

Protandric hermaphroditism in the bivalve *Arca noae* (Mollusca: Arcidae)

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Abstract

Following the histological analysis of *Arca noae* samples from the south-western Adriatic Sea, five hermaphroditic specimens were found out of 168 sexed individuals (3.0%). The hermaphrodite gonads showed the co-occurrence of male and female germ cells within the same acini, i.e. both spermatozoa in the lumen and oogonia lining its wall. Gonia increased in size through winter, thus suggesting that the direction of sex change is from male to female. Both the biometrical analyses and theoretical considerations strongly suggest that *A. noae* is an obligate protandric species.

Keywords: Bivalvia, Arcidae, Adriatic Sea, reproduction, hermaphroditism, histology.

Introduction

The Noah's ark, *Arca noae* Linnaeus, 1758 (Mollusca: Arcidae), is a commercial bivalve distributed throughout the Mediterranean and the eastern Atlantic Ocean from Portugal to Angola that lives on both rocky and gravel ("pralines") grounds, at a depth ranging from a few to more than 50 m (Poutiers, 1987), where it holds on to solid substrates using its robust byssus. Within this range, it is mainly fished in the Adriatic Sea and is highly prized in Italian, Slovenian and Croatian marketplaces (Poutiers, 1987). Most available literature on *A. noae* deals with its Adriatic population(s) (e.g. Hrs-Brenko, 1980; Valli & Parovel, 1981; Bello & Paparella, 2001; Peharda *et al.*, 2002, 2003, 2006 and 2009). All of these papers are in agreement that the observed Adriatic population(s) along the northern-middle east coasts and the south-western coasts are overfished and thus require management. Such a situation requires investigations on the ecology and biology of *A. noae*, including its reproduction.

The number of papers on the reproduction of Noah's ark is limited. According to Valli & Parovel (1981) *A. noae* is a dioecious bivalve that, in the Gulf of Trieste, reproduces throughout a long season spanning from spring to autumn, although spawning has two peaks, in March and September. As for the south-western Adriatic Sea, Bello & Paparella (2001) reported that the Gulf of Manfredonia population also has two spawning peaks per year; by contrast, the population from Bari has just one spawning peak in summer (present authors' personal ob-

servations). Peharda *et al.* (2006), who examined Noah's ark banks off the southern Croatian coast over two different years, also described a comparatively long reproductive season that peaks in summer.

Valli & Parovel (1981) found one hermaphroditic specimen of *A. noae*. According to Valli (pers. comm.) the gonadic acini of this specimen contained male gametes in their lumina and developing female germ cells in their wall; hence an individual that was going to change sex from male to female. This hermaphrodite had been collected in winter, during the resting phase of the reproductive cycle. No percentage corresponding to this only specimen was reported; in any case, it can be deemed as very low, in the order of magnitude of the percent unit. Peharda *et al.* (2006) also found hermaphrodites among the observed Noah's arks; in all 13 specimens out of 590, collected between autumn and spring, which amounts to 2.2%. These authors concluded that "some *Arca noae* may exhibit functional protandric dioecy with some [male] individuals changing to females in subsequent years."

This paper, prompted by the finding of some hermaphroditic Noah's arks in the south-western Adriatic Sea, intends to provide a description of the progress of sex change in hermaphroditic specimens of *A. noae* and to discuss the significance of sequential hermaphroditism in this species.

Material and Methods

A total of 177 specimens of *Arca noae* were hand-

collected by SCUBA diving (collection roughly distributed from December 2004 to November 2005) (Table 1), on a rocky bottom off Bari (south-western Adriatic Sea) at a depth of between 5 and 6 metres. Because of the sampling method, very small Noah's arks might have been overlooked and not collected, so the sample is possibly biased toward larger specimens.

Soon after sampling, the molluscs were transported to the laboratory where shell length was measured to the nearest 1 mm below, and their gonads were removed and fixed in 10% buffered formalin, dehydrated in ethanol and embedded in paraffin wax. Five-micrometer-thick sections were de-paraffinized in xylene, re-hydrated using graded ethanol solutions and stained with haematoxylin-eosin. Digital fields were photographed with 2.5, 10 and 40X objectives on a digital camera (DC 300, Leica, Cambridge, U.K.) connected to a light microscope (DMRBE, Leica, Cambridge, U.K.), using an image analysis software application (QWIN, Leica, Cambridge, U.K.).

Raw data of the individuals sampled in 2002 in the eastern Adriatic (*cf.* Peharda *et al.*, 2006) were kindly provided to us by M. Peharda and used in the evaluation of the hermaphroditism incidence.

The mean shell lengths of the two sexes were compared using Student's *t*-test; the parameters of the predictive regression of male percentage ($\frac{M}{M+F} \times 100$), on shell length classes (SL) were computed by the least squares method (Sokal & Rohlf, 1981). In order to extrapolate the percentages of the shell length classes inferior to 55-60 mm, the exponential curve equation describing the population's theoretical survival, $N_t = N_0 e^{rt}$ was used (Wilson & Bossert, 1971).

Table 1. Size (*N*), mean shell length (\overline{SL}) and associated standard deviation (*s*) of *Arca noae* monthly samples.

month	<i>N</i>	\overline{SL} (mm)	<i>s</i> (mm)
December	18	44.2	7.7
January	11	45.6	2.7
February	7	50.0	5.8
March	14	49.1	3.7
May	10	52.3	5.4
June	24	42.8	10.2
July	20	39.9	5.9
August	20	34.2	5.1
September	18	47.3	4.0
October	18	46.7	7.7
November	17	42.1	4.6

Results

Histological analysis of specimens from the south-western Adriatic *Arca noae* population showed that 80 individuals were males, 83 females and 5 hermaphrodites (Table 2). Nine specimens, in the size range of mature individuals, were sexually inactive, so their sex could not be determined; all had been collected in September and October, i.e. soon after the reproductive season. The hermaphrodites had been collected from December to March (1 in December, shell length: 43 mm; 1 in January, 41 mm; 1 in February, 49 mm; 2 in March, 46 and 48 mm), concomitantly with the intergenetic period or resting phase of the reproductive cycle and with the early maturing processes, i.e. the recrudescence period.

In maturing and mature individuals, gonads are whitish and purple/red in males and females, respectively. The hermaphroditic Noah's arks were macroscopically identified as females. However, histological examination showed the simultaneous presence of both male and female germ cells. The gonads of these individuals showed an apparently normal ovarian structure composed of acini whose wall was apparently formed by oogonia; however, in the lumen of some acini, male germ cells at different development stages were observed (Figs 1-2); the latter acini were mostly located in the gonad periphery (Fig. 1a, 1b). Progress was observed from winter to spring in the acini of hermaphrodite specimens collected in subsequent months. In December, gonia of hermaphrodites, localized in the acinus wall, had a flat shape (Fig. 2a), with a height of 12-15 μ m and a width of 20-22 μ m; in February-March, gonia increased in height and showed a rounded or polygonal shape with a diameter/major axis of 30-35 μ m (Fig. 2b). Male germ cells observed within the lumen of hermaphroditic individuals were mostly spermatids and flagellated spermatozoa.

The length frequency distributions of both sexes were unimodal. The female distribution appears somewhat moved to the right with respect to the males, despite the fact that the modes of both sexes, as well as that of the hermaphrodites, coincided with each other (modal length class: 45-50 mm) (Fig. 3). The mean length (46.2 mm) of females was significantly higher than the mean length (41.6 mm) of males (Student's *t* = 3.55; *df* = 161; *P* < 0.001). The hermaphrodite mean was 45.4 mm.

The overall male percentage, $\frac{M}{M+F} \times 100 = 49.1$, was very close to the theoretical value of sex equivalence, al-

Table 2. Overall sample size (*N*), shell length range and mean size (\overline{SL}) per sex of *Arca noae*.

