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Respond to review:	1. We would firstly thank your evaluation. We highly realize the limitation of this finding due to the small sample size despite having good consistency with previous studies. So we agree to put this paper as a short paper or short communication. 2. We have edited it to be more concise and short. For this, we took out the map and replaced it with the geographic position of the raft localities in the Molucca Sea where the samples were obtained.

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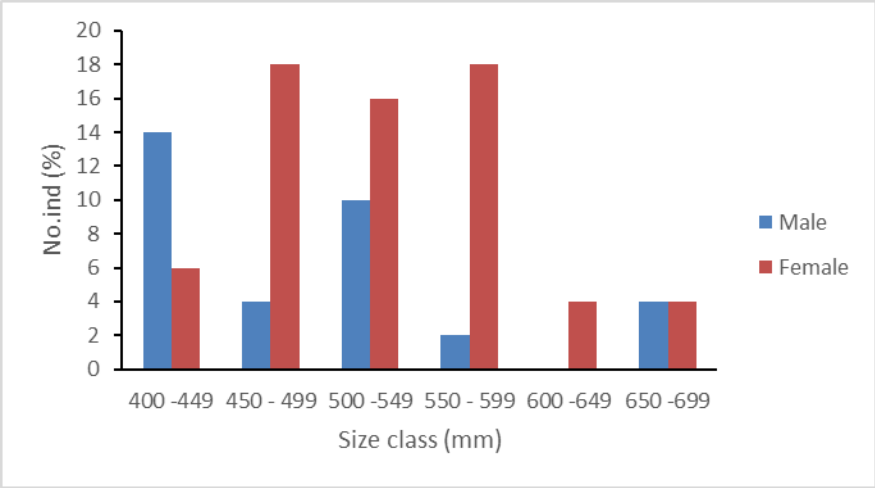
Table 1. Gonad maturity characteristics

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 and translucent, and the 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, and 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. Length frequency distribution of dolphinfish *C. hippurus*



Estimation of first maturity size of dolphinfish Coryphaena hippurus Linnaeus in the Molucca Sea, North Sulawesi, Indonesia

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Abstract. This study aims to estimate the smallest size of mature individuals that can be exploited. Fish samples of *Coryphaena hippurus* were collected from Kalinaun fishermen's catches in the Molucca Sea. They were sexed, then the fork length (FL) and maturity stage were recorded. Results showed that *C. hippurus* in the Molucca Sea had a sex ratio of 1: 1.94 ($P<0.05$). Males had a length range of 499 – 831 mm FL and females were in 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 the Molucca Sea has passed the size at first maturity, while the individual size also declines far below the maximum size. This study provided basic information for future management needs of the dolphinfish, especially in the Molucca Sea.

Keywords (3 to 5):
sex, fork length (FL), maturity stage, Kalinaun, fishermen.

Introduction

Fisheries management must be directed to maintain the fish populations remain sufficiently abundant to minimize extinction risk and sustain intact ecosystems (Freshwater et al., 2020). Fish reproduction is an important aspect in maintaining the equilibrium of the fish stock population in the water since stock recovery is highly dependent upon reproductive success. The reproductive cycle of fishes is closely related to environmental changes particularly temperature, photoperiod, and food supply (Bagenal, 1978). Thus, fecundity, sexual maturity, 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 and maintain the recovery rate (Das et al., 1989; Brown et al., 2003).

Dolphinfish, *Coryphaena hippurus* (Linnaeus, 1758) (Coryphaenidae), known as mahi-mahi, is a commercially important species in tropical and temperate waters worldwide that generally inhabit open waters, and less frequently

occurs in coastal waters (Benyamin & Kurup, 2012). They also live in Indonesian waters and have become an important fisheries commodity in line with the tuna catch decline in the Indian Ocean since 2007 (IOTC 2012).

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

C. hippurus is a long-range and fast swimming fish that displace with time and is an opportunistic epipelagic predator and preys on biota associated with a fish aggregating device (FAD) and floating debris, such as fish, squids, and shrimps (Oxenford, 1999; Malone et al., 2011; Whitney et al., 2016). *C. hippurus* can stay several days in association with a raft (Taquet et al., