ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 56(2): 233 - 244, 2015	ORIGINAL SCIENTIFIC PAPER

Towards a better management of *Nassarius mutabilis* (Linnaeus, 1758): biometric and biological integrative study

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Fishing of Nassarius mutabilis *is by far the most important activity carried out by artisanal fisheries in the central and northern Adriatic Sea, accounting for more than 35% of total fishing effort and yielding from 2000 to 3000 t of landings each year. This gastropod is targeted from the beginning of autumn to the end of spring using basket traps. Despite its importance under a socio-economic point of view, the scientific studies on the biology and ecology of this species are very scarce and the failure of the current management measures could also be attributed to the scarce knowledge on the biology of this species in the area. Taking this statement into consideration, the results of the present study contribute to fill a few existing gaps and may be useful to improve the management measures currently in force. Samples were collected from March 2006– March 2008 during monthly fishing surveys and carried out using basket traps in the central Adriatic Sea. A total of 383 males (size range 7–29 mm SH) and 504 females (size range 15-32 mm SH) were caught. Mean SH (± SD) of males was 15.84±3.57 mm and mean SH of females was 25.31±3.06 mm. A significant difference in the biometric characteristics between the two sexes was discovered. The condition index (CI) showed similar seasonal trends for both sexes, with a decrease from late winter to spring followed by an increase from summer to fall - early winter. The minimum values were recorded in summer and the maximum ones in winter, following an opposite trend in respect to water temperature. In the males this trend was observed starting from size classes larger than 11 mm SH, hence this size might be very close to the size at first sexual maturity. The size at first maturity hypothesized for females (16-20 mm SH) agrees with the minimum landing size (MLS) currently established for this species (20 mm SH). The absence of small females (SH < 15 mm) and large males (SH > 23 mm) leads to suppose a sex reversal from male to female at around 20 mm SH. On this basis, the management measure of MLS leads to the selective retention of females and, hence, to a sexual gap between the two sexes with possible consequences on the biology of the species and the resilience of the stock. The peaks of CI observed for males and females confirmed that the spawning season of this gastropod occurs in late winter – early spring, according to the abundant presence of egg capsules attached to submerged substrates in that period at sea. Consequently, from a management point of view, it would be advisable to shorten the fishing season that at present extends from fall to later spring.*

Key words: *Nassarius mutabilis,* biometrics, condition index, sexual maturity, Adriatic Sea

INTRODUCTION

Nassarius mutabilis is a gastropod of the family *Nassariidae* which represents an important resource for small-scale fisheries in the central and northern Adriatic Sea. Around 250 fishing vessels (from 6 to 12 m Loa) target *N. mutabilis* in this area using basket traps from the beginning of autumn to the end of spring and the fishing season is established year by year by the Local Maritime Authorities (SOLUSTRI *et al.*, 2002). This is by far the most important activity carried out by artisanal fisheries, accounting for more than 35% of total fishing effort (number of vessels x days at sea) and yielding from 2000 to 3000 t of landings each year (GRATI *et al.*, 2010).

Analysis of the trend of Catches per Unit Effort (CPUEs) in the last 10 years gave evidence of a decrease in catch rates (GRATI *et al.*, 2010).

Despite its importance under a socio-economic point of view, the scientific studies on the biology and ecology of *N. mutabilis* are very scarce and dated (CRISP, 1978; PLESSI *et al.*, 2001; SALVATO *et al.*, 2001).

This species inhabits fine sands and fine muddy sands at depths between 2 and 15 m along the Mediterranean coasts (FISHER, BAUCHOT & SCHNEIDER, 1987), but its distribution is not homogeneous and no particular bathymetric preference has been observed (PICCINETTI & PICCINETTI MANFRIN, 1998).

It is a gonochoristic species with possible sequential hermaphroditism (BALDUCCI *et al.*, 2006). Its reproductive period goes from late winter to spring. Fertilization is internal and eggs are laid between March and May, enclosed in conical, transparent egg capsules which are attached to hard substrates (FABI & GIANNINI, 1983). When the eggs hatch an indirect development occurs with the production of a veliger larva (RIEDL, 1991).

N. mutabilis spends most of the day buried into the substrate with its proboscis protruded from it. During the night, also thanks to its well-developed sense of smell, it emerges to search for food (DIMON, 1905; COPELAND, 1918; KOHN, 1961; BEDULLI, 1976; CRISP, 1978).

The smallest specimens (Shell Height <15 mm) are attracted by substrata rich in detritus as they feed on it, while the largest ones are carnivorous (RELINI, BERTRAND & ZAMBONI, 1999) and feed on dead organisms or live animals which suffocate by means of their foot (TORELLI, 1982).

The general goal of the present study is to partially fill existing knowledge gaps on the biology of *N. mutabilis* in order to improve the management measures for a better exploitation of this resource.

MATERIAL AND METHODS

From March 2006 to March 2008 monthly fishing surveys using basket traps were carried out along the coast of the Marche Region (Adriatic Sea; Fig. 1) for a total of 25 samplings. The basket traps used in the survey had same structure as those employed by professional fishermen (Fig. 2), but smaller mesh size. They were

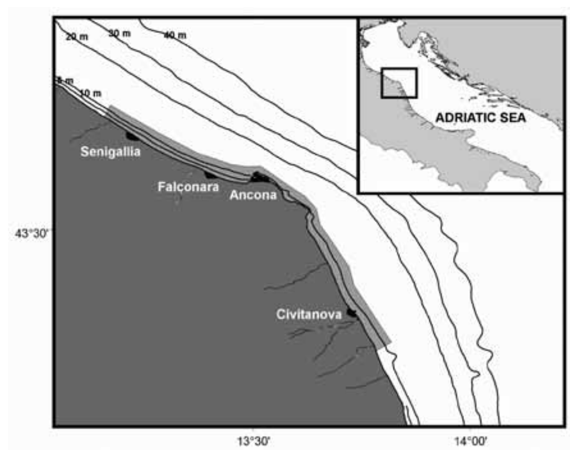

Fig. 1. Map of the area (shaded area) where fishing surveys using basket traps were carried out

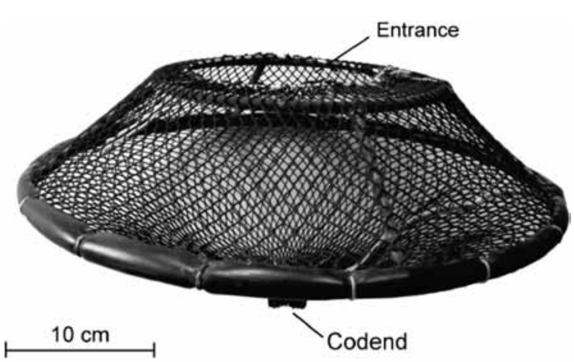

Fig. 2. Picture of a basket trap for Nassarius mutabilis

made of a truncate-cone steel frame (Ø = 6 mm) covered by a cylindrical panel of net (stretched mesh size = 5 mm) hanged either on the small hoop (upper base) and on the large hoop (lower base), and tied in the lower part creating a sort of cod end. The trap entrance is on the upper base.

A total of 20 basket traps baited with frozen fish were used during each fishing trip. The fishing time was around 24 hours and all the catches were sorted in the laboratory. At each fishing trip the bottom temperature was recorded using a CTD.

Specimens belonging to the target species *N. mutabilis* were separated from the by-catch and measured for Shell Height (SH) to the lowest 0.1 mm.

Six size classes were considered (6-10 mm; 11-15 mm; 16-20 mm; 21-25 mm; 26-30 mm; 31-35 mm) and, when available, 10 individuals of N. *mutabilis* belonging to each size class were randomly chosen at each fishing survey for a total of 887 specimens. The shell of each specimen was shattered using a vice, to allow the determination of sex (it was assumed the presence/absence of penis as distinctive character for sex determination) and the separation of the meat from the shell.

The following parameters were recorded on each specimen: shell fresh weight (FWS; 0.0001 g), soft tissue fresh weight (FWM; 0.0001 g); shell dry weight (DWS; 0.0001 g), soft tissue dry weight (DWM; 0.0001 g); shell ashes weight (SAW; 0.0001 g), soft tissue ashes weight (MAW; 0.0001 g); maximum shell diameter (D; 0.1 mm), last whorl height (W; 0.1 mm); aperture height (A; 0.1 mm). Dry weight was obtained by drying specimens for 24 h in an oven at 100°C, while ash weight was obtained by incinerating individuals in a muffle furnace for 24 h at 550°C (VALLI, BUSETTO & BORGHESE, 2000).

Outliers' values were identified by means of Cook's Test and not considered in the subsequent analysis.

Linear regression was used to compare the four morphometric measures (SH, D, A, W), as well as the four weights (DWM-MAW, FWM, DWM, MAW) separately for males and females. Slopes of the linear regressions obtained for males and females were compared using a Student's t-test. Prior to apply linear regression, the respect of the principal assumptions (statistical independence, homoscedasticity and normality of the errors), which justify the use of linear regression models for purposes of inference or prediction, have been explored.