Sex	<i>N</i> (%)	<i>SL</i> range (mm)	\overline{SL} (mm)
Male	80 (47.6)	17 - 61	41.6
Female	83 (49.4)	28 - 70	46.2
Hermaphrodite	5 (3.0)	41 - 49	45.4

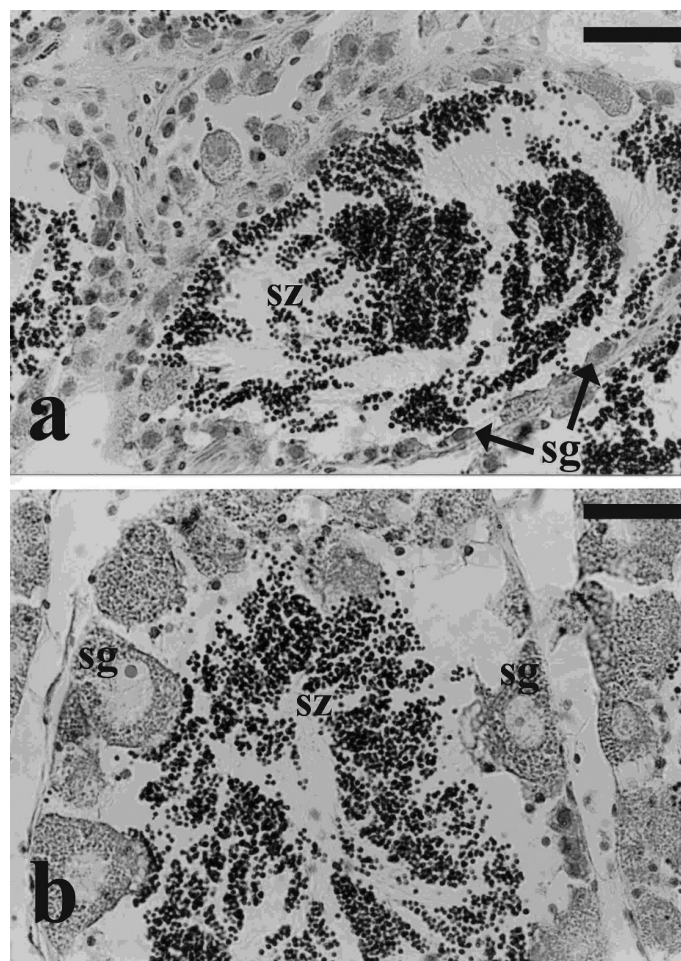


Fig. 1: Micrographs of the gonad from a hermaphrodite *Arca noae* specimen sampled in March. a) Spermatozoa (sz) are visible in several acini in a peripheral area at the left of the micrograph. b) Higher magnification of a particular of a) showing loose acini, some of which contain flagellated spermatozoa (sz) Haematoxylin-eosin staining. Bar = 500 μ m in a) and 150 μ m in b).

though this parameter varied significantly according to size, ranging from 100% in the smallest size classes to 0 in the largest one (Fig. 3). The regression line fitting the male percentage distribution along the size classes (SL) was highly significant: $\frac{M}{M + F} \times 100 = 131.44 - 1.86 \text{ SL}$;

$r = 0.962$; $P < 0.0001$. Based on this straight line, male / female equality was reached at a length of 44.5 mm, which is quite similar to the mean hermaphrodite length.

The percentages of the 168 sexed individuals were: 47.6% males (M), 49.4% females (F) and 3.0% hermaphrodites (H). When taking into account the months of occurrence of hermaphroditism only, the percentage of sex-changing individuals rose to 11.6%, which represented a 4-fold increase with respect to the overall 3.0%. Yet, in order to establish the actual extent of hermaphroditism, the incidence of hermaphroditic individuals was calculated on the mature male fraction of the population, since only mature males can change sex in protandrous animals, including *A. noae*: $[H / (M + H)] = 20.0\%$. On the basis of the same criterion, the re-calculated percentage for the eastern Adriatic population sampled in 2002 by Peharda *et al.* (2006) also provided higher values for both the incidence

of hermaphroditism in the months of sex change (15.0% = 6 H / 40 sexed specimens; almost 7-fold the overall year-around one) and the incidence of hermaphroditism in the male fraction apt to change sex (35.3%).

