greece). Sampling was carried out on a monthly basis between June 1999 and May 2001. The results showed that the shrimp Penaeus (Melicertus) kerathurus reproduction period spans between late April and late September. The size-at-maturity was estimated at a size of 45.23 mm carapace length (or 156.2 mm in total length). The smallest mature female in the samples was found to have 30 mm CL or 113.95 mm TL. Maximum gonadosomatic index (GSI) was estimated to be 9.62% for female shrimps at stage IV gonad maturity stage. The population gonadosomatic index peaks in May with an average value of 6.895%. Potential fecundity was estimated to be 154600 of oocytes per g of gonad tissue at the stage IV (mature female). Monthly sex ratios (males/females) were found greater than 1 throughout the year with maximum values reaching 2.5. A new life cycle pattern is proposed for this particular population.

Key words: Penaeus kerathurus, reproduction, sex ratio, gonadosomatic index, fecundity, Greece

#### INTRODUCTION

Penaeus (Melicertus) kerathurus is a valuable commodity in the rural Mediterranean region. The total Mediterranean production is rather small in relation to other coastal stocks along the northern Mediterranean coast (FISCHER *et al.*, 1987). Most of the production of the north Mediterranean countries comes from Italian waters, Amvrakikos Gulf in western Greece and along the Greek coast of the northern Aegean Sea (CONIDES *et al.*, 1990; LUMARE & SCORDELLA, 2001). Other major producer coun-

tries in the Mediterranean are Tunisia and Libya though the fisheries in these countries are not significantly developed (FAO, 2000). The species in Greece is considered a target species of the coastal fishery segment and its fishing is carried out using a specific shrimp double trammel net (CONIDES, 2001). A small quantity is also landed as by-catch from the trawler segment. Previous research on the species was focused mainly on its artificial reproduction, larval rearing and on-growing to marketable size for the support of commercial cultivation efforts (KLAOUDATOS, 1984; RODRIGUEZ, 1976; SAN FELIU *et al.*, 1976; LUMARE, 1998).

Even though there exists a significant amount of information on the population dynamics and fishery of other Mediterranean commercial shrimps, such as Parapenaeus longirostris, Aristeus antennatus, Aristaeomorpha foliacea and Palaemon spp. (e.g., CASCALHO & ARROBAS, 1987; CONIDES et al., 1988; DEMES-TRE & LLEONART, 1993; DEMESTRE & MARTIN, 1993; CAU et al., 2002), there is limited and scarce information on the state of the fishery and dynamics of this species in the northern Mediterranean and especially in Greek waters. There have been only a few studies on *Penaeus* (Melicertus) kerathurus in Amvrakikos Gulf, dealing with aquaculture and migration patterns (CONIDES et al., 1990, 2006; KLAOUDATOS et al., 1992).

The present study contributes to rectifying this situation by reporting data on the reproduction of *Penaeus (Melicertus) kerathurus* in western Greece and especially the monthly distribution of the gonadosomatic index, the distribution of the population maturity stages, the monthly sex ratio, the fecundity, and elaborates on the life cycle of the species.

#### MATERIAL AND METHODS

# Description of the fishing area

Amvrakikos Gulf is a semi-enclosed gulf located on the western Greek coastline. The gulf has an area of 530 km<sup>2</sup> with a maximum length of 32-35 km and maximum width of 10-15 km (Fig. 1). The maximum depth of the gulf is 63 m at the centre. A narrow channel connects the gulf to the Ionian Sea with a length of 4 km, width of 600 m and maximum depth of 15-17 m. The water quality in the gulf is heavily affected by the flow of 2 rivers on the north side; the rivers Louros with an average flow of 19 m<sup>3</sup> sec<sup>-1</sup> and Arachthos with an average flow of 71 m<sup>3</sup> sec<sup>-1</sup>. At those river deltas, 4 lagoons have been created over the years, named Rodia, Tsoukalio, Avleri and Logarou, with a total area of 50 km<sup>2</sup>. Evidence of anoxic water layers below the 35 m