The morphometric measures and the weights were also analysed separately using a multivariate approach. A Draftsman plot was used to evidence variables correlation and in the case of highly correlated variables we excluded them from the following analysis. Prior to apply the Principal Component Analysis, normalise operation (the values for each variable have their mean subtracted and are divided by their standard deviation.) was employed, this is usually necessary for data where variables are often on different scales with arbitrary origins as in the present case. A PCA was carried out in order to provide a bi-dimensional representation. To test the null hypothesis of no differences in dispersion between the two sexes, a test for homogeneity of multivariate dispersion based on Euclidian distance was applied. Following this, morphometric data was analysed using the per mutational multivariate analysis of variance (PERMANOVA; ANDERSON, 2001) based on Euclidean distances with 4999 permutations to identify differences in a 2-way fixed term design, were the main factors were sex and season. Because of the interaction of the previous terms, a one way design was performed with sex as the main factor and for each sex using season as its main factor. In this case a pair wise test was also applied.

Thirteen condition indices (CI) were computed on a monthly basis for males, females and total sample (BODOY, PROU & BERTHOME, 1986; VALLI, BUSETTO & BORGESE, 2000):

1. $$\frac{(DWM - MAW)}{(SH + D + W)^3}$$

2. $$\frac{FWM}{(SH \cdot D \cdot W)}$$

3. $\frac{DWM}{(SH \cdot D \cdot W)}$

4. $\frac{(DWM - MAW)}{(SH \cdot D \cdot W)}$

5. $\frac{FWM}{(SH + D + W)^3}$

6. $\frac{DWM}{(SH + D + W)^3}$

7. $\frac{(FWM + FWS)}{(SH \cdot W)}$

8. $\frac{FWM}{SH^3}$

9. $\frac{DWM}{SH^3}$

10. $\frac{(DWM - MAW)}{SH^3}$

11. $\frac{FWM}{(SH + D + A)^3}$

12. $\frac{DWM}{(SH + D + A)^3}$

13. $\frac{(DWM - MAW)}{(SH + D + A)^3}$

Condition index is a way to measure the overall health of an organism, it depends on how much it is eating compared to the energy it has to use to live, migrate, reproduce, and do its other activities.

Variability of indices was calculated following the method suggested by VALLI, NODARI & CASTENETTO (1988) and the Coefficients of Variation (CV) and were compared by means of Friedman test. The indices were set following an increasing order of variability and pairwise comparisons were performed by means of Wilcoxon test. Subsequently, the Kruskal-Wallis test was applied to the indices to evidence monthly sensibility in the samples.

The best CI was chosen on the basis of the lowest variability and highest sensibility.

Eventual correlation of the previously selected index by sex with the bottom temperature was investigated using Spearman's rho test.

RESULTS AND DISCUSSION

Demography of catches shows that the traps used in the experimental surveys are provided with a very small mesh size and, hence, the catches could be considered representative of the population at sea.

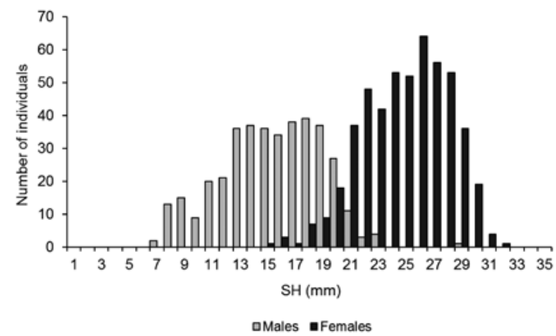

Fig. 3. Shell height frequency distributions of males and females of Nassarius mutabilis selected for the analysis

A total of 383 males and 504 females were measured for SH. Males belonged to the size range 7–29 mm SH and females to the size interval 15-32 mm SH (Fig. 3). The mean SH (± SD) of males was 15.84±3.57 mm and the mean SH of females was 25.31±3.06 mm. The absence of small females and large males might be due to a sex reversal from male to female at a SH of around 18-20 mm. Indeed, hermaphroditism occurs in 3% of Prosobranchia Gastropods and most of the hermaphrodite genera belong to the Cocculiniformia, Eulimiodea and Calyptraeoidea (HELLER, 1993). HELLER (1993) reported sequential hermaphroditism for 17 Genus of Prosobranchia Gastropods. Sequential hermaphroditism, contrary to simultaneous hermaphroditism, is considered to be selectively advantageous when the reproductive success of males is less sensitive to size (or age) than that of females (HOAGLAND, 1978; WARNER, 1978; GHISELIN, 1987; WRIGHT, 1988). Obviously, deeper analysis of the gonads would be necessary to confirm the occurrence of sequential hermaphroditism also in *N. mutabilis*.