Discussion

Although the occurrence of hermaphroditism in *Arca noae* has been established previously (Valli & Parovel, 1981; Peharda *et al.*, 2006), this paper reports on the histological evidence showing that sex change occurs during the winter months, i.e. during the resting and recrudescence phases of the reproductive period. The seasonal finding of hermaphroditism, together with the changes occurring in the gonad size from December (resting phase) to March (recrudescence period) may be explained by the following hypothesis: a pool of male stem gonad maintain their bipotentiality (female and male) and when reproductive activity resumes in spring, they have the capability to differentiate to male oogonia. The presence of a pool of stem (undifferentiated and pluripotent) gonad throughout the reproductive life has already been suggested for both invertebrates (for a review see

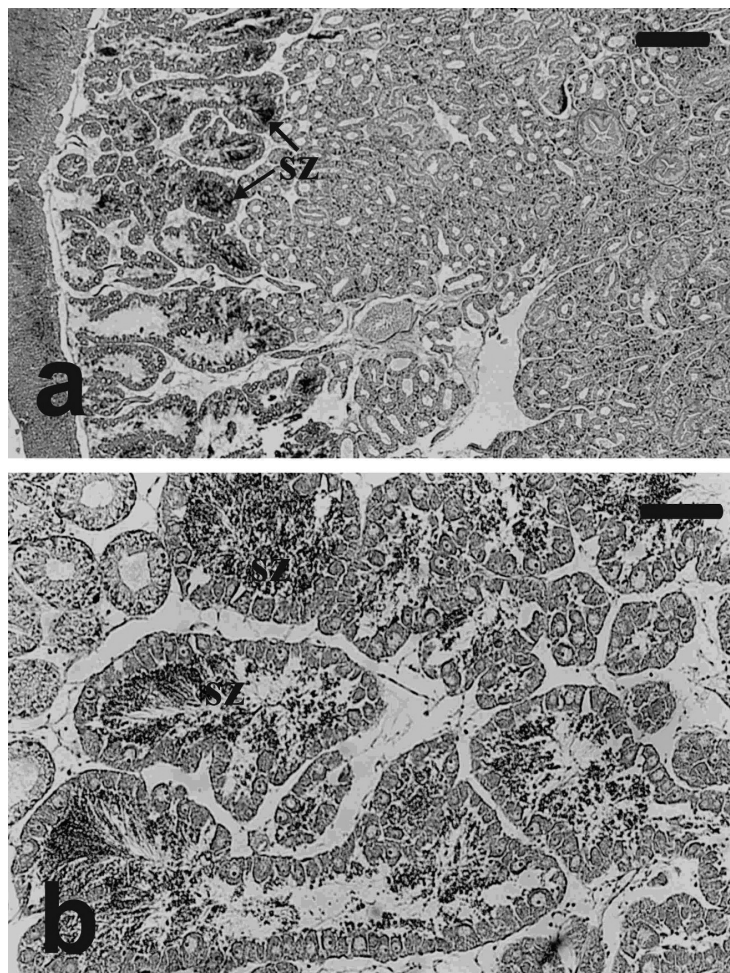


Fig. 2: Micrographs of two gonad acini from hermaphrodite *Arca noae* specimens. a) Acinus from an individual sampled in December showing a wall constituted by a single layer of flattened gonia and a lumen containing flagellated spermatozoa. (b) Acinus from an individual sampled in March showing a wall constituted by rounded or polygonal gonia and a lumen containing flagellated spermatozoa. Haematoxylin-eosin staining. Bars = 50 µm.

Isaeva *et al.*, 2008) and vertebrates (e.g. Schultz *et al.*, 2010). Alternatively, it is theoretically possible that gonia already differentiated as spermatogonia, at the onset of the new reproductive season, de-differentiate to stem gonia and then differentiate again towards the female line. Whatever the mechanism of hermaphroditism, the individuals that change sex at the beginning of a new reproductive season still have in their gonads advanced stages of male cells (spermatids and spermatozoa) residue of the past reproductive season. Various models of gonad structural organization have been reported for different hermaphroditic bivalve taxa. *Arca noae* fits the category of consecutive sexuality, according to the Kasyanov (2001) classification, where once in the life of the bivalve, the animal undergoes a single gender switch, usually from male to female.

