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DEFINITION OF A NEW FORMULA FOR THE CALCULATION OF THE TOTAL HEIGHT OF THE FAN SHELL *PINNA NOBILIS* IN THE MIRAMARE MARINE PROTECTED AREA (TRIESTE, ITALY)

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ABSTRACT

Four years of monitoring of Pinna nobilis *population in Miramare Marine Protected Area (Trieste, Italy) have led to the development of a new equation for indirect calculation of total shell height (H_{tot}) for this bivalve, endemic to the Mediterranean Sea. As the formulas listed in the cited literature are not corroborated by* in situ *measured growth data, it was necessary to describe a specific formula for Miramare population. This exponential equation was called MirExp. A specific growth curve has also been created with this formula and the data collected every year from 2008 to 2011. This curve shows that in Miramare* P. nobilis *grows faster in its early years than what is reported in other studies carried out in Croatia and Spain. Population density is also the highest among those reported in consulted papers, which make reference to studies conducted in various Mediterranean protected and non-protected areas.*

Key words: *Pinna nobilis,* population dynamics, growth, marine protected area, Adriatic Sea

DEFINIZIONE DI UNA NUOVA FORMULA PER IL CALCOLO DELLA LUNGHEZZA TOTALE DEGLI ESEMPLARI DI *PINNA NOBILIS* PRESENTI NELLA AMP DI MIRAMARE (TRIESTE, ITALIA)

SINTESI

Dopo 4 anni di monitoraggio del popolamento di Pinna nobilis *all'interno dell'Area Marina Protetta di Miramare (Trieste) si è giunti alla definizione di una nuova equazione per il calcolo indiretto della lunghezza totale (H_{tot}) della conchiglia di questo bivalve endemico del Mediterraneo. Le formule riportate in bibliografia non trovano riscontro nei dati di accrescimento misurati in situ e quindi è stato necessario descrivere una formula specifica per il popolamento di Miramare, denominata MirExp trattandosi di una equazione esponenziale. Grazie a questa formula e ai dati raccolti annualmente dal 2008 al 2011, si è definita anche una curva di accrescimento specifica che ha evidenziato come questo bivalve si accresca più velocemente nei primi anni a Miramare rispetto a quanto riportato in altri studi svolti in Croazia e Spagna. La densità di popolamento, inoltre, è la più alta tra quelle riportate nei lavori consultati effettuati in varie aree del Mediterraneo, protette e non.*

Parole chiave: *Pinna nobilis*, dinamica di popolazione, accrescimento, area marina protetta, Adriatico

INTRODUCTION

Pinna nobilis L., 1758 is an organism of the phylum Mollusca, class Bivalvia and subclass Pteriomorphia, endemic to the Mediterranean Sea, where it is present since the end of the Miocene (Gómez-Alba, 1988). It is one of the largest living bivalves and among these, like other Pinnids, shows the fastest growth of the shell (Richardson *et al*., 2004): specimens with a length of 120 cm have been found (Zavodnik *et al*., 1991; Richardson *et al*., 1999; Garcìa-March *et al*., 2007). The fan shell live partially buried upright in the sandy and pebbly substrata particularly in association with marine phanerogams such as *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* e *Nanozostera noltii* (Zavodnik *et al*., 1991).

Despite the interest of the scientific community for this species and its conservation, knowledge about the ecology and population dynamics of *P. nobilis* are still fragmentary. Although in France and in Spain there are several studies on various aspects of this bivalve, such as its ecology (Combelles *et al*., 1986; De Gaulejac & Vicente, 1990; Butler *et al*., 1993), morphology, growth and age assessment (Moreteau & Vicente, 1980; García--March & Ferrer, 1995, Richardson *et al*., 1999; Hendriks *et al*., 2012), gametogenesis and reproduction (De Gaulejac, 1995; De Gaulejac *et al*., 1995a, b), there are only sporadic publications on the ecology and morphology of this species in the Adriatic Sea (Mihailinović, 1955; Zavodnik, 1967; Zavodnik *et al*., 1991; Peharda *et al*., 2002; Šiletić & Peharda, 2003; Prestinenzi, 2009).