Positive allometric relationships were obtained for both sexes either between the morphometric measures (SH vs D, SH vs W, SH vs A, D vs W, D vs A, and W vs A; Fig. 4) and

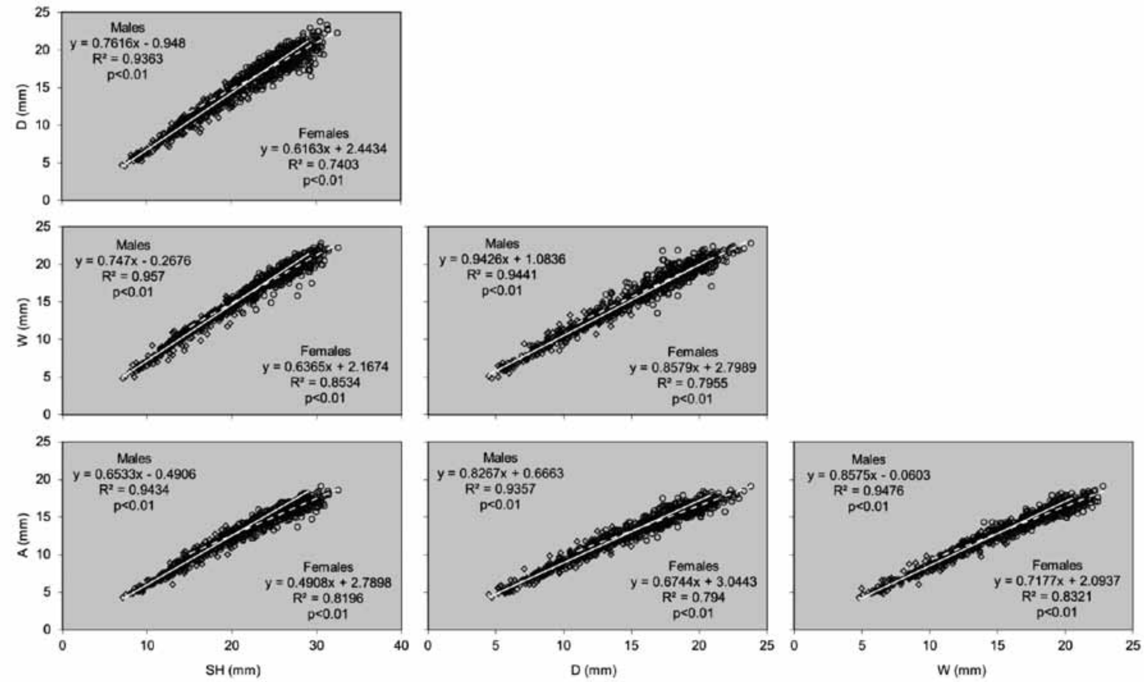

Fig. 4. Linear regressions of the biometric parameters of Nassarius mutabilis. D = maximum diameter of shell; W = last whorl height; A = aperture height; SH = shell height. Males are represented by diamonds and white lines; females are represented by circles and white dotted lines