Despite the ostensibly small proportion of hermaphrodites – viz. 2.2% in the eastern Adriatic Sea (Peharda *et al.*, 2006) and 3.0% in the south-western Adriatic Sea (present results) – the concomitance of other facts, such as a) the significant modification of the sex ratio through-

out the size range (strongly biased towards the males for the smallest sizes and towards the females for the largest ones) (Peharda *et al.*, 2006; present results); b) the significantly larger mean size of females with respect to males (Peharda *et al.*, 2006; present results); c) the mean size of hermaphrodites in-between the male and female mean sizes (Peharda *et al.*, 2006; present results); d) the first maturity size; smaller in males than in females (Peharda *et al.*, 2006); and e) the hermaphrodite seasonal range confined to late autumn and winter i.e. the inter-genetic part of the reproductive cycle (Peharda *et al.*, 2006; present results); all indicate that *A. noae* hermaphroditism is neither exceptional nor occasional.

The biological substance and meaning of hermaphroditism in the Noah's ark are open to discussion. Two alternative hypotheses can be confronted. First hypothesis: *A. noae* is an obligate protandric hermaphrodite mollusc, which signifies that all individuals start their sexual life as males and afterwards change sex at a given age or size. Second hypothesis: *A. noae* is a partially sex changing species, where a number of females (either a

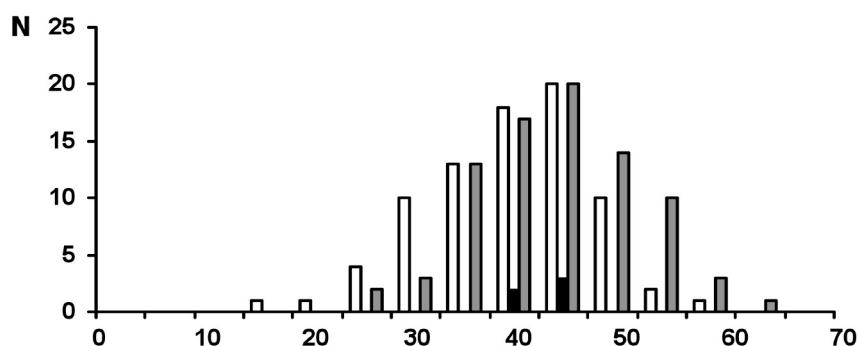


Fig. 3: Length frequency distributions of males (white), hermaphrodites (black) and females (grey) in the *Arca noae* sample from the south-western Adriatic Sea.

small or a large fraction of them) mature directly from the juvenile stage and the remaining females derive from adult males through sex change. Regardless of the ostensible extent of the hermaphrodite phenomenon in *A. noae*, which appears limited to a low fraction of the sexually mature population, computation of the hermaphroditism incidence in mature males only, i.e. the fraction of the population suitable to change sex, shows that a fairly high proportion of the population is indeed involved in sex-changing, viz. 20.0% or 1/5 of the sample and 35.3% or 1/3 of the sample in the south-western and eastern Adriatic samples, respectively. If one extrapolates these figures to the entire Adriatic population, it follows that all the males can change sex over a three- to five-year lapse of time, i.e. 1/3 to 1/5 per year. This is a very rough estimate, since it is based on comparatively small samples; nevertheless, it is supported by the fact that *A. noae* is a long-lived animal (up to 16 years [Peharda *et al.*, 2002]) that reaches sexual maturity in its 2nd year of life (smallest mature male length = 12.3 mm [Peharda *et al.*, 2006]; mean 2-year-old length = 16.8 ± 0.67 mm [Bello & Paparella, 2001]) and changes sex over a wide size, i.e. age, range (31.3 - 67.4 mm [Peharda *et al.*, 2006]).