The importance of studying this bivalve mollusc is shown, *inter alia*, by its protection status under official documents such as the Barcelona Convention (1995), ratified by the Italian government with Act no. 175 of 25/05/1999, and the EU Habitats Directive (43/92). In particular, *P. nobilis* is listed under Annex II of the Barcelona Convention including endangered species requiring a specific conservation strategy, and under Annex IV of the Habitats Directive on the conservation of natural and semi-natural habitats and of wild flora and fauna, where species requiring strict protection measures are listed.

Although protective measures have been introduced already in the early nineties, there is still uncertainty on the conservation status of *P. nobilis* populations at Mediterranean scale (Addis *et al*., 2009). The amateur collection of shells, the destruction due to anchoring and fishing both commercial and recreational, but especially the regression of the seagrass meadows as its elective habitat, have contributed to the decline of the population of *P. nobilis* (Zavodnik *et al*., 1991; Centoducati *et al*., 2007; Katsanevakis, 2007b). On the contrary, an increase of the number of individuals of this species has been reported in the Gulf of Trieste (Northern Adriatic Sea) in recent years (Prestinenzi, 2009), particularly in Miramare Marine Protected Area as for the juveniles is concerned. Therefore marine reserves - which, regardless of their size, are associated to high values of density, biomass, individual size and specific diversity (Halpern, 2003) - can be considered essential to protect a breeding stock that can increase marine living resources through spill-over (Tewfik & Béné, 2003). Estimates of *P. nobilis* recruitment and spill-over rates on populations in small-size MPAs (Mljet National Park, Croatia) have already been made by using models (Peharda & Vilibić, 2008), which however need further refinement through *in situ* experiments and systematic monitoring.

To better understand this new positive trend in the presence of *P. nobilis*, a monitoring was carried out on a non-stop basis in Miramare MPA for 4 years, from 2008 to 2011. Its aim was to assess the population structure and dynamics inside the Reserve by counting and measuring the size of individuals, estimating their density and studying their spatial distribution. Data collected have been compared over four years thanks to the use of tagging techniques, through which monitored individuals were identified. Measurements were made *in situ* by SCUBA divers as this is a method of non-destructive sampling and therefore an excellent tool for the analysis of species or habitats protected or rare (Katsanevakis, 2007a).

MATERIALS AND METHODS

The sampling methodology follows the indications provided by the Protocol to study and monitor *Pinna nobilis* populations within marine protected areas written by MEPA (Malta Environment and Planning Authority) in the framework of the MedPAN project (García-March & Vicente, 2006). Data were collected through measurements made by SCUBA divers. In Miramare, a round area of nearly 5.6 m radius (approximately 100 m^2) was defined: the area was sampled by two SCUBA divers swimming from its central point - whose GPS coordinates were recorded - outwards and stopping whenever they saw a *P. nobilis* individual. Its distance from the centre was marked by using a tape, degrees to the north were noted down and a plastic numerical tag was put at the base of the shell bonded with a nylon cable tie. The height of the unburied shell (H), maximum width at the point of maximum dorso-ventral length of the shell (L_{max}) and minimum width at the bottom (L_{min}) were then measured. Morphometric measurements were performed by using a 0.1 cm tape, whereas the total antero-posterior shell height (H_{tot}) was estimated by using equations to relate the measurements of unburied shell height and total height. During the dives unbroken shells of dead individuals were collected to validate total height data resulting from equations.

Double sampling

In 2011, two monitoring actions were carried out with the help of the Coast Guard Divers Team: *P. nobilis*

specimens were surveyed both in May by coast guard divers and in August by Miramare biologists. Each individual was therefore counted and measured twice in a period of three months, and the same morphometric measurements were performed. The comparison between the two sampling periods showed a difference in the measured minimum width at the bottom (L_{min}), that leads to a statistically significant difference (Mann-Whitney test U = 964, z = -1.974, p(same)< 0,05). Statistical analysis was performed using the open source data analysis package PAST ver. 2.17 (Hammer *et al*., 2001). L_{min} is necessary to calculate total shell height (H_{tot}) with García-March & Ferrer equation (García-March & Ferrer, 1995), which was chosen in the past as its results were the closest to the measurements made on dead individuals with unbroken shells collected during the dives.

Miramare's new formula for H_{tot} calculation

Hence a different formula was created to calculate H_{tot}, which could better adapt to Miramare's measurement method. So, the new formula derived directly from the measurements on Miramare's specimens. In order to avoid any mistakes due to the difficulty of measuring minimum width at the bottom, the new formula considered parameters that can be better measured such as the height of the unburied shell (H) and maximum width at the point of maximum dorso-ventral length of the shell (L_{max}), thus neglecting L_{min}. The new formula was calculated following the indications given by Katsanevakis (2006), who obtained the formula to convert the size-at-age and growth-rate results into total shell height from the relationship between H_{tot} and L_{max} of some dead individuals with unbroken shell.

The formula was based on the data of 10 individuals with unbroken shells and no concretions that were collected in Miramare during the samplings in 2007, 2008 and 2010. Total shell height and maximum width were measured by using a 0.1 cm tape and data were put in a graph with H_{tot} values on the y-axis and L_{max} values on the x-axis. Probability measures (r) showed that the equation best describing the relationship between H_{tot} - L_{max} is the exponential equation (MirExp) given below:

$$\text{(MirExp)} \qquad y = 10.259\, e^{0.0809x} \qquad R^2 = 0.9118$$

where y is H_{tot} value in cm and x (to be inserted) is L_{max} value in cm, both measured *in situ*.

From the specimen measured during the four year sampling period, a growth curve was elaborated according to the Šiletić & Peharda (2003) methodology applied to a study of *P. nobilis* in the Mljet National Park (Croatia). Total shell height data used were so derived from MirExp resulting values. Šiletić & Peharda (2003) estimated *P. nobilis* growth parameters by using von Bertalanffy equation:

$$L_t = L_\infty (1-e^{-k(t-t0)})$$

where L_t is H_{tot} at a given time, L_∞ is the x-intercept on Gulland-Holt plot, k is the regression line slope on Gulland-Holt plot and $t-t_0$ is the time since the first measurement made in t_0 (Gulland & Holt, 1959).

The Gulland-Holt plot was constructed by using the data resulting from the measurements performed on the 24 tagged shells over two years (2008 and 2011). In the plot, the growth rate expressed in cm/year (*i.e.*, the difference between the values measured over the two years) is shown in the y axis, whereas the average total shell height obtained from the mean value of H_{tot} of each individual is shown in the x axis.

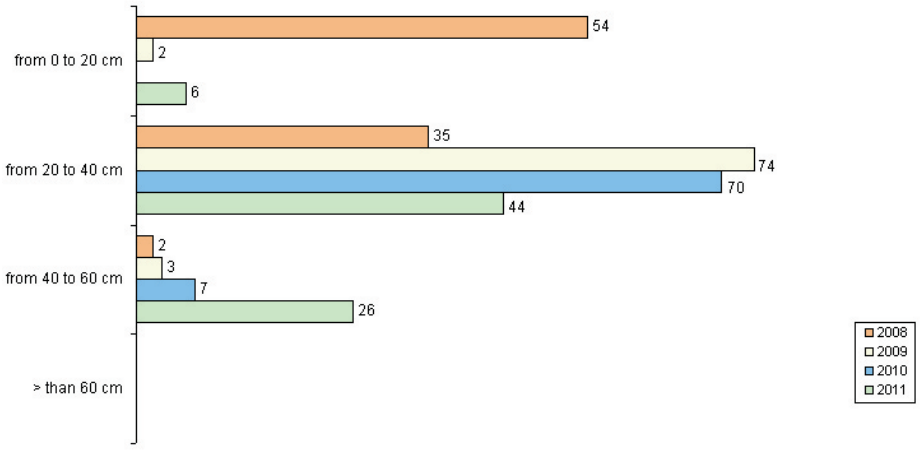

Fig. 1: Size frequency distribution of** Pinna nobilis **over four years of sampling (2008, 2009, 2010 and 2011)
Sl. 1: Velikostna porazdelitev leščurjev v obdobju 2008-2011