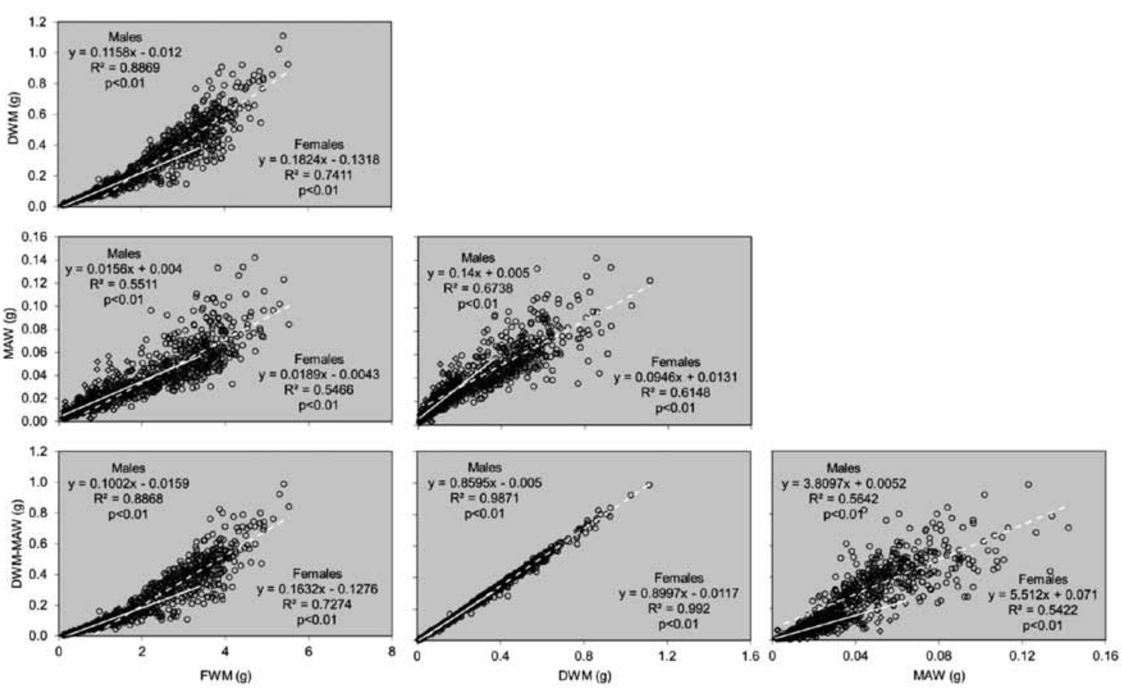

Fig. 5. Linear regressions of the weight parameters of Nassarius mutabilis. DWM = soft tissue dry weight; MAW = soft tissue ashes weight; FWM = soft tissue fresh weight. Males are represented by diamonds and white lines; females are represented by circles and white dotted lines

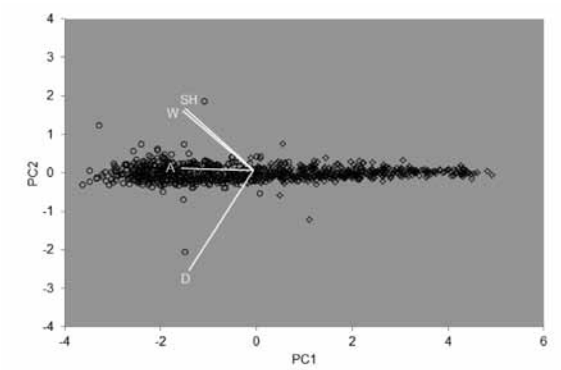

Fig. 6. Principal Component Analysis bi-plot ordination of four biometric indicators of Nassarius mutabilis specimens. D = maximum diameter of shell; W = last whorl height; A = aperture height; SH = shell height. Males are represented by diamonds and females by circles

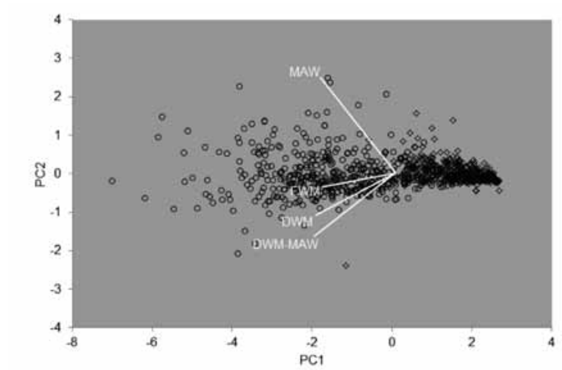

Fig. 7. Principal Component Analysis bi-plot ordination of four weight indicators of Nassarius mutabilis specimens. DWM = soft tissue dry weight; MAW = soft tissue ashes weight; FWM = soft tissue fresh weight. Males are represented by diamonds and females by circles

*Table 1. Results of the PERMANOVA analyses. d. f.: degree of freedom; MS: mean square; *: significant; **: highly significant; ≠: significant difference*

BIOMETRIC DATA					
Two-way PERMANOVA					
Factors	d. f.	MS	Pseudo F	p (perm)	
Sex	3	4.5	3.4	0.013*	
Seasons	1	1706.8	1275.7	0.001**	
Sex x Seasons	3	10.1	7.6	0.001**	
One-way PERMANOVA Males					
Factor	d. f.	MS	Pseudo F	p (perm)	Pairwise test
Seasons	3	21.5	5.6	0.001**	3 ≠ 1, 2, 4
One-way PERMANOVA Females					
Factor	d. f.	MS	Pseudo F	p (perm)	Pairwise test
Seasons	3	13.7	3.5	0.012*	3 ≠ 1, 2
WEIGHT DATA					
Two-way PERMANOVA					
Factors	d. f.	MS	Pseudo F	p (perm)	
Sex	1	1517.7	1004.4	0.001**	
Seasons	3	28.2	18.7	0.001**	
Sex x Seasons	3	34.9	23.1	0.001**	
One-way PERMANOVA Males					
Factors	d. f.	MS	Pseudo F	p (perm)	Pairwise test
Seasons	3	1.6	2.9	0.019*	3 ≠ 1, 2, 4
One-way PERMANOVA Females					
Factors	d. f.	MS	Pseudo F	p (perm)	Pair-wise test
Seasons	3	83.2	36.5	0.001**	3 ≠ 1, 2, 4; 2 ≠ 1; 4 ≠ 1

the weights (FWD vs DWM, FWM vs MAW, FWM vs DWM-MAW, DWM vs MAW, DWM vs DWM-MAW, MAW vs DWM-MAW; Fig. 5).

The R^2 values of the linear regressions between the length parameters of males ($0.936 \leq R^2 \leq 0.957$) were higher than those observed for females ($0.740 \leq R^2 \leq 0.853$; Fig. 4).