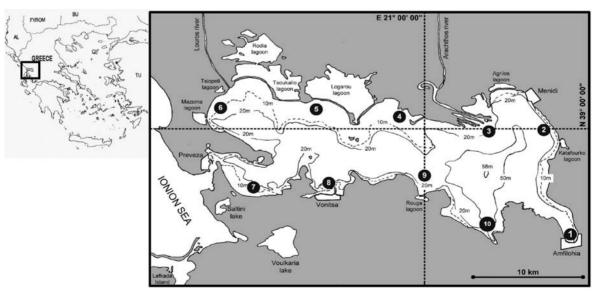


Fig 1. Map of the study area and main sampling stations in Amvrakikos Gulf, western Greece

depth contour has been gathered from previous oceanographic research (N.C.M.R., 1989).

# **Experimental fishery**

The experimental fishery of the shrimps was organised on a monthly basis during the period 1999-2001 in the area of Amvrakikos Gulf. A total number of 24 samplings was organised from a grid of pre-selected stations. During the sampling campaign a total number of 5,505 specimens were collected (3186 males; 2319 females). Fishing was carried out using the local traditional gear, the double trammel net (average length - 1000 m; height - 2 m; internal mesh - 22 mm; external mesh - 110 mm). Sampling was carried out using local traditional coastal fishing vessels equipped with a 25-hp inboard petrol engine. Specimens were kept on ice until transported to the laboratory and were stored in a freezer at -20°C until they were measured.

#### Measurements

The measurements of the shrimps included the carapace length (CL; from the base of the rostrum to the end of the carapace, in mm), the total length (TL; from the tip of the rostrum to the tip of the telson with the body shrimp extended, in mm) and the total wet weight (in g). In addition, the sex was identified in all specimens. Finally, the female gonad maturity stage was identified and the female gonad weight (in g) was measured. Length measurements were made using digital callipers while weight was measured by electronic balance to the nearest 0.01 g. The gonadosomatic index was estimated as the gonad weight to somatic weight ratio as follows:

$$GSI(\%) = \frac{Gonad \text{ weight (g)}}{Total \text{ Wet Weight (g)}} 100$$

A total number of 200 female individuals (at stage IV of maturity) were used for measuring gonadosomatic index and fecundity. Gonad development was classified as one of the Stages I–V (LUMARE 1998). Female fecundity (total number of oocytes in stage IV mature individu-

als) was studied by counting the total number of oocytes on small pieces of ovarian tissue (0.1-0.3 g) using a stereomicroscope and then relating the total number of oocytes in this sample to the total weight of the ovary. The relationship between potential fecundity and total length was fitted to a power equation:

Number of oocytes =  $a + [Total Length, mm]^b$ 

Sex ratio was estimated as the ratio of males to females (males/females).

#### RESULTS

# Maturity stages of the population

The reproduction period of the species *Penaeus (Melicertus) kerathurus* in Amvrakikos Gulf was considered as the period of the year during which stage IV shrimps (shrimps with fully mature gonads but which have not yet released oocytes) can be found in the samples. The reproduction period spans between May and September, with stage-IV females comprising between 59.1% and 72.7% of the population. The reproduction process showed a peak in August with 72.7% percent of stage-IV shrimps in the population. The monthly fluctuations of

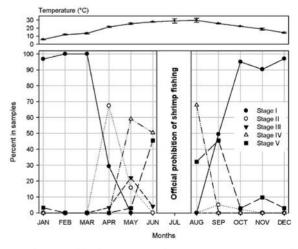


Fig. 2. Monthly fluctuations of average temperature and the maturity stages of females Penaeus (Melicertus) kerathurus in Amvrakikos Gulf

the percentages of the various gonad maturity stages are illustrated in Fig. 2 as averages from the tow years sampling.

# Gonadosomatic index (GSI)

From the analysis of a sub-sample of the female population and the anatomical extraction of gonads in various maturity stages, the gonadosomatic index was estimated. The average values and ranges of GSI are 0.77% (0.23-2.94) for stage I, 2.1% (0.72-5.96) for stage II, 3.95% (1.07-6.08) for stage III, 9.62% (4.20-21.98) for stage IV and 2.51% (0.61-4.92) for stage V females. GSI increases with the ovary development stage, reaching an average maximum value in Stage IV mature females of 9.62%. Another important observation is that the standard deviation of the average GSI estimation increases with the development stage. This indicates that there is relative instability in the size of the gonads in relation to the body size and the maturation stage and a higher range of values can be found in the population. This ultimately makes distinguishing the maturity stage difficult simply by weighing the gonads. GSI peaks in the period of May and August reached a value of 6.895% (Fig 3).

# **Fecundity**

The results show that the average number of oocytes in a Stage IV gonad is 1546±249 oocytes

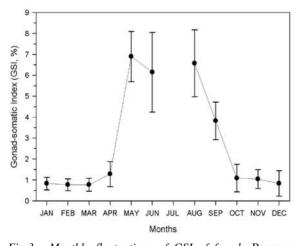


Fig 3. Monthly fluctuations of GSI of female Penaeus (Melicertus) kerathurus in Amvrakikos Gulf

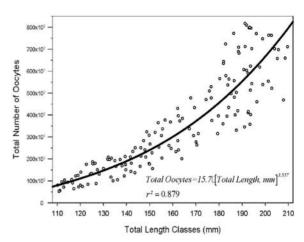


Fig 4. Relationship between Total Number of Oocytes (in ovary) and Total Length (mm) of Penaeus (Melicertus) kerathurus in Amvrakikos Gulf

per 0.01 g of Stage IV gonad or approximately 154,600 oocytes per g of ovary. The relationship between Total Length and Total Oocyte number was found (Fig. 4):

Total Oocytes = 15.7[Total Length, mm]<sup>3.557</sup>  $r^2$ =0.879, Std. error= $\pm$ 8894.5 oocytes.

#### Sex ratios

Monthly sex ratios (males/females) greater than 1 were found throughout the year, except in September, and reaching a maximum value of 2.1±0.5% in May and August (Fig. 5).

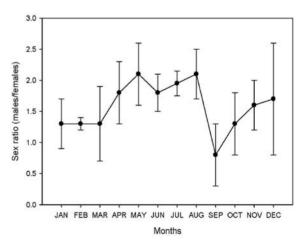


Fig. 5. Overall monthly sex ratios of Penaeus (Melicertus) kerathurus population in Amvrakikos Gulf (Vertical limits denote standard deviation)

# **DISCUSSION**

# Reproduction period

The monthly distribution of the Stage IV (gonad maturity) female shrimps shows that the reproduction period spans from late April to late September (Fig. 2). In addition to this, the increase of percentage of Stage V females (spent) during the same period indicates that the release of oocytes starts almost immediately (approximately within 15 days) after the first appearance of Stage IV females. In addition to that, the plotting of average temperature of the water in Amvrakikos Gulf clearly shows that one month before the first appearance of stage IV females (March), the temperature increases rapidly by 7.7°C (from  $13.53\pm0.6$ °C to  $21.23\pm0.4$ °C). The response of the shrimps to this rapid increase of temperature is their massive maturation and the initiation of reproduction. KLAOUDATOS (1984) reports for the period of 1974-1980 in Amvrakikos Gulf that there exists a rapid increase of temperature by 4-6°C (depending on the area of the gulf). The same occurred during that study in the period 1980-1984; (KLAOUDATOS, 1984). N.C.M.R. (1989) reported a similar increase by 4-5°C in the same period and finally, ETANAM (1999) reported a similar increase by 5-6°C in surface waters and 4-6°C increase of temperature close to the sea bottom in Amyrakikos Gulf in the same time period during the year. The thermal stimulus had been recognised as the main stimulus (together with photoperiod) for the initiation of reproduction (maturation of gonads and migration to the reproduction grounds for copulation) and it is extensively used in intensive aquaculture practices (LUMARE, 1976, 1979; KLAOUDATOS, 1984; LAUBIER-BONICHON & LAUBIER, 1976; BEARD et al,. 1977; LAUBIER-BONICHON, 1978; SHIGUENO, 1976; SAN FELIU et al., 1976). In the following 30-40 days, both sexes arrive at the reproduction grounds and copulation occurs (transfer of spermatophore). After that, the reproduction period starts.