In addition, the assumption that the overall sex ratio is close to 1 is wrong; indeed the sex ratios reported by Peharda *et al.* (2006) and in this paper only concern the samples taken into consideration and not the entire population to which they belong. It is evident that SCUBA sampling of *A. noae* is very strongly biased because small specimens are quite cryptic and are not noticed by the divers (Bello & Paparella, 2001; Peharda *et al.*, 2009). When better sampling procedures are adopted, such as the one used by Peharda *et al.* (2009), the male/female ratio increases significantly e.g. $311/142 = 2.2$ (Peharda *et al.*, 2009). However, the latter sex ratio value is still far removed from the actual population ratio. For instance, it is evident that in the length frequency distribution reported by Peharda *et al.* (2009), the 1-year cohort is completely missing and the 2- and 3-year cohorts are rather underrepresented (cf. Bello & Paparella, 2001, about 1- and 2-year cohort mean lengths), which cohorts consist mainly if not exclusively of males. The theoretic

cal survival of any wild population, following settlement, for instance, in the *A. noae* case, is expected to be best represented by a negative exponential curve (e.g. Wilson & Bossert, 1971). Therefore, in the length frequency distribution graphs reported by Peharda *et al.* (2006 and 2009) and in the present results, respectively, the size classes inferior to 55-60 mm, 40-41 mm, and 45-50 mm are underrepresented [note that the least underrepresented distribution is that reported by Peharda *et al.* (2009), viz. the one that used the best collected sample]. As for the south-western Adriatic sample (present results), when one fits the theoretical negative exponential curve to the frequency distribution of the supposedly population representative size classes, viz. 45-50 mm and larger, a completely different picture comes out. The equation of the fitted curve allows to extrapolate the estimated percentages of overall mature individuals (M + F + H) per size class and, in turn, the male and female percent compositions of each size class and, hence, the overall estimated M/F = 12.8 (details of computations available on request). This value is in the range of other obligate protandric molluscs; for instance Rivera-Ingraham *et al.* (2011) found the sex ratios equal to 13.1 and 25 in harvested and non-harvested populations, respectively, of the gastropod *Patella ferruginea*. How rough this estimate can be, is indicative of the fact that the actual *A. noae* sex ratio (M/F) is much higher than 1 and, thus, fits the requirement of the sex allocation theory whereby - in species where sex change occurs - the sex ratio is biased toward the first sex (Charnov, 1982 & 1989; Allsop & West, 2004). Incidentally, the use of L_{50} (size at which 50% of the animals have changed sex) is considered to be the most statistically rigorous estimate of the mean 'size at sex change' of a population (Allsop & West, 2003). In our case $L_{50} = 44.5$ mm, which corresponds very closely to the average hermaphrodite length (45.4 mm).

To sum up, in our opinion the present results and a careful re-interpretation of the data available in the literature support the hypothesis that *A. noae* is an obligate protandric hermaphrodite mollusc better than the alternative hypothesis, viz. that it is a partial sex changer. Moreover, the variability in the size of *A. noae* hermaphrodites

(Peharda *et al.*, 2006) may indicate that sex change is affected by social factors, namely population density and composition by size and sex (*cf.* Rivera-Ingraham *et al.*, 2011). Indeed, the overexploitation of Adriatic Noah's ark populations (see Introduction) alters the population structure both size-wise and sex-wise, since females, which are larger than males, are proportionally more harvested than males. Therefore it is not possible, in these circumstances, to establish actual "natural" parameters, such as the age and size of sex change and the sex ratio.

Several cases of hermaphroditism have been reported in arciid bivalves, all of them described as protandric (Yankson, 1982; Pérez-Medina, 2005; Mzighani, 2005; Afiati, 2007; Flores & Licandeo, 2010). The overall data about *A. noae* discussed in this paper and the above cited literature about several species of *Anadara* lead us to believe that protandric hermaphroditism is quite a common feature of the Arciidae family.

While discussing the present data on *A. noae* together with the examination of the relevant literature, it became apparent that two main drawbacks should be avoided in future research on this important bivalve. First: since macroscopically examined hermaphrodites mimic females, histological examination of the gonads is necessary to detect sex-changing individuals in order to avoid underestimation of their incidence for the entire population; this is an easily surmountable problem. Second: representative sampling should include small and very small specimens for the purpose of obtaining reliable data to describe the actual populations and, in turn, derive sound biological parameters; this is quite a difficult task indeed.

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