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Manuscript Title: Size at first maturity of dolphinfish Coryphaena hippurus Linnaeus in Molucca Sea, North Sulawesi, Indonesia

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Respond to review: Responses to Review Results:

We would firstly appreciate the reviewers who have given very good point of view on our manuscript. We are very confident that all these valuable comments and suggestions have enriched this article for future improvement. There, we provide also some opinion regarding the field condition during the study period.

Insufficient number of samples. In general, I fully agree that the number of samples is very small. However, since Coryphaena hippurus is a migratory species, high number of catches is difficult to obtain. Their occurrence is quite seasonal. During the sampling period, May to July 2021, only these samples were obtained, and after these months until now, there is no dolphinfish catch in this area due to bad weather conditions. These were also collected from local fishermen who caught 1-5 individuals, because they are dependent upon line fishing.

More samples could change the sex ratio result of this species. It is not really true. Several previous studies have also shown the same finding even though they applied much more number, so that our finding is consistent with theirs as explained in the discussion section that there is probably sex segregation in certain period of the life cycle. In fact, during the sampling period, there was higher possibility to catch females than males, particularly in this waters. For this, I have added more information in the discussion.

Maximum size of dolphinfish in the paper is small. This is not right, and there is a misunderstanding of the reviewer 2, because I have put the maximum size of the fish in the introduction section (line 35-36) as expected by the reviewer. Moreover, due to adding more basic information on the introduction section, this part has been moved to line 37.

Size at first maturity estimation. The statistical analysis designed by Udupa (1986) has emphasized on the coverage of all possible fish sizes including immature and mature individuals to obtain mean size at first maturity in the fish population, so that the use of Udupa's statistical method is reasonable. This method has been much adopted for this purpose. Even though our results found smaller size at first maturity than previous report (Benseddik et al., 2019), our sample data have covered all immature and mature individuals. The difference may be caused by different environmental conditions with locations.

Regarding the photo, we do not have good photographs. We also do not have egg samples right now, and to take other samples, there is no fishing activity until now because the ocean weather condition is very bad and the fish catch depends upon the local fishermen relying on traditional fishing.

Despite the limitation of this finding due to the difficulty of obtaining large number of samples, this study has provided good basic information on this species for future research and management.

Finally, I would give the decision to the FAS Publishing of Korean Society in terms of publication types whether as short communication or research article.

Table 1. Gonad maturity characteristics

Maturity stage	Note	Female	Male
I	Immature	Small ovary up to ½ the length of the body cavity. It is translucent. Oocyte does not appear.	The testis is small up to ½ the length of the body cavity. It is whitish.
II	Maturing	The ovary is about half the length of the body cavity. It is orange, translucent, and oocyte cannot be seen by the naked eye.	The testis is about ½ the length of the body cavity. It is white and about symmetrical.
III	Ripening	The ovary is about 2/3 the length of the body cavity. Ovary yellow-orange, oocyte appears. Ovary with blood vessels on the surface. No transparent eggs or translucent, eggs are still dark.	The testis is about 2/3 the length of the body cavity.
IV	Ripe	The ovary is about 2/3 up to full of the body cavity. The ovary is orange-pink with blood vessels on the surface, eggs are apparent.	The testis is about 2/3 up to fulfilling the body cavity. It is white-soft cream.
V	Spent	Ovary shrinks down to ½ the body cavity. Wall is thick. There may be dark and mature eggs in the ovary that disintegrate from absorption, dark or translucent.	Testis shrinks down to ½ the body cavity. Wall is thick. The testis is soft.

Table 2. Gonad maturity stage of *C. hippurus* recorded in this study.

Size class (mm)	Gonad Maturity Stage (N=50)							
	I		II		III		IV	
	Male	Female	Male	Female	Male	Female	Male	Female
400-449	0	0	4	0	3	3	0	0
450-499	1	0	0	0	1	6	0	3
500-549	1	0	3	0	0	7	1	1
550-599	0	0	0	0	0	8	1	1
600-649	0	0	0	0	0	2	0	0
650-699	0	0	0	0	1	2	1	0