The study of the Penaeid reproduction period has difficulties (MINAGAWA et al., 2000). The study of the reproduction period based on the proportion of stage IV females in the general population does not appear to be a good indicator. The ratio between spawning individuals may vary spatially (deep-shallow waters) and temporally (MINAGAWA et al., 2000). For example, in Penaeus semisulcatus the proportion of spawning individuals varies by month and area, and the peak was shown to differ between years (CROCOS & VAN DER VELDE, 1995). Further, more spawning individuals are found in deep waters than in shallow waters (SHLAGMAN et al., 1984). This trend is also evident in Penaeus (Fenneropenaeus) merguiensis (CROCOS & KERR, 1983). This situation is not evident for Amvrakikos Gulf. The gulf is shallow and small in area while there is evidence that the prawns do not migrate towards the Ionian Sea, spending all their lifecycle in the gulf (KLAOUDATOS et al., 1992). Therefore such temporal or spatial differences do not occur and the prawn population exhibits uniform movements around the gulf.

Penaeus (Melicertus) kerathurus species is a closed-thelycum penaeid, therefore the spermatophore is implanted (copulation) immediately after the female moults while spawning occurs after the cuticle of the exoskeleton has hardened. Since this occurs in a period of 27 days (CROCOS & KERR, 1983; DALL et al. 1990) and the spermatophore is viable for 15-20 days, then spawning will occur about 20-30 days after copulation. This indicates that the copulation may occur in May (10-20 of May) and the first stage IV individuals appear in late May and in June. The reproduction moulting of the females, therefore, starts around 10-15 of March.

# **Gonadosomatic index**

The average values and ranges of GSI were found to be 0.77% for stage I, 2.1% for stage II, 3.95% for stage III, 9.62% for stage IV and 2.51% for stage V of gonads. Earlier studies (KLAOUDATOS, 1984) showed that the respective values of GSI per stage are 2%, 2-7%, 7-12%, 12% and 3% respectively. These values are not in accordance with the present study and this may be ascribed to the fact that the GSI values reported by KLAOUDATOS (1984) originate from

cultured shrimps and not from wild stock directly. There have been reported great differences in the GSI values between reared and captured shrimps Penaeus (Melicertus) kerathurus (MEDI-NA et al., 1996). Moreover, the reported ranges are different. In the present study, the maximum GSI (peak of reproduction) was evident during May and reached 6.9%, which is very low in comparison with other authors. MEDINA et al. (1996) reported that GSI in July reached 11.3% for Penaeus (Melicertus) kerathurus in the Gulf of Cádiz (Spain). RODRÍGUEZ (1985) found a maximum value of 10.52% in the same region. On the other hand, KLAOUDATOS (1984) working in Amvrakikos Gulf found that the GSI reaches a maximum of 11.73% during June. This proves that the reproductive performance of the shrimps has been lowered significantly and that the reproduction peak has shifted by one month (from June to May).