Tab. 1: Total height (H_{tot}) in cm measured in 2008, 2009, 2010 and 2011 on 24 individuals tagged in 2008 and re-measured every year
Tab. 1: Celotna višina (H_{tot}) v cm, izmerjena leta 2008, 2009, 2010 in 2011 pri 24 primerkih, označenih leta 2008 in na novo izmerjenih vsako leto

No.	Tag No.	H_{tot} 2008	H_{tot} 2009	H_{tot} 2010	H_{tot} 2011
1	17	29.4	35.9	37.4	43.3
2	15	34.5	40.6	44.0	46.9
3	14	34.5	39.0	37.4	39.3
4	2	37.4	39.0	44.0	51.7
5	4	19.6	25.0	34.5	42.3
6	21	25.0	29.4	34.5	37.4
7	5	15.4	24.0	30.6	35.9
8	7	15.4	25.0	31.8	34.5
9	29	15.4	24.0	31.8	35.9
10	20	14.2	23.0	29.4	37.4
11	32	27.1	34.5	37.4	37.4
12	18	29.4	34.5	34.5	39.0
13	67	29.4	33.2	37.4	38.7
14	74	29.4	31.8	34.5	34.0
15	58	27.1	31.8	34.5	36.8
16	65	29.4	35.9	37.4	40.6
17	39	18.1	28.2	34.5	42.6
18	230	16.0	23.0	30.6	34.5
19	11	16.7	27.1	31.8	40.6
20	100	18.1	29.4	34.5	41.9
21	61	19.6	37.4	39.0	43.3
22	97	40.6	47.7	37.4	44.0
23	175	21.2	31.8	40.6	42.3
24	189	19.6	25.0	36.2	44.0

RESULTS

Over four years, a total of 323 individuals were tagged counted and measured (91 in 2008, 79 in 2009, 77 in 2010 and 76 in 2011). The 26 % of the 2008 tagged pool, 24 specimens, maintain the numerical tag along the four years and so were re-measured year after year, thus providing an indication of their *in situ* growth.

Figure 1 shows the values of average total shell height calculated with the new MirExp equation, with shells divided into 4 size classes of 20 cm each. According to literature, 20 cm of H_{tot} is the threshold under which individuals should be considered juveniles (Combelles *et al*., 1986; Butler *et al*., 1993; Richardson *et al*., 1999). Classes are divided into 0-20 cm (juveniles), 20-40 cm (adult juveniles), 40-60 cm (adults) and more than 60 cm (old individuals). It is evident that in 2008 most individuals were juveniles, whereas in 2009 and 2010 they were adult juveniles and in 2011 adults. There are no individuals longer than 60 cm, which at present is the maximum height for specimens found in Miramare. The comparison between four sampling years shows a general increase in H_{tot} values of individuals from one year to the next, as well as a different size distribution as juveniles were only present in 2008 and 2011, whereas recruitment and the birth of new shells almost did not occur in 2009 and 2010. As a whole, a shift can be seen if size class frequencies for all sampled specimens are considered: in 2008, 59 % of the population consisted of individuals smaller than 20 cm in total height; in 2009 and 2010, 94 % and 91 % respectively consisted of shells ranging from 20 to 40 cm, which became only 58 % in 2011.

Population density distribution was marked by fluctuations over the years. The highest average density was recorded in 2008 with 20.84 individuals/100 m^2, followed by 2009 with 18.88 ind./100 m^2, 2010 with 15.69 ind./100 m^2 and 2011 with 13.57 ind./100 m^2.

Growth

As the sampling lasted 4 years, the growth curve was calculated by using only the specimens that were found and re-measured all four years, from 2008 to 2011. In particular, 2008 saw the presence of many juveniles that presumably settled in that year and, consequently, were likely to be at least age four in 2011.

The morphometric data from the pool of the same 24 specimens were collected *in situ* in 2008, 2009, 2010 and 2011 (Tab. 1) and were then inserted in Miramare's exponential formula (MirExp) to calculate H_{tot}. The resulting total shell height values for 2008 and 2011 were used to obtain a growth curve, according to the method used by Šiletić & Peharda (2003) in their population study of *P. nobilis* in Mljet National Park (Croatia). The Gulland-Holt plot created with the Miramare's shells data on 2008 and 2011 (Fig. 2), shows that the point where the line crosses the x axis corresponds to the L_∞ value (52.6 cm) and the line slope corresponds to a k value of 0.3408. Von Bertalanffy equation - adjusted to the measurements performed on *P. nobilis* individuals found in Miramare MPA - was therefore as follows:

$$L_t = 52.6\ (1\text{-}e^{-0.3408(t\text{-}t0)})$$

With this equation it was possible to establish a relationship between size and age of Miramare's *P. nobilis* individuals and to construct a specific growth curve. Figure 3 shows that the growth curve calculated for Miramare specimens grows faster than the curve of the Croatian population study, but both curves cross in year six. Then Miramare curve slowly tends to its maximum value, whereas the Croatian curve continues to increase.

The study conducted by Šiletić & Peharda in 2003 estimates that *P. nobilis* reaches a total shell height of approximately 11 cm in year one, 20 cm at age two and 28 cm at age three. The authors also state that *P. nobilis* can exceed 65 cm in 15 years and specimens can grow fast (nearly 10 cm a year in their early years), to then

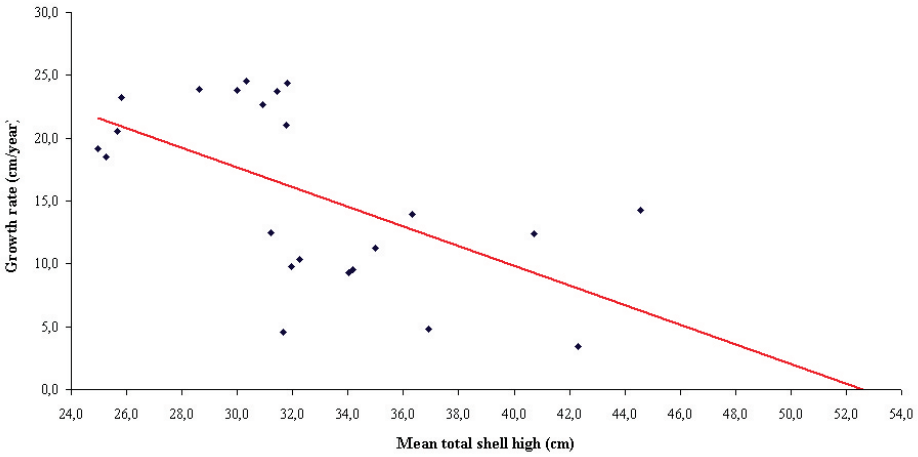

Fig. 2: Correlation between mean total shell height and growth rate (cm per year) for Miramare's** P. nobilis **specimens
Sl. 2: Korelacija med povprečno višino in stopnjo rasti lupine (cm/leto) za miramarske leščurje

slow down growth from year four onwards. If that is compared to the data collected in Miramare from 2008 to 2011, it can be seen that growth over the first years is higher in Miramare, where growth is equal to 15.2 cm at age one, 26.0 cm at age two and 33.7 cm at age three. After that it slows down and the shell is 52.3 cm high at age fifteen, which is 10 cm less than what is reported by Šiletić & Peharda (2003).

With a view to validating Miramare's growth curve, growth values obtained in the first years were compared with the values measured on specimens, which in 2008 were about 15 cm high, *i.e.* that presumably settled in 2008 and therefore were at least age four.

As shown by deviations resulting from the difference between measured values and estimated values, differences range from a minimum of 0.2 cm to a maximum of 4.6 cm. In general, differences are overestimated as the curve overestimates growth especially at age three and four (2010 and 2011), when the shells reach about 30 cm in size and their growth becomes increasingly slower over time.

DISCUSSION

Results show it was necessary to create a new formula for indirect calculation of total height by using data of dead shells collected in Miramare. As a matter of fact, the data of unburied shell height and minimum width at the bottom are needed to use García-March & Ferrer equation (García-March & Ferrer, 1995) that in the past was used for monitoring activities. But these measurements can significantly vary due to substrate conditions as they are influenced by the amount of sand cover, the presence of obstacles due to pebbles and concretions as well as other factors that can make difficult the action of measuring during the sampling. However, if this equation is applied to the data collected in the two sampling periods, there are significant differences in H_{tot} of specimens measured in May and August, which cannot be justified by a three-month growth. According to Hendriks *et al*. (2012), the average growth rate for *Pinna* juveniles is of 0.28 mm/day; this leads, if apply to in a 3 month period, to an average growth of 2.5 cm for each young individuals. The data of total shell heights calculated with the August data for Miramare showed, on the contrary, in some cases negative growths if compared with the H_{tot} of specimens measured in May or in other cases positive growths of more than 3, 4, 6 and 8 cm for some individuals. That is why Miramare's new equation

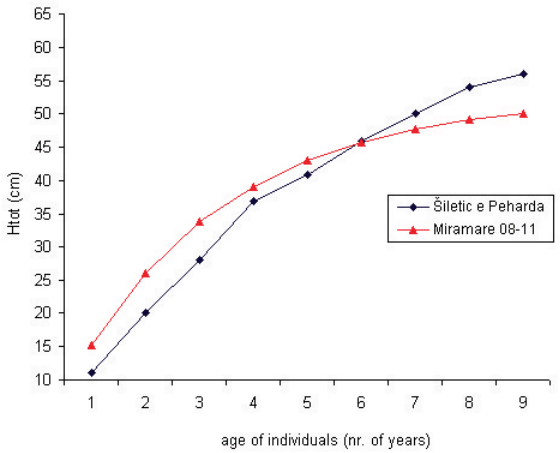

Fig. 3: Graph of relative von Bertalanffy growth equation for** P. nobilis **in Šiletić & Peharda (2003) and in Miramare MPA, representing growth data obtained by measurements in 2008 and 2011
Sl. 3: von Bertalanffyjeva rastna krivulja za leščurje iz študije Šiletić & Peharda (2003) in iz te raziskave (zavarovano morsko območje Miramare, 2008-2011)

takes account of *in situ* measurements related to unburied shell height and maximum shell width, and is based on an exponential equation formula.

In 2008, average density reached the highest value (20.84 individuals/100 m^2) followed by 2009 with 18.88 ind./100 m^2, 2010 with 15.69 ind./100 m^2 and 2011 with 13.57 ind./100 m^2. In any case, all values are very high and corroborated by the cited literature (Guallart, 2000; García-March, 2006; García-March & Kersting, 2006; Katsanevakis, 2006; Centoducati *et al*., 2007) only with the counts made in Mljet National Park (Peharda *et al*., 2002; Šiletić & Peharda, 2003), where density ranges from 2 to 20 ind./100 m^2 and transects were used in the sampling. If a comparison is made with other studies using circle sampling, it can be noted that in Malta, for example, average density in the sampled stations ranges from 0 to 5.1 ind./100 m^2 (García-March & Vicente, 2006) in a recently set-up MPA (from Rdum Majjiesa to Ras ir-Raheb).

Estimated annual growth seems to be different from what is reported in literature, as shown by the individuals tagged in 2008 that were recounted and re-measured every year up to 2011 included. According to the monitoring carried out in Mljet National Park (Šiletić & Peharda, 2003) and some studies conducted in Spain by Richardson *et al*. (1999), *P. nobilis* annual growth reaches an average of 10 cm/year. When von Bertalanffy equation is used to analyse Miramare's growth curve, growth is higher in year one (15.2 cm). Even in the following years, growth in Miramare seems to be 5-6 cm higher than in Mljet for the first three years. The growth curve is a useful tool to gain indications of the age classes of individuals, although starting data usually provide some constraints. It should be highlighted that growth curves as set out above are significantly influenced by starting data. As a matter of fact, when total shell height values of a set of adult individuals are used, the resulting growth rate is lower, as growth decreases with age and consequently juveniles' growth is underestimated. Similarly, if only a dataset of juveniles is used, the resulting rate is very high due to rapid growth in year one, thus overestimating real growth. To address this situation, the specimens sampled over all four years were used so as to compare "actual" growth result from *in situ* measurements and validate the growth curve.