Slightly lower R^2 coefficients were obtained for the weight parameters of both sexes, due to a higher variability especially associated to the largest specimens. Also in this case, males ($0.551 \leq R^2 \leq 0.987$) showed R^2 coefficients generally higher than females ($0.542 \leq R^2 \leq 0.992$; Fig. 5).

The Student's t-test evidenced high significant differences between males and females between all the linear regressions calculated for morphometric measures and weight parameters ($p<0.01$), with the exception of DWM vs DWM-MAW ($p > 0.05$) and FWM vs MAW ($p > 0.05$). Comparison of morphometric parameters' regressions between males and females indicated that the sex reversal induces significant changes in the shape of shell. In fact, the development of shell height in respect to the other parameters (W, A, and D) is more consistent for females than for males. This can be related to the shape and position of the female gonad that develops longitudinally along the shell height.

The multivariate analyses showed that the differences between the two sexes in terms of biometric (Fig. 6) and weight data (Fig. 7) were mainly driven by aperture height and soft tissue fresh weight, respectively. In both cases the PCA biplots provided a good representation of the data set variability, with the first axis (PC1) explaining 97% and 93% of the variance associated to biometric and weight data, respectively (Figs 6 and 7). The PERMDISP showed that the dispersion of biometric data around the centroid was homogenous for the two sexes ($F = 3.097$; p (perm) = 0.094). On the contrary, the same analysis carried out on the weight data evidenced dis-homogenous dispersions, being the males' data less disperse than those of females ($F = 290.84$; p(perm) = 0.001). The two-way PERMANOVA applied to biometric and weight data gave significant interactions between the factors "sex" and "seasons" (Table 1). The same analysis applied separately for each sex evidenced in males significant differences in both length and weight data, being summer always different from the other seasons. As already observed by other authors, this is probably due to the fact that

*Table 2. Condition indices with results of statistical analysis. n.s. = not significant; * = significant (p < 0.05); ** = highly significant (p < 0.01)*

Condition Index	Rank Average	Wilcoxon test	Kruskal-Wallis test
(DWM-MAW)/(SH+D+W)^3	1.1044	**	**
(DWM-MAW)/(SH+D+A)^3	2.3624	*	**
DWM/(SH+D+W)^3	2.5824	*	**
DWM/(SH+D+A)^3	3.9970	*	n.s.
FWM/(SH+D+W)^3	5.0041	n.s.	n.s.
FWM/(SH+D+A)^3	5.9899	n.s.	n.s.
DWM/SH^3	7.0344	n.s.	*
(DWM-MAW)/(SH·D·W)	7.9952	n.s.	*
DWM/(SH·D·W)	9.1198	*	*
(DWM-MAW)/SH^3	10.0296	n.s.	*
FWM/SH^3	10.8244	*	*
FWM/(SH·D·W)	11.9703	n.s.	*
(FWM+FWS)/(SH·W)	12.9858		*

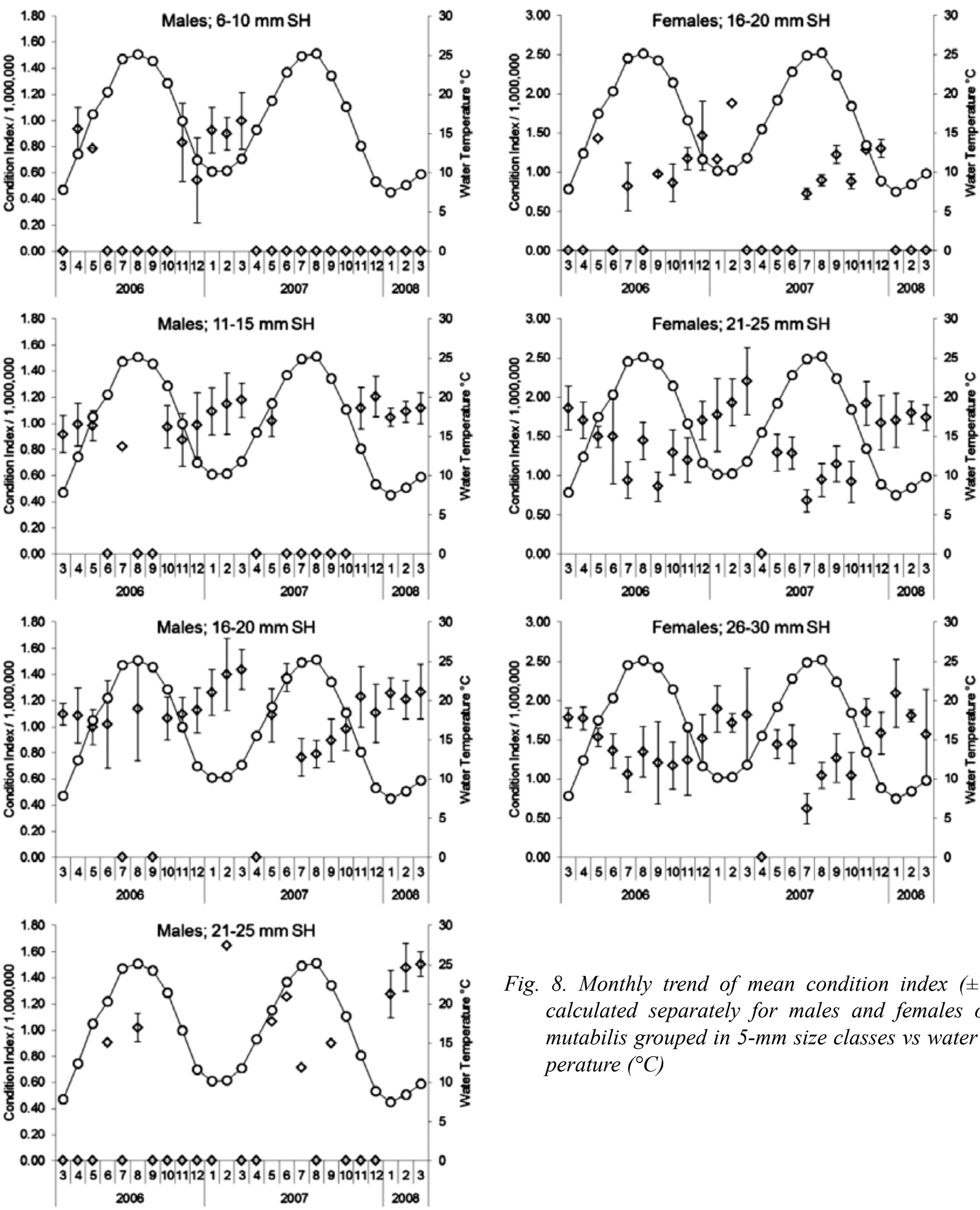

Fig. 8. Monthly trend of mean condition index (± SD) calculated separately for males and females of N. mutabilis grouped in 5-mm size classes vs water temperature (°C)

in summer the growth rates are higher in respect with the winter ones (CESPUGLIO, PICCINETTI & LONGINELLI, 1999). Differently, females showed a lower degree of differentiation in biometrics data, while weight data were characterized by high significant seasonal differences (Table 1).

According to what was observed by VALLI, BUSETTO & BORGHESE (2000) for *Nassarius reticulatus*, statistical analysis evidenced that the best CI tested among 13 different ones was $(DWM – MAW)/(SH+D+W)^3$ (Table 2) because it showed the lowest variability and the highest monthly sensibility in the samples. The monthly trend of CI evidenced two main findings. The CI showed an evident seasonal trend only for the SH classes larger than 11 mm; hence this value might be very close to the size at first sexual maturity of males. Obviously, also in this case further analysis on the gonads, at a microscopic level, could confirm this finding.

This CI followed similar trends for both sexes, with a decrease from late winter to spring followed by an increase from summer to fall-early winter, confirming that the spawning season of this gastropod occurs in late winter – early spring. This is in accordance with the abundant presence of egg capsules attached to submerged substrates in that period at sea. Consequently, from a management point of view, it would be advisable to shorten the fishing season that at present extends from fall to later spring (Fig. 8). The lowest values were recorded in summer and the highest ones in winter, with an opposite trend in respect to water temperature (Spearman test: $p << 0.01$). This trend was less evident for males belonging to the size classes 6-10 mm SH, and for females belonging to the size classes 16-20 mm SH. On this basis, the size at first maturity hypothesized for females agrees with the minimum landing size currently established for this species (20 mm SH). On the other hand, taking into account the demography structures of the catches and the eventual sex reversal at around 20 mm SH, this management measure leads to the selective retention of females and, hence, to a sexual gap between the two sexes with possible consequences on the biology of the species and the resilience of the stock.

CONCLUSIONS

Nassarius mutabilis represents an important resource for the small-scale fishers of the central and northern Adriatic Sea who exploit this species from fall to late spring by means of specific basket traps baited with dead fish (GRATI *et al.*, 2010). The management measures adopted up to now include minimum landing size (20 mm SH), daily quota per vessel (from 100 kg to 180 kg depending on the crew size), and fishing season (from November to May). However, these measures have not been sufficient to assure a rational exploitation of this resource and to maintain the stock consistent over time, as showed by the gradual decrease of catches which have occurred in the last 10 years (GRATI *et al.*, 2010). On the other hand, despite its importance under the socio-economic point of view, the scientific literature on *N. mutabilis* is very poor (CRISP, 1978; PICCINETTI & PICCINETTI-MANFRIN, 1998; SALVATO *et al.*, 2001; PLESSI, BERTELLI & MONZANI, 2001; SOLUSTRI *et al.*, 2002) and the failure of the current management measures could be also attributed to the scarce knowledge on the biology of this species in the area.

The main findings of the present study contribute to partially fill existing gaps and may be useful to improve the management measures currently in force.

In particular, the following highlights have been identified:

- sex reversal from male to female at around 18-20 mm SH;
- sex reversal induces significant changes in the shape of shell;
- size at first sexual maturity for males at around 11 mm SH;
- size at first sexual maturity for females at around 20 mm SH;
- in summer the growth rates are higher in respect with the winter ones;
- the spawning season occurs in late winter – early spring;

On this basis, from a management point of view it would be advisable to:

- shorten the fishing season (autumn – spring) from autumn to end of winter;
- revise the minimum landing size (20 mm SH) to avoid the selective retention of females, with possible consequences on the spawning stock structure and dynamics.

ACKNOWLEDGEMENTS

The authors wish to thank Marco BERLUTI, captain of the fishing vessel Vulcano, who helped us during samplings at sea.

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Received: 17 May 2013
Accepted: 13 February 2015

Biometrijska i biološka integrativna studija o boljem gospodarskom upravljanju morskim pužem, *Nassarius mutabilis* (Linnaeus, 1758)

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SAŽETAK

Lovljenje morskog puža *Nassarius mutabilis* je daleko najvažnija djelatnost malog priobalnog ribolova u središnjem i sjevernom Jadranu, što čini više od 35% ukupnog ribolovnog napora s prinosom od 2000 do 3000 tone svake godine. Ovaj puž se lovi od početka jeseni do kraja proljeća pomoću vrše. Unatoč njegovoj društveno-ekonomskoj važnosti, znanstvene studije o biologiji i ekologiji ove vrste su vrlo rijetke, stoga se neuspjeh trenutnih mjera upravljanja također može pripisati oskudnom znanju o biologiji ove vrste u navedenom području. Rezultati ovog istraživanja pridonose rješavanju nekoliko postojećih nedostataka i mogu biti korisni za poboljšanje mjera upravljanja koje su trenutno na snazi. Uzorci su prikupljeni pomoću vrše (košare; pletene stupice) u razdoblju od ožujka 2006. do ožujka 2008. godine tijekom mjesečnih istraživanja u središnjem Jadranu. Ukupno su uhvaćena 383 mužjaka (raspon veličina 7-29 mm SH) i 504 ženke (raspon veličina 15 - 32 mm, SH- visina školjke-puža). Srednja visina puža (± SD - značajna razlika) mužjaka iznosila je 15,84 ± 3,57 mm, a srednja visina kućice ženki je iznosila 25.31 ± 3.06 mm. Otkrivena je značajna razlika biometrijskih karakteristika između dva spola. Indeks stanja (CI) pokazao je slične sezonske trendove za oba spola, s padom od kraja zime do proljeća, nakon čega slijedi porast od ljeta do jeseni odnosno početkom zime. Najmanje vrijednosti zabilježene su u ljeto, a maksimalne zimi, što predstavlja suprotni trend u odnosu na temperaturu vode. Kod mužjaka je ovaj trend uočen počevši od kategorije visine kućice veće od 11 mm, pa bi stoga ove veličine mogle biti vrlo blizu veličini puža kod prve spolne zrelosti. Hipotetski veličina ženki kod prve spolne zrelosti (visina kućice 16-20 mm) se slaže s veličinom minimalnog ulova (MLS) trenutno uspostavljenog za ovu vrstu morskog puža (visina kućice 20 mm). Odsutnost malih ženki (visina kućice <15 mm) i velikih mužjaka (visina kućice > 23 mm) dovodi do pretpostavke da se preokret kod muškog i ženskog spola odvija kada kućica dosegne visinu od oko 20 mm. Na temelju toga, mjera upravljanja bi trebala dovesti do selektivnog zadržavanja ženki, a time i do spolnog razdvajanja s mogućim posljedicama na biologiju vrste i održivost stocka. Vrhunac indeksa stanja uočenog kod mužjaka i ženki je potvrdio da se mriještenje ovog mekušca javlja krajem zime - početkom proljeća, u skladu s obilnom prisutnošću kapsula jajašaca pričvršćenih na potopljene supstrate u tom razdoblju. Prema tome, s gledišta gospodarskog upravljanja, bilo bi poželjno da se skrati ribolovna sezona koja se u ovom trenutku proteže od jeseni do kasnog proljeća.

Ključne riječi: *Nassarius mutabilis,* biometrija, indeks stanja, spolna zrelost, Jadransko more