# **Fecundity**

The fecundity in Penaeids has been evaluated by estimating the total number of oocytes in dissected ovaries (CROCOS & KERR, 1983; CHOY, 1987). Fecundity is significantly related to size/ age (DALL et al., 1990) although there are studies that showed a very low correlation between size and fecundity (especially in egg bearing species; CONIDES et al., 1988). In the present study, Penaeus (Melicertus) kerathurus showed a maximum fecundity of 800,000 oocytes at a maximum natural size of 210 mm (TL; = 53 mm CL) in the ovaries or approximately a ratio of 156,000 oocytes/g of ovary. The Penaeus (Melicertus) kerathurus species shows great similarity with Penaeus (Farfantepenaeus) duorarum, Penaeus esculentus, Penaeus (Fenneropenaeus) indicus, Penaeus (Melicertus) latisulcatus, Penaeus (Fenneropenaeus) merguiensis and Penaeus semisulcatus in terms of number of oocytes per individual female shrimp. Earlier studies have reported higher values of fecundity with more than 1000000 oocytes per female for Penaeus (Melicertus) kerathurus (HELDT, 1938; DALL et al., 1990). Differences in estimates of fecundity among studies might be artificial, as the result of differences in technique, or natural, as the results of differences in fecundity with geographical location or climatic conditions. Additionally, different geomorphological, trophic and fishing efforts in the different areas could explain such differences (MARTOSUBROTO, 1974).

#### Sex ratios

The sex ratios obtained in this study can be considered as skewed. The overall sex ratio (males/females) was found to be 1.37. TURKMEN & YILMAZYERLI (2006) found an overall sex ratio of 0.72 in Izmir Bay, Turkey. In most cases, the sex ratios (expressed as males/females in numbers) are rarely close or equal to the generally considered as normal 1:1 ratio. In most cases, the males far outnumber the females and in some cases show 2 or 3 times higher numbers.

The sex ratio profile of the prawn in Amvrakikos Gulf provides indications of the pattern of movement. The sex ratio is found to be close to 1-1.5 between January and April. This is the period of simultaneous appearance of males and females in shallow waters for copulation and spermatophore exchange. Immediately afterwards, the sex ratio increases again to 2-2.5 as the females move to the centre of the gulf for spawning while the males seem to remain along the coasts. Afterwards, the sex ratio decreases rapidly to 0.5-1.2 as the females appear again close to the coasts for feeding due to energy exhaustion after reproduction (September-October) and then it increases again (December-January) during the wintering migration or dispersion in the gulf to reach areas with more suitable temperatures.

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# Razmnožavanje kozice Penaeus (Melicertus) kerathurus u zaljevu Amvrakikos, zapadna Grčka

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# SAŽETAK

Razmnožavanje kozice, Penaeus (Melicertus) kerathurus (Forskål, 1775), je istraživano na populacijama iz prirodne sredine na području zaljeva Amvrakikos (Jonsko more, zapadna Grčka). Uzorkovanje je obavljeno svakog mjeseca u razdoblju između lipnja 1999. godine i svibnja 2001. godine. Rezultati istraživanja pokazuju da se kozica mrijesti u razdoblju travanj-rujan. Veličina pri kojoj kozica dosiže spolnu zrelost iznosi 45.23 mm dužina karapaksa (156.2 mm ukupna dužina). Utvrđena je najmanja spolno zrela ženka u uzorku pri dužini 30 mm dužina karapaksa (113.95 mm ukupna dužina). Najveća izračunata vrijednost gonadosomatskog indeksa iznosi 9.62% za ženke pri stadiju IV zrelosti gonada. Gonadosomatski indeks za čitavu populaciju ima svoj maksimum u svibnju pri srednjoj vrijednosti od 6.895%. Izračunati potencijalni fekunditet iznosi 154600 oocita po gramu tkiva gonada pri stadiju IV (zrela ženka). Mjesečni omjer spolova (mužjak/ženka) ima vrijednosti veće od 1 tijekom cijele godine uz maksimalnu vrijednost 2.5. Iznesen je i predložak obrasca novog životnog ciklusa populacije na istraživanom području.

Ključne riječi: Penaeus kerathurus, razmnožavanje, odnos spolova, gonadosomatski indeks, fekunditet, Grčka