CONCLUSIONS

Thanks to its systematic monitoring, Miramare MPA was able to apply equations to its population in order to calculate total shell height and growth curves that were created on purpose and validated by on-field measurements.

The data provided by regular monitoring activities over four years show significant variability, especially in terms of recruitment: in some years (2008) juveniles accounted for most of the population (60 %), whereas in others (2009, 2010 and 2011) they represented a very low percentage or were not even present.

Growth seems to be faster than in other areas of the Mediterranean, but von Bertalanffy equation does not seem to be a very reliable tool as it changes significantly according to the starting size of individuals. As a matter of fact, if the population is mostly made up of juveniles (see 2008-2009 data), its growth rate is much higher than in a population whose average size is higher (see 2009-2010 data). The resulting information can therefore be misleading: this is why better information is obtained by comparing actual growth data of the 24 shells collected with *in situ* measurements over four years, which seem to be closer to the values given by Miramare's curve.

In any case, considering the H_{tot} of 24 individuals representing various size classes and measured over four years (2008 and 2011), there is an increase in size of about 15 cm at age one, 11 cm at age two and 7 cm at age three. With this growth rate, in 2011 almost 75 % of individuals ranged from age 3 to age 5 and their size from 30 to nearly 45 cm. According to Gosling (2003), adult individuals have the best reproductive capacity and following Combelles *et al*. (1986), Butler *et al*. (1993) and Richardson *et al*. (1999) in this species specimen longer than 20 cm can be considered young adults and adults. If this is combined to the high density of individuals found in the sampled stations, it can be stated that Miramare MPA is an important breeding area.

It is still unclear why there are not many large-sized individuals (over 55 cm) as there is no direct source of disturbance in the area since fishing, anchoring and/or shell collection are forbidden inside the MPA.

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DEFINICIJA NOVE ENAČBE ZA IZRAČUN CELOTNE VIŠINE LEŠČURJEV, *PINNA NOBILIS*, V ZAVAROVANEM MORSKEM OBMOČJU MIRAMARE (TRST, ITALIJA)

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POVZETEK

V članku so povzeti rezultati štiriletnega spremljanja stanja in populacijske dinamike leščurja, Pinna nobilis, *v zavarovanem morskem območju Miramare (Trst) in opisana izpeljava nove enačbe za posredni izračun celotne višine lupine (H_{tot}). Z uporabo enačb, navedenih v citirani strokovni literaturi, smo dobili vrednosti H_{tot}, ki niso bile v skladu s terenskimi meritvami rasti pri 24 primerikih leščurjev, ki smo jih označili leta 2008 in letno spremljali njihov razvoj vse do leta 2011. Novo enačbo smo zasnovali z uporabo podatkov, pridobljenih z morfometričnimi meritvami nezlomljenih lupin mrtvih primerkov, ki smo jih našli na miramarskem območju. Eksponentno enačbo, zasnovano posebej za miramarsko populacijo, smo poimenovali MirExp, z njeno pomočjo pa lahko izračunamo tudi rastno krivuljo. Stopnjo rasti, izračunano na podlagi rastne krivulje, smo preverili z morfometričnimi podatki označenih primerkov, ki smo jih izmerili med potopi. Iz krivulje je razvidno, da primerki, ki živijo na zavarovanem območju, v prvih letih rastejo hitreje, kot je navedeno v citirani strokovni literaturi (15 cm v prvem letu, medtem ko so podobne študije, izvedene na Hrvaškem in Španiji, poročale o 10-cm rasti). Na zavarovanem območju je zelo visoka tudi gostota, in sicer znaša njeno letno povprečje 17,25 osebkov/100 m^2, medtem ko se velikost leščurjev v povprečju giblje med 30 in 45 cm. Miramarsko populacijo tako večinoma sestavljajo odrasli primerki, stari od 3 do 5 let. Podatki o populacijski dinamiki, vključno s podatki o visoki populacijski gostoti, pričajo, da je miramarsko območje pomembno rastišče te endemične školjke v Sredozemskem morju, zaščitene z mednarodnimi konvencijami.*

Ključne besede: *Pinna nobilis,* populacijska dinamika, rast, zavarovano morsko območje, Jadransko morje

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