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The Growth of Appendix Masculina of the Norway Lobster (*Nephrops norvegicus*) in the Central Aegean Sea (e. Mediterranean)

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Abstract

The objective of the present research is to study the seasonal morphometric variations of the secondary sexual characters of *Nephrops norvegicus* males. Samples were collected from March 2014 to September 2014, except the summer months, using a commercial bottom trawl in a depth range of 446-728 m in the Central Aegean Sea (Mediterranean Sea). This is the first attempt to resolve the morphometry of the appendix masculina in the Mediterranean basin of this species. Carapace length, surface and perimeter of appendix masculina were measured and the relationships among them. A positive allometric growth among these structures is observed. The possible role of this appendix in copulation of Norway lobster is suggested

Keywords: *Nephrops norvegicus*, appendix masculina, Aegean Sea, allometry.

Introduction

Norway lobster (*Nephrops norvegicus*), one of the main representatives of Nephropidae family is the crustacean with the highest commercial value in Europe. The species is distributed in the NE Atlantic and the Mediterranean (Farmer, 1975). Its overall depth range extends from 15 to 800 m, where in the Mediterranean particularly, has been reported to be mainly between 200 and 800 m depth (Maynou and Sardà, 1997). In Greek waters, *N. norvegicus* is one of the highest value species in mixed demersal fishery.

The morphometric characteristics of this commercially and ecologically important decapod have been scarcely studied in the Mediterranean basin and only some sporadic studies from the Catalan Sea (Maynou and Sardà, 1997; Sardà and, 1981), the N. Tyrrhenian Sea (Mori *et al.*, 1994) have attempted to shed light on the biology of the Norway lobster. Studies of reproduction in the Mediterranean were developed mainly in the eighties (e.g. Froglia and Gramitto, 1981; Orsi Relini and Relini, 1989; Sardà, 1991). The species has visible external organs for reproduction and it spawns once per year. The spermatogenesis in males occurs throughout the year, whereas the ovaries of the females mature in spring when the moulting also occurs after the egg-hatching stage. The post-moult period in late spring seems to be the only period available for copulation (Farmer, 1974). Rotllant *et al.* (2012) described the gross morphology of the male reproductive system of *N. norvegicus* and demonstrated that it is similar to that of other decapods species, as reviewed by Krol *et al.* (1992).

Appendix masculina is a dominant accessory that distinguishes males from females in decapods and it is located on the endopod (inner side) of the second pleopods of the species (Williams, 1984). Appendix masculina is a visible rounded body structure, completely absent from the females, which along with the petasma, is associated with the sperm transfer and copulation. In particular, during the copulation period the first pair of pleopods creates an open-ended tube that its tip is inserted inside the sperm receptacle of the female. Then, the appendix masculina, with the appendix interna, functioning similarly to a piston facilitates the sperm transfer inside the tube created by the first pleopods (Cobb and Phillips, 1980). According Bauer (1996) males with partially ablated petasmata or with ablated appendices masculinae did not copulate with females. The maximum dimensions for both appendices have been measured in the copulation period of some other few decapods, like *Aristaeomorpha foliacea* and *Aristeus antennatus* (Kapiris *et al.*, 2002) and *Parapandalus narval* (Thessalou-Legaki, 1989).

The aim of this work is to provide the first morphometric data on the appendix masculina

(surface, perimeter) of *Nephrops norvegicus* individuals caught in the region of Southern Aegean Sea (E. Mediterranean) and to study its possible role during the copulation period. The relative growth of the two measurements (surface, perimeter) compared with carapace length, which is treated as a reference dimension, is also examined. This is the first investigation of the role and both measurements of appendix masculina in *Nephrops*, since in previous studies the measurements of its appendix masculina have been only used for the estimation of the size at onset of maturity (McQuaid *et al.*, 2006) and the ratio appendix masculina:carapace length for separation of immature from mature males of this species in the Atlantic Irish waters (Hillis, 1981). Lastly, the current investigation will aid in providing more information on when exactly the copulation period occurs for the Norway lobster population in the Eastern Mediterranean region, since biological gaps exist concerning this subject.

Materials and Methods

All the specimens were collected monthly using a commercial bottom trawl net with a mesh size of 18 mm at the cod end, from March to September 2014, in the frame of the National Fishery Data Collection Project (EPSAD) programme. The samples were caught mainly from Evoikos and Argosaronic Gulf (Central Aegean Sea, Eastern Mediterranean Sea) within a depth range of 446 to 728 m (Figure 1).

In totally 492 males and 822 females were studied in the present study. The carapace length (CL) was measured with the use of calipers to the nearest 0.01 mm and the sexual maturity in males was identified, according to the absence or presence of the petasma´s fusion (I. Immature and II: Mature, respectively). Female *Nephrops*' individuals are classified into ovary maturation stages which have been established based on the ovarian color and size, according to the scale given by Farmer (1974): Stage I (immature), Stage II (developing/recovering), Stage III (maturing), Stage IV (mature), Stage V (spent) and the berried females (ovigerous females with eggs attached to pleopods) have been also recordered.

The second pleopods were removed at the base to ensure the intact removal of the endopodite and the appendix masculina. The pleopods were stored in 70% alcohol and the total surface and the perimeter of the appendix masculina of mature males (Stage II) were estimated based on the images taken from that body part using the Image analysis Pro Plus software (Figure 2). The perimeter and surface measurements were given in mm and mm^2**,** respectively. The relationships between the appendices' surfaces vs. CL and among the appendices in each species were studied using the linearized allometric model, log y=log a + b log x, which has a slope of b, the allometric growth constant. If b>1, then the dimension y increases relatively more rapidly than does the reference dimension x, and the growth is considered as positively allometric. Otherwise, b<1 indicates negative allometry, and b=1 indicates a condition of isometry. The type of allometry was determined by testing the slope (b) of the obtained regression equations against the isometric slope of 1

Figure 1. Sampling area.

Figure 2. Appendix masculina of a *N. norvegicus* male individual

using Student's t-test (Sokal and Rohlf, 1981). The comparison of the appendages' slopes b and carapace length between the sampling months was carried out by one-way ANOVA. All the above statistics were applied using STATGRAPHICS Plus software. Statistical significance was indicated by $P<0.05$.

Results

The mean CL of the studied male individuals of *Nephrops* ranged from 33.40 mm (September) to 47.32 mm (July) (Table 1). The minimum carapace length of the mature males was 21.05 mm. The surface (range: 0.42-16.49 mm^2) and the perimeter (3.01-18.98 mm) of appendix masculina of the sampled specimens presented the highest mean values in March and April while the lowest in August and September (Table 1). Significant differences of CL, surface and perimeter with time were observed in the monthly surveys (one-way ANOVA, $P<0.05$, in all cases).

The measurements of the appendix masculina showed strong positive correlation to respective CL values (Table 2, Figure 3). In all the cases, the regressions were statistically significant (regression ANOVA, $P<0.01$), and the allometric growth of the surfaces and perimeters proved to be positive in almost all cases (t-test results, $b>1$).

Reproduction showed a clear seasonality both in terms of ovarian maturation and spawning period. Although females with immature (Stage I) and developing/recovering ovaries (Satge II) of *N. norvegicus* were present in March and April, an increased presence of maturing (Stage III) individuals was also found in the same months (Table 3). In this decapod, the ovarian maturation process (appearance of stages III and IV) seemed to be rapid, beginning from late spring (April and May), but the maximum values were found in early and middle summer (June, July). The highest proportion of spent individuals was found in August and September and, during these months, the lowest surface and perimeter values of the males' appendix masculina have been measured, as already written above.

Discussion

The average carapace length of the caught individuals of *N. norvegicus* is similar to those found in other Mediterranean areas (e.g. Sardà *et al.*, 1998). The lengths of both appendices that take part in decapods' copulation (appendix masculina and appendix interna) differ among the decapods. However, the appendix masculina is usually longer than appendix interna (Noël, 1976; Thessalou-Legaki, 1989; Kapiris *et al.*, 2002).

The values of the parameters of the regression (a: y-intercept; b: slope) of the surface and perimeter of the appendix masculina with the carapace length of the present study differ than those values obtained from the regression of appendix masculina length-carapace length in males specimens caught in the Irish Sea (McQuaid *et al.*, 2006). Hillis (1981) suggested that in the future the ratio appendix masculina-carapace length in *N. norvegicus* could be used for separating age-groups and could be proved that this ratio could be efficient for separating age-groups in areas where length-frequency modes are not reliable. Our findings do not show any discontinuities in the relationships between these measurements during growth for both species. The positive allometry at a high level of statistical significance is an evidence for the non-degeneration of the appendix masculina in *N. norvegicus*.

The mechanics of copulation in *N. norvegicus* have been described by Lüling (1958) based on the morphology of the pleopods and the thelycum, although already figured by Höglund (1942) without precise details. Copulation only occurs between mature males and newly-moulted females (Farmer, 1975). The observed highest mean values of the appendix masculina's surface and perimeter in March and April, the start of the mating of this species (Relini *et al.*, 1998), supports the hypothesis that this appendix is most likely involved in copulation. In addition to this, mating in *Nephrops* occurs annually in the Irish Sea, following female ecdysis and just after the previous egg batch has hatched which is during April–May (Nichols *et al.*, 1987). In the same

Table 1. Descriptive statistics of the monthly samples of male of *N. norvegicus* used in the analyses (surface in mm^2 and perimeter of appendix masculine and carapace length (CL) in mm (all values are significant at P<0.05)

Measure	March		April		May		June		July		August		September	
	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
Surface, (mm^2)	8.56±2.54	69	9.33±2.68	40	7.78±2.16	17	7.01±1.98	45	6.82±1.67	38	5.98±2.01	29	5.25±1.40	254
Perimeter, (mm)	13.44±3.1	69	14.00±3.2	40	12.92±2.9	17	12.24±2.7	45	13.50±1.3	38	11.92±1.7	29	10.25±1.7	255
CL (mm)	42.09	69	43.66	40	39.77	17	44.36	45	47.32	38	46.97	29	33.40	254

Table 2. Allometry of the surface and perimeter of the appendix masculina measured in *N. norvegicus*. CL: carapace length; a: y-intercept; b: slope; r: correlation coefficient; standard error of slope, t: Student's t-test statistic. In all cases P<0.05

Dimension Measured/CL	Sampling month	a	b	r	Standard error of slope	t-test	Allometry
Surface	March	-2.41	2.04	0.97	0.06	33.46	Positive
Perimeter	March	-0.42	0.95	0.97	0.0004	32.24	Negative
Surface	April	-2.68	2.21	0.97	0.087	25.47	Positive
Perimeter	April	-0.65	1.099	0.97	0.043	25.44	Positive
Surface	May	-3.60	2.80	0.99	0.002	36.89	Positive
Perimeter	May	-1.07	1.36	0.99	0.018	39.33	Positive
Surface	June	-1.23	2.04	0.98	0.07	36.22	Positive
Perimeter	June	-0.93	1.23	0.96	0.004	29.87	Positive
Surface	July	-2.66	2.34	0.97	0.063	33.12	Positive
Perimeter	July	-0.98	1.58	0.98	0.015	29.15	Positive
Surface	August	-1.87	2.36	0.99	0.046	30.87	Positive
Perimeter	August	-1.01	1.98	0.98	0.012	24.76	Positive
Surface	September	-3.26	2.57	0.98	0.035	71.49	Positive
Perimeter	September	-0.85	1.22	0.97	0.017	70.40	Positive
Surface	Total	-3.18	2.54	0.98	0.02	94.91	Positive
Perimeter	Total	-0.83	1.20	0.98	0.01	93.70	Positive

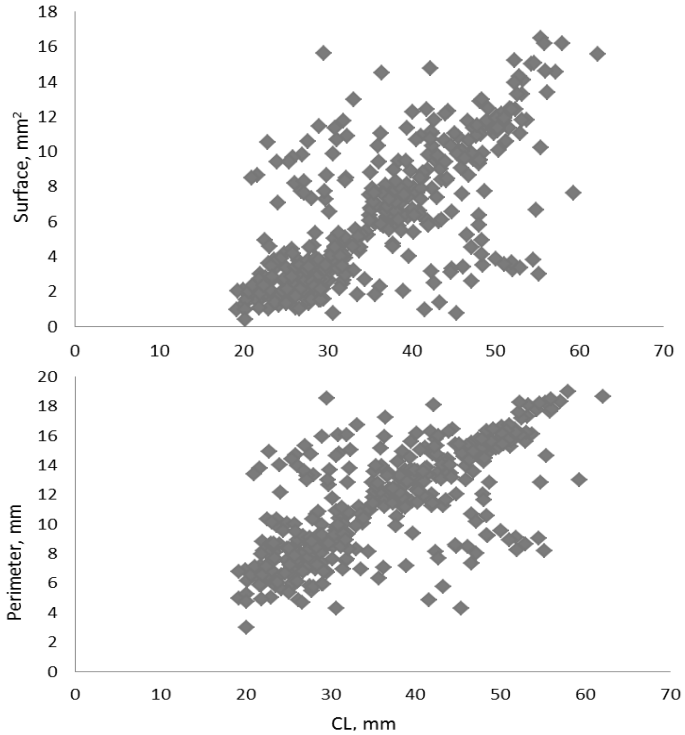

Figure 3. Relative growth of the appendix masculina's surface and perimeter of *N. norvegicus.*

Table 3. Monthly occurrence of ovarian maturity stages of *N. norvegicus* females from the C. Aegean Sea

Sampling month	Maturity stage				
	1	2	3	4	5
March	30.72 (N=51)	17.47 (N=29)	42.77 (N=71)	1.20 (N=2)	7.84 (N=13)
April		13.41 (N=12)	62.20 (N=51)	21.95 (N=18)	2.44 (N=2)
May		22.72 (N=5)	50 (N=11)	13.64 (N=3)	13.64 (N=3)
June		8.45 (N=15)	20.02 (N=36)	69.19 (N=126)	2.34 (N=4)
July			30.85 (N=10)	69.15 (N=24)	
August	2.24 (N=4)	3.25 (N=7)	18.22 (N=39)	51.84 (N=110)	24.45 (N=52)
September	5.24 (N=6)	35.86 (N=44)		14.58 (N=18)	44.32 (N=56)

period (March, April), the highest percentage of mature males was found in the present study area, whereas the lowest percentage occurred in September.

In conclusion, the current study is a basic description of the morphometric relationships of the appendix masculina in mature males' specimens of *N. norvegicus*. Our results indicate that since the biggest values of the appendix masculina were found during the copulation period of *N. norvegicus*, this accessory male organ plays a significant role in the copulation process by accommodating the sperm transfer into females. However, a more extended study, with samples collected throughout the year, is required to clarify the relationship between the reproductive process and the measurements of the appendix in *N. norvegicus*.

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