

Sex ratio and minimum size at sexual maturity of *Haliotis asinina* in the seagrass vegetation of Tapulaga, Southeast Sulawesi, Indonesia

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Abstract. Abalone *Haliotis asinina* is also found in seagrass vegetation. The aim of this research was to determine sex ratio and minimum size at sexual maturity of *H. asinina*. The sampling duration was seven months (from April to October 2018) in Tapulaga, Soropia District, Konawe Regency, Southeast Sulawesi (S 03° 56'19.7" and E 122° 38' 41.6"). Random sampling in seagrass vegetation was conducted using a traditional tool called "ganco" (traditional fishing spear). Male and female abalone ratio of 1.15:1 (balanced condition) was found. The minimum size of sexual maturity was 32.94 mm for male and 33.42 mm for female individuals. R square was 0.99 for both male and female *H. asinina*.

Key Words: gastropod, marine herbivorous, intertidal zone, abalone, reproduction biology.

Introduction. Abalones are herbivorous marine gastropods belonging to genus *Haliotis*. *Haliotis madaka*, *H. gigantea*, *H. discus discus*, and *H. discus hannai* have been reported to occur in Korea (Lee & Min 2002), *H. iris* and *H. australis* in New Zealand (Wilson & Schiel 1995), *H. tuberculata* in Europe (Mgaya 1995), *H. rubra* and *H. cylobates* in South Australia (Shepherd 1973; Lindberg 1992). There are seven species of abalone distributed in Indonesia i.e. *H. asinina*, *H. varia*, *H. diversicolor*, *H. ovina*, *H. glabra*, *H. planata*, and *H. squamosa* (Dharma 1988). Those species were found in Sulawesi, Maluku, Lombok, Sumbawa, and Papua (Setyono 2009). More than 20 species are classified as commercially important (Jarayabhand & Paphavasit 1996). The main aim of abalone aquaculture is to enhance growth rate, nutritional value of feed and high survival (Iba 2008). *H. asinina* is a species that has been cultured (Zhang et al 2004) because it has a high average growth rate (Fermin et al 2000). *H. asinina* is also found in the Philippines (Capinpin et al 1998; Maliao et al 2004).

In nature, abalone populations are generally found at intertidal zone with substrate conditions covered with coralline algae (Clavier & Chardy 1989; Day & Branch 2002). Abalones are found almost worldwide, living in rocky coastal waters, coral exposure, and hiding in rock holes and gullies (Imai 1982). They also inhabit colony coral substrate to a depth of 60 m (Najmudeen & Victor 2004), some abalones are also found in seagrass areas (Shepherd 1973).

Tapulaga waters have seagrass vegetation and this location is very close to the fisherman residence. Seagrass species such as *Thalassia hemprichii* and *Enhalus acoroides* are abundant in this area with sandy substrates and rubble shells substrates. The seagrass species provides natural food for abalone *H. asinina* i.e. benthic algae diatoms (Nahrullah et al 2016), especially for the larval phase (Setyono 2005).

Abalone larvae are produced through external fertilization in the water column (Stephenson 1924) as the reproductive cycle beginning. A quality adult produces a good amount of recruitment within suitable environmental conditions (Wilson & Schiel 1995).

Both male and female adults play an important role in reproductive stages. Sex ratio and minimum size at sexual maturity are one of the factors affecting reproductive potential. Abalones reproduction biology is influenced by extern and intern factors. The present study on *H. asinina* at seagrass vegetation was carried out to provide information on (1) sex ratio and (2) minimum size of sexual maturity.

Material and Method. *H. asinina* samples were collected from the wild using a traditional tool named "ganco" (traditional fishing spear) (Figure 1B). The abalone was collected randomly from seagrass vegetation in Tapulaga, Soropia District, Konawe Regency, Southeast Sulawesi (S 03° 56' 19.7" and E 122° 38' 41.6") from April to October 2018 (Figure 2).



Figure 1. Equipment used for collecting abalone in Tapulaga.

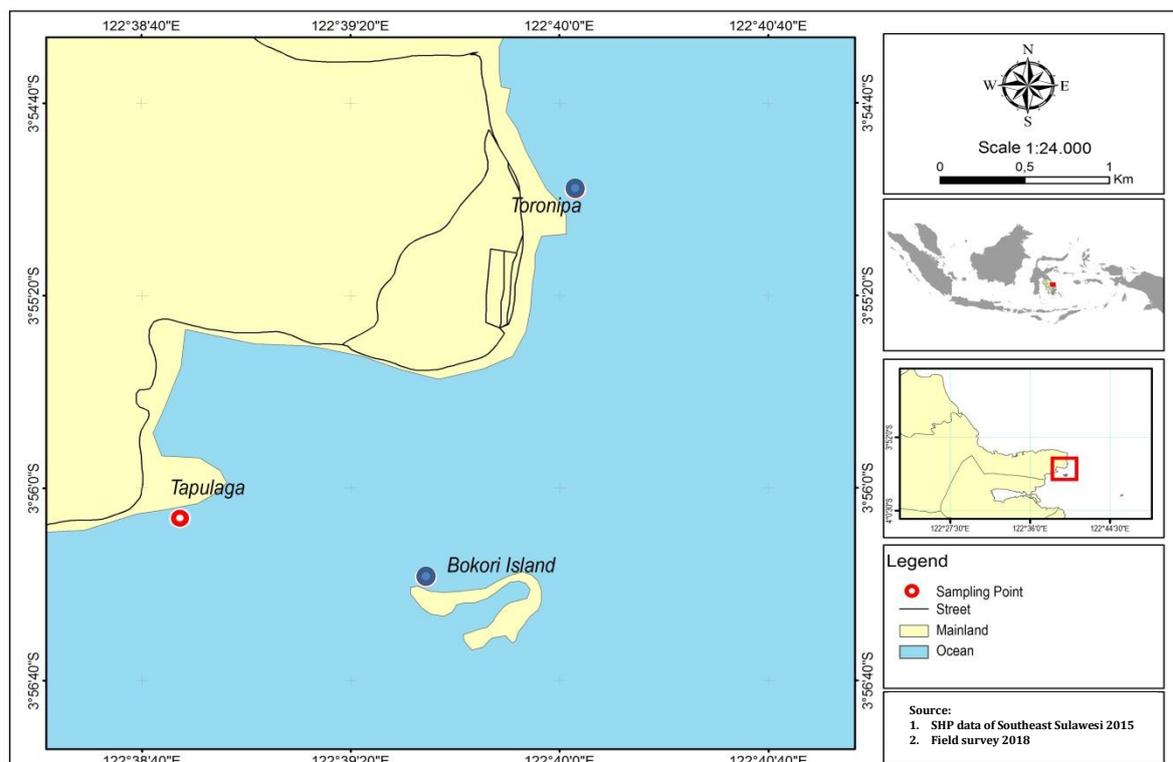


Figure 2. The sampling location of abalone *Haliotis asinina* at Tapulaga, Konawe Regency, Southeast Sulawesi.

Sex ratio. Sexes were determined by the color of gonads (testis and ovaries). Mature male abalone present milky white surface of conical appendage and mature female abalone a dark green color (Capinpin et al 1998; Litaay & Silva 2003).

Minimum size at sexual maturity. The shell length (SL) of each abalone was measured, using digital calipers to the nearest millimeter (accuracy: 0.01 mm). Gonad development stage in *H. asinina* was determined macroscopically according to Leighton (2008) and Singhagraiwan & Doi (1992) (Table 1).

Table 1

Characteristics of each gonadal development stage (GDS) of *Haliotis asinina* (modified from Singhagraiwan & Doi 1992)

GDS	Characteristics
0	Gonad is not visible. Abalone is either a juvenile or a spent mature abalone
1	Pre-mature gonad covering a small portion of the hepatopancreas
2	Partially mature gonad covering about 25% of the hepatopancreas
3	Fully mature gonad covering about 50% of the hepatopancreas

Data analysis. Initially, identification was made to distinguish male and female abalones. After that, the number of male and female abalone was compared. If there was a variation between the two sexes, analysis was continued using chi-square test to find significant variation from 1:1 ratio. Minimum size at sexual maturity was determined by opportunity 50% of the mature gonad abalone using a nonlinear regression function on the logistic curve with Sigma plot 12.0 software. Equation:

$$y = \frac{a}{1 + e^{-\left[\frac{x-x_0}{b}\right]}}$$

Where y is opportunity for abalone gonad maturity (%), e is exponential natural number, a is intercept, b is slope, and x, x₀ is shell length size to-i (mm).

Results and Discussion

Sex ratio. A total of 188 abalone samples were collected during the study, consisting of 76 males and 66 females. There were 46 abalones in immature stage with indeterminate sex and digestive gland visible as grey brown mass (Leighton 2008). The sex ratio of total *H. asinina* was approximately 1.15:1. The "Chi-Square" test indicated no significant difference (balanced condition, P>0.05).

Minimum size at sexual maturity. Gonadal development stage 2 (GDS 2) (large gonad, tip rounded but not swollen) (Leighton 2008; Najmudeen & Victor 2004) was mostly observed in the shell length class of 31.09-37.09 mm, 38.09-44.09 mm, and 45.09-51.09 mm (SL<50.00 mm). Mature stage (GDS 3) (very large gonad, tip rounded and swollen, bulging at shell edge) was found mostly in the in shell length class of 52.09-58.09 mm, 59.09-65.09 mm, and 66.09-72.09 mm (SL>50.00), respectively. In shell length class of 73.09-79.09 mm, male abalone tended to be in the early maturing stage (GDS 1) (sex determinable, small gonad, creamy or milky white color, and tip pointed). Meanwhile, for female abalone, late maturing stage (GDS 2) was mostly observed in shell length class of 33.42-38.42 mm, 39.42-44.42 mm, and 45.42-50.42 mm (SL≤50.00 mm). GDS 4 was mostly found in the shell length class of 51.42-56.42 mm, 57.42-62.42 mm, and 63.42-68.42 mm (SL>50.00) (Figure 3).

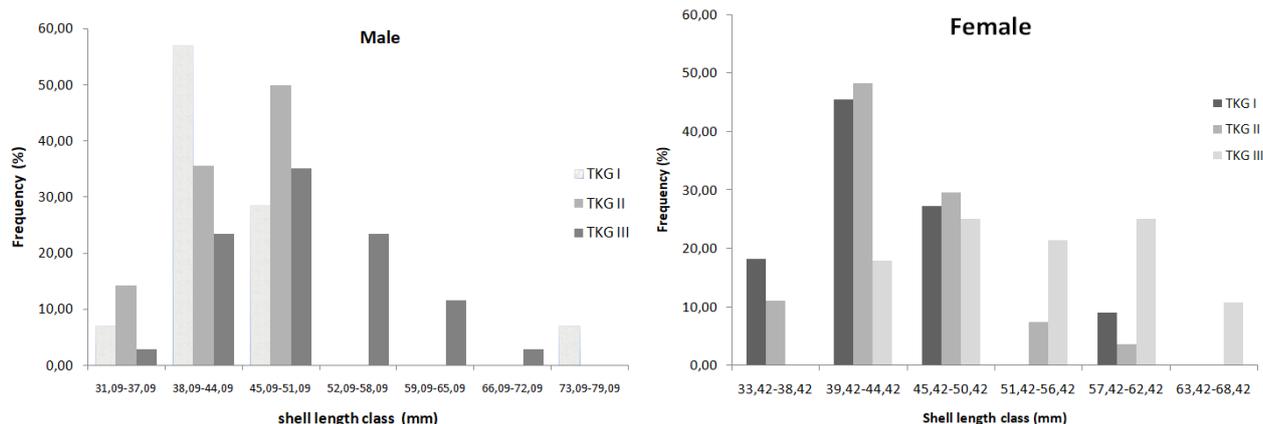


Figure 3. Frequency of individual attendance at length size class.

Minimum size at sexual maturity was found 32.94 mm and 33.42 mm for male and female abalone, respectively in seagrass vegetation. R square (r^2) was 0.9946 for males and 0.9933 for female abalones. Gonads maturation stages of *H. asinina* is presented in Table 2.

Table 2

Testis and ovaries maturation stages of *Haliotis asinina* in seagrass vegetation

Month	Sexes	Number	Stages		
			I (%)	II (%)	III (%)
April	Male	17	29.41	64.71	5.88
	Female	10	50.00	50.00	-
May	Male	9	11.11	11.11	77.78
	Female	10	20.00	10.00	70.00
June	Male	7	14.29	-	85.71
	Female	13	7.69	15.38	76.92
July	Male	12	8.33	16.67	75.00
	Female	7	-	-	100.00
August	Male	5	-	40.00	60.00
	Female	12	-	83.33	16.67
September	Male	10	50.00	50.00	-
	Female	6	16.67	83.33	-
October	Male	16	12.50	37.50	50.00
	Female	8	25.00	50.00	25.00

Sex ratio. *H. asinina* at Tapulaga coastal area was found in seagrass habitats. Other species of abalone that utilize seagrass such as *H. laevigata*, *H. rubra*, *H. roei*, *H. fulgens* (Shepherd 1973; Serviere Zaragoza et al 1998), *H. cyclobates* interact with *Pinna bicolor* living among seagrass (Keasing et al 1995). The sex ratio showed that the male and female abalone in seagrass vegetation was 1.15:1 (balanced ratio 1:1), as was reported in previous studies (Capinpin et al 1998; Jarayabhand & Paphavasit 1996; Maliao et al 2004; Najmudeen & Victor 2004; Setyono 2006), as well as in other abalone species, like *H. cracherodii*, *H. rufescens*, *H. rubra* (Booolootian et al 1962; Litaay & Silva 2003). This shows that distribution between male and female abalone is same in the population. Sex ratio in a balanced condition (ratio 1:1) has similarities in the structure of size, growth, and mortality rate (Capinpin et al 1998).

Nevertheless, data from field surveys on length size groups showed that the number of male abalones attaining gonadal maturity (GDS 4 / ripe) was more commonly found than that of female abalone for monthly sampling time. Male abalone individuals

grow faster than females because it attempts to use more energy during the reproduction process (gamete maturation) (Setyono 2009).

Minimum size at sexual maturity. The compatibility of the regression model shows better model because r^2 value (R square) was 0.99, which is close to +1 (Figure 4). Shell length contributed to 99% on size at sexual maturity, both in male and female abalones. The relationship between shell length values and size at sexual maturity for both sexes shows a very close and positive relationship.

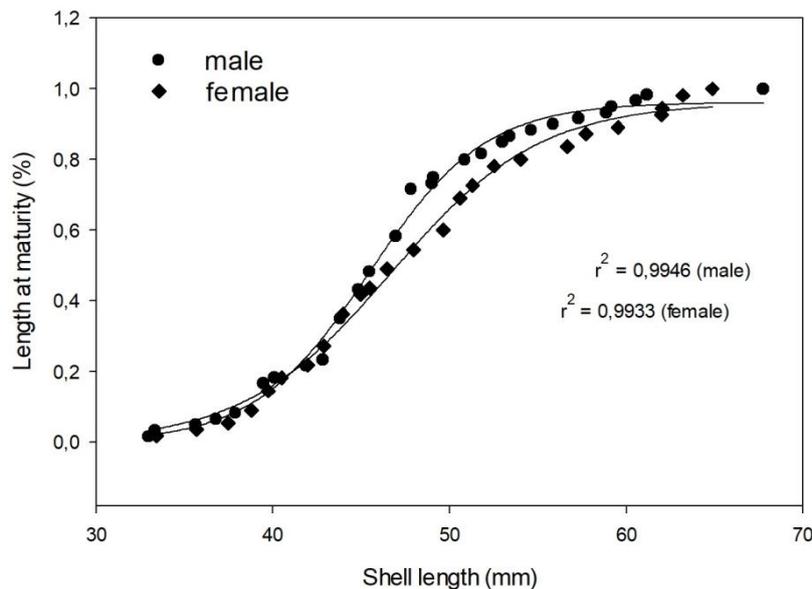


Figure 4. Size at sexual maturity of male and female *Haliotis asinina*.

In the present study, the sexual maturity of abalone in seagrass vegetation in Tapulaga was attained at shell length of 32.94 mm for male and 33.42 mm for female. This size is smaller than that of size founded by Capinpin et al (1998), where sexual maturity for both male and female *H. asinina* was at 40.60 mm shell length in wild-caught. In the Guernsey population, minimum size of sexual maturity was attained at 40 mm and 49 mm for males and females, respectively (Hayashi 1980). In the same case, in Southern Lombok waters, abalone *H. asinina* attain first sexual maturity at size length of 35.10-40.00 mm for male and 40.10-45.00 mm in female, respectively (Setyono 2009). Our study found that generally large amounts of small size abalone (<40.00 mm) were distributed in seagrass vegetation. Nevertheless, the type of abalone found had mostly ripe gonad, both sexes, in a relatively small shell length class. The possibility is caused by the presence of several factors including ecological pressure such as fishing intensity carried out every day during low tide, and also because of the distance of abalone habitat locations close to local settlements, making it easier for fisherman to catch abalone every day.

Habitat conditions and fishing pressures can also affect size variation of sexually mature abalone (Capinpin et al 1998; Maliao et al 2004). Broodstock density also contributes to low recruitment rates (Maliao et al 2004). Competition, commensalism, predation, parasitism, and climate (Day & Branch 2002; Jenkins 2004; Leonart et al 2003; Shepherd & Clarkson 2001) become phenomena affecting the development of abalone in the wild. These factors force abalones to fasten gonad maturation even though at relatively small size of shell length. This is due as a response of the body to maintain the sustainability of its population. This finding indicated that the reproductive strategy plays an important role in maintaining the presence of the species in nature (Effendie 1997). One effort to maintain the population is applying the r and K strategies (Reznick et al 2002).

Other factors that also affect gonadal maturation are food availability, water quality, and ecological pressure. Availability of food supports the development of gonads. The availability of optimal nutrition in food can induce higher reproductive performance in terms of spawning, fecundity, and hatching rates (Teruel & Fermin 2001). Recovery and recruitment of abalone populations is also influenced by climatological phenomena (Maliao et al 2004).

The size at first sexual maturity of *H. asinina* in South Lombok waters is bigger than that those found in Tapulaga waters, Southeast Sulawesi. This might also be caused by the habitat of the abalone. Abalone commonly prefer rocky waters overgrown by micro and macro algae (Setyono 2009), *H. tuberculata* for example select rock habitats in sublittoral regions (Forster 1962; Forster et al 1982) some are found in seagrass beds, such as *H. cylobates* in Southern Australia (Lindberg 1992). Abalone feeding on macro algae (*Gracillaria* sp.) shows a better growth rate of 76%, survival rate of 97.5% (Hayati et al 2018), and affects egg quality such as increased egg diameter (Banne et al 2017).

Abalone inhabiting seagrass beds, use the grass as their food (Shepherd 1973). Seagrass provide various benthic algae diatoms, including *Navicula* sp., *Nitzschia* sp. (Nahrullah et al 2016; Padang 2011). Benthic diatoms are consumed by larvae and juvenile or postlarva abalone (Setyono 2003; Ding et al 2017). Therefore, *H. asinina* found in the seagrass vegetation at Tapulaga was mostly smaller in size. This factor can be one of the causes that *H. asinina* caught in Tapulaga waters had smaller shell size than *H. asinina* in the habitat of coral rocks.

Conclusions. The sex ratio value between male and female abalone at seagrass vegetation Tapulaga was in a balanced condition of 1.15:1. The minimum size at sexual maturity for male *H. asinina* was 32.94 mm and for female individuals 33.42 mm, respectively.

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