Figure 1. Fishing ground

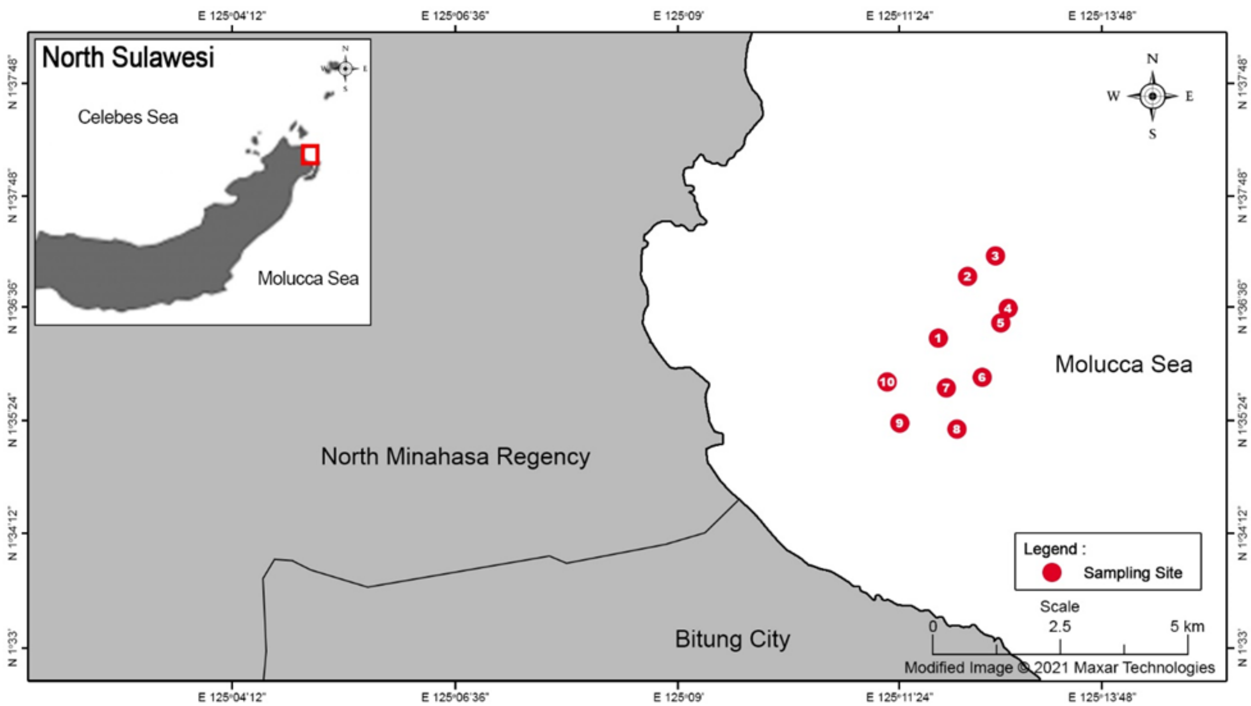
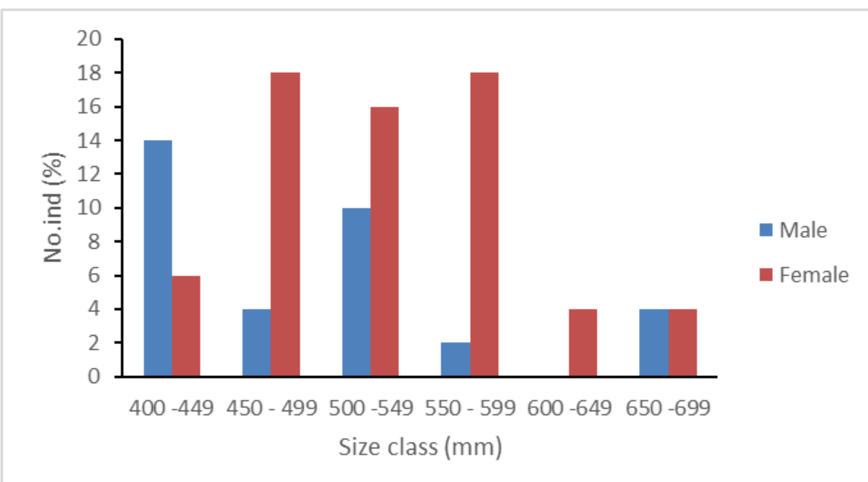


Figure 2. Length frequency distribution of dolphinfish *C. hippurus*



Responses to Review Results:

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2. **Insufficient number of samples**. In general, I fully agree that the number of samples is very small. However, since *Coryphaena hippurus* is a migratory species, high number of catches is difficult to obtain. Their occurrence is quite seasonal. During the sampling period, May to July 2021, only these samples were obtained, and after these months until now, there is no dolphinfish catch in this area due to bad weather conditions. These were also collected from local fishermen who caught 1-5 individuals, because they are dependent upon line fishing.
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6. **Regarding the photo**, we do not have good photographs. We also do not have egg samples right now, and to take other samples, there is no fishing activity until now because the ocean weather condition is very bad and the fish catch depends upon the local fishermen relying on traditional fishing.
7. Despite the limitation of this finding due to the difficulty of obtaining large number of samples, this study has provided good basic information on this species for future research and management.
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FAS (Fisheries and Aquatic Sciences) TITLE PAGE

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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article, Review article or Short Communcation
Article Title (within 20 words without abbreviations)	**Size at first maturity of dolphinfish *Coryphaena hippurus* Linnaeus in Molucca Sea, North Sulawesi, Indonesia**
Running Title (within 10 words)	**Size at first maturity of dolphinfish *Coryphaena hippurus***
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Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Ethics approval and consent to participate	This article does not require IRB/IACUC approval because there are no human and animal participants.

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(Un-structured) Abstract (up to 350 words)

Abstract. This study aims to estimate the smallest size of mature individuals that can be exploited. Fish samples *Coryphaena hippurus* were collected from Kalinaun fishermen's catches in Molucca Sea. They were sexed, then the fork length (FL) and maturity stage were recorded. Based on these two parameters, the size at first maturity was estimated. Results showed that *C. hippurus* in Molucca Sea had sex deviation with male-female ratio of 1 : 1.94 ($P<0.05$). Males had length range of 499 – 831 mm FL and females were at the length range of 481-813 mm FL. Size at first maturity was estimated as 529 mm FL for males with a range of 475-588 mm FL and 405 mm FL for females. Thus, *C. hippurus* caught in Molucca Sea has passed the size at first maturity, while the individual size also declines far below the maximum size. This study had provided basic information for future management needs of the dolphinfish, especially in Molucca Sea.

Keywords (3 to 5):

sex, fork length (FL), maturity stage, Kalinaun, fishermen.

Introduction

Long-term fish stock availability is always expected to meet human need as animal protein source. Therefore, fisheries management must be directed to maintain the populations remain sufficiently abundant to minimize extinction risk and sustain intact ecosystems (Freshwater et al., 2020). Fish reproduction is important aspect in maintaining the equilibrium of fish stock population in the water, since stock recovery is highly dependent upon the reproductive success. The reproductive cycle of fishes is closely related with the environmental changes particularly temperature, photoperiod and food supply (Bagenal, 1978). Determination of fecundity and the development of sexual maturity is also a fundamental to fishery science (Brown et al., 2003). Fecundity and spawning habits must be understood to explain the variation of the level of population to make efforts to increase the amount of fish harvest (Das et al., 1989). Hence, an appropriate management strategy can be applied to maintain the harvest level and the recovery rate.

Coryphaena hippurus (Linnaeus, 1758) (Coryphaenidae) is a commercially important species in tropical and temperate waters worldwide that generally inhabit in open waters, and less frequently occurs in coastal waters (Benyamin & Kurup, 2012). They are also known as mahi-mahi, one of the fisheries resources living in Indonesian waters and have become important fisheries commodity in line with tuna catch decline in Indian Ocean since 2007 (IOTC 2012). Since 1999, the export of this species is rising. Most dolphinfish in the United States are imported from Taiwan (34%), Peru (26%), Ekuador (21%), Panama (6%), Vietnam (5%), Costa Rica (3%), and other countries, such as Brazil, China, the Philippine, Indonesia, Japan, Colombia, South Africa Selatan, Chili, Thailand, Nicaragua, Argentina, Singapore, Mexico, and Oman (Marsh & Mazurek, 2007).

C. hippurus has sufficiently large size, the young one is about 30 cm long and the adults can reach 200 cm long with body weight up to 50 kg. Mean individual weight of fish caught ranges from 7 to 13 kg, rarely reaches 15 kg. *C. hippurus* is caught as bycatch in several types of fishing gears, such as purse seine, longline, and trolling, targeted for tuna, skipjack, and eastern little tuna (Chodrijah & Nugroho, 2016).

C. hippurus is a long-range and fast swimming fish (Hudson, 2014) that displace with time and an opportunistic epipelagic predator and preys on biota associated with fish aggregating device (FAD) and floating debris, such as fish, squids, and shrimps (Oxenford, 1999; Malone et al., 2011; Whitney et al., 2016), and can stay several days in association with raft (Taquet et al., 2007), Therefore, Japanese fishermen benefit bamboo raft "*tsukee*" to catch *C. hippurus* (Sakamoto & Kojima, 1999). Dolphinfish spend >80% of daytime activity and 40% of night time activity near the surface, where variability in diving patterns was more pronounced (Lin et al., 2020) and inhabit warmer sea water temperature of 24ºC- 30ºC (Palko et al., 1982). This species occupy temperature from 17 to 32ºC but spent 95% of their time between 25º and 29ºC, and when surface sea temperature (SST) rises, dolphinfish use behavioral thermoregulation by moving deeper up to 250 m (Schlenker et al., 2021). Based catch data, the highest dolphinfish CPUE occurs at 24◦C and chlorophyll-a concentration of <0.2 mg m^{-3} for the longline fishing and at 27◦C for recreational fishing when chlorophyll-a concentration is <0.1 mg m^{-3} with a peak at 0.02 mg m^{-3} (Farrell et al., 2014). Dolphinfish also do more vertical movements to deeper water column at night than daytime, whereas in the regions with the warmest SSTs they stay deeper during daytime hours than in regions with cooler SSTs, and nighttime activity increased with increasing lunar illumination, while conversely, below that temperature the opposite condition tends to occur (Schlenker et al., 2021).

The IUCN status of dolphinfish is least concern (Carlson et al., 2020). Many studies have been done on this species in several oceanic waters, such as India, Mexico, Mediteranian, Spain, Atlantic, and United States. Only several studies are carried out on biological characteristics and exploitation of this fish resources (Chodrijah & Nugroho, 2016) in Indonesia waters. This study is aimed at estimating the size at first maturity of Dolphinfish *C. hippurus* caught in Molucca Sea, North Sulawesi.   Knowledge on length at maturity and spawning season is important for the proper management and conservation of fish stocks (Nandikeswari, 2016). Size at first maturity is the smallest size of mature legally taken, the size at which 50% of the individuals are sexually mature (Farley et al., 2013). Size at 50% maturity (*l*) is commonly evaluated for wild populations as a point of biological reference. It has been utilized in various exploited animals, such as crustaceans (Skud & Perkins, 1969; Carlucci et al., 2006; Otieno et al., 2014; Peixoto et al., 2018)), fish population (White et al., 2011; Tesfahun, 2018), mollusks (Galimany et al., 2015; Pratasik et al., 2015). Size at first maturity estimation can be used to ensure that a sufficient number of juveniles reaches maturity (Roa et al., 1999), and there is strong belief that only fishing those individuals which have reached maturity is one of the basic rules that should be followed to ensure sustainability (Ilkyas et al., 2018). Proper estimation of size at first maturity is very useful for fish stock management (Karna et al., 2011). These data provide basic information on fish biology that is crucial for dolphinfish fisheries management in Indonesian waters and other neighborhood countries.

Materials and Methods

Dolphinfish *Coryphaena hippurus* samples were mainly collected from fishermen in Kalinaun coast, East Likupang district, North Minahasa, North Sulawesi. The fish samples were obtained in May to July 2021, because there was no catch after this period. Fishing activity was conducted near man-made Fish Aggregating Device (FAD) in Molucca Sea (Figure 1) located in the northern part of the village. In fishing operation, local fishermen usually used live bait-handline. Live baits were obtained in the multi-hooks handline fishing before daybreak. Additional fishing operations were also carried out in the same fishing ground using by trolling around the FAD to obtain more samples.

Figure 1. Fishing ground

The fish were sexed on the beach. The fork length and weight were also recorded, then the gonads were removed and brought to the Laboratory of the Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado, for further observation. The estimation of sex ratio used non parametric comparative test Chi-Square ($\chi 2$, $\alpha = 0.05$). Gonadal maturation was observed under a dissecting microscope. Maturation cycle is morphological changes of gonads to attain full growth and ripeness (Brown et al., 2003). The fish maturity stage was identified following Effendie (2002) (Table 1).

Table 1. Gonad maturity characteristics.

Estimation of first gonad maturity was carried out by setting the size class intervals, from the smallest to the largest one. Length distribution analysis followed Sturges (1926) as follows:

$$k = 1+3.3 \log n$$

where k is number of classes and n is number of data. Class interval was estimated as

$$C = \frac{Xn - X1}{k}$$

where C is class interval, Xn is the largest data value, X1 is the smallest data value, and k is number of classes.

Spearman-Karber equation was applied to estimate the size at first maturity of the fish (Udupa, 1986) as follows:

$$\mathbf{m} = x_k + \frac{\mathbf{x}}{\mathbf{2}} - (\mathbf{x}\sum \mathbf{p1})$$

where x_k = log last size in which 100% fish are fully mature

x = log size increment = $x_{l+1} - x_{l,}$ $l = 1, 2, \ldots k-1$

and x_o = log last size in which no fish are fully mature

r_l = number of fully mature fish in size group i

pi = proportion of fully mature fish in size group i

$p_l = r_l/n_l$, if $n_l \neq n_{l+1}$ for $i = 1, 2, \ldots k-i$

and $p_l = r_l/n$, if $n = n_l = n_{l+1}$ for $i = 1, 2 \ldots k-i$

Size at first maturity was obtained with antilog (m) = M.

antilog [m $\pm$ 1.96 $\sqrt{}x^2$ $\boldsymbol{\Sigma}$i $\{\frac{(pi - qi)}{ni - 1}\}$

Results

During the study, 50 fish individuals were collected from local fishermen in Kalinaun, East Likupang District, North Minahasa Regency, North Sulawesi. Males had size range of 405 mm - 674 mm FL with a weight range of 670 – 1,640 g, and females were at the length range of 431 mm - 687 mm FL with weight range of 725 – 2,650 g. Based on Sturges (1926), the length distribution was divided into 6 size class intervals.

Figure 2. Length frequency of *C. hippurus* caught in Molucca Sea.

Sex ratio, maturity stage, and size at first maturity

Sex ratio information is useful to maximize reproduction. The present study found sex ratio of 1 : 1.94 (P<0.05) represented by 17 males and 33 females. Gonad maturity of this species shows that more females are mature at smaller size than males (Table 2).

Table 2. Gonad maturity stage

Size at first maturity was estimated as 529 mm FL for males with a range of 475 – 588 mm and 405 mm FL for females.

Discussion

This low number of catches could result from that *C. hippurus* is not a target species. Local fishermen in this area go fishing for yellowfin tuna, marlin and sharks, whereas *C. hippurus* is optional when the target fish are not found. It could result from that the market value of this species is still low. Field observations also revealed that the occurrence of *C. hippurus* in this region is seasonal. Besides, although the fish *C. hippurus* are around, they are not bites at all in trolling or live bait fishing. Only few individuals of *C. hippurus* are caught, usually 1-5 individuals per boat. However, there is still no study on fishing season of *C. hippurus*, particularly in this area.

Previous study on dolphinfish landing in the Bitung Fisheries Port found 4,160 individuals of *C. hippurus* at the size range of 300 mm FL – 1,210 mm FL with mean length of 598 ± 13.9 mm FL (Chodrijah & Nugroho, 2016) reflecting small size dominance. Our present study found narrower size distribution than the previous in Molucca Sea, but inside the range of that landed in Bitung Fisheries Port. Difference in this size composition could result from less number of samples obtained in the present study due to high dependence on local artisanal fishermen who caught *C. hippurus* using handline, whereas in the previous report (Chodrijah & Nugroho, 2016), the fish samples came from catches of many kinds of fishing gears, such as purse seine, longline, and trolling.

Furthermore, this size range is far below the maximum individual size previously reported (Chodrijah & Nugroho, 2016) reflecting that mean individual size of *C. hippurus* has been declining. The recovery rate of a population is related to mortality rate, the closer the mean individual size to the maximum, the lower the mortality rate (ECTF, 2004). The present finding revealed that the dolphinfish population has high mortality rate. However, there are so many factors influencing fish population availability in the ocean. This condition is supported by Goldstein et al. (2007) that life-history traits are vulnerable to environmental stress and fishing pressure that result in smaller mature fishes as a response for survival. Fish mortality could occur at the specific stages and species and the causes may be single or cumulative pressure from a range of sources, such as pollutants, anthropogenic climate change or natural variability (Olsen et al., 2019) and fishing activities. Recruitment patterns with time can also influence the population size as well, and therefore, mortality events in the early life stages may have severe and long-lasting effects on the population (Langangen et al., 2017). Climate change is other factor causing changes in fish populations, in which it can affect the distribution of particular species and the fish susceptibility to particular fishing fleets (Rijnsdorp et al., 2009). This condition could occur because population size has probably fallen below some threshold level of abundance so that the rate of recovery is not able to well respond to the fishing rate.

This sex deviation is similar to that reported in the western and central Mediterranean (Potoschi et al., 1999; Benseddik et al., 2019) reflecting sex segregation in *C. hippurus* until reaching the mature stage. This result is also in agreement with Perle et al. (2020) that sex segregation occurs in *C. hippurus* or males are more susceptible to fisheries than females, even though our finding found more female than male individuals. Reports on the sex ratio of dolphinfish population from North Carolina, Gulf Stream, Florida Current, Puerto Rico, Virgin Islands, Gulf of Mexico, and Barbados have been consistent with the present finding with higher proportion of females than males (Oxenford, 1999). According to Benseddik et al. (2019), higher proportion of females from FADs captures could result from greater availability of females, higher natural mortality in males, or differential growth of both sexes. Moreover, males and females show different maturity stages with size class (Table 2). Both sexes show bigger individual size than 400 mm FL with more females at mature stages (III and IV). It indicates that males need bigger size to reach gonad maturity or females reach gonad maturity earlier than males. These data are consistent with Beardsley (1967) that female dolphinfish begin to mature (reach stage II) at about 350 mm FL (about 6-7 months old), 50% are mature at 450 mm FL, and 100% are mature at 550 mm FL, whereas males are mature at slightly larger size (427 mm FL). In the present study, females above 400 mm FL had reached maturity stage III and IV. The present study showed the same condition and suggested that mature individuals seem to gather in the same area for spawning and feeding around the rafts. Therefore, more females were caught than males around the FAD.

Although the present estimate of size at first maturity is smaller than previous report (Benseddik et al., 2019), 553 mm FL for females and 605 mm FL for males, both studies have suggested that females are mature earlier than males. This difference could result from different environmental conditions with localities. It means that 50% of mature individuals occurs at this size, particularly in Molucca Sea population, could be set as minimum legal size of this species to meet the sustainability criteria and avoid economic loss due to fishing immature individuals. The use of minimum legal size in fisheries is basically intended to protect juveniles, let them grow to adult and spawn at least once before caught, and therefore, this minimum legal size estimate is considered as management tool to maintain the spawning stock and control the fish size caught. The size range of *C. hippurus* caught in Molucca Sea reflects adult mature individuals and has mostly passed the size at first maturity. Nevertheless, since fishing is a major factor reducing size and age at first maturity (McIntyre & Hutchings, 2003) and decline in age and size at maturity may have negative effect on fish recovery (Hutchings, 2002), it needs to be controlled. The individual size decline of *C. hippurus* far below the maximum size could have indicated reduced population size and should not be ignored. Earlier maturity can be associated with reduced longevity, increased post-reproductive mortality, and smaller sizes at reproductive age. Populations composed of small individuals will reduce reproductive potential (Scott et al., 1999), increase variance in offspring survival (Hutchings & Myers, 1993), and eventually negatively affect the population growth.

Mesh size control and escapement could be an alternative to maintain or increase the individual size range or even increase the longevity, and the reproductive potentiality of dolphinfish. Larger fish have higher fecundity and can produce more eggs. So far, commercial purse seiners (< 30 GT) for small pelagic fish have fished any fish schools encountered in the open sea using small mesh size. As a result, small yellowfin tuna, skipjack, and dolphinfish are also caught (field obs.). Mesh size control and escapement could be done by redesigning the fishing gear that enables sufficient number of smaller fish passing through the mesh. Also, fishing gear separation should be established for commercial small pelagic and large pelagic fisheries in order to maintain stock availability and prevent individual size decline. This effort limitation could help reduce the risk of population collapse and become one of the remedies to population recovery. Nevertheless, all the management efforts need to be supported by strong regulations to force fishers to obey. Fish population recovery, therefore, requires institutional structures that either entice fishers to leave the business, through expensive buyout schemes of fishing boats and licenses, or else force them to reduce fishing activity (Hutching & Reynolds, 2004).

The present study has contributed to providing important biological information for future management, especially dolphinfish *C. hippurus* of Molucca Sea. Long term study on biology and ecology of this species is required to well describe the population status of *C. hippurus*, so that the management policy could be strengthened. The fisheries committee among neighborhood countries that take advantages of the resources should also participate in sustainable resource utilization programs by maintaining the exploitation level and the ecosystem equilibrium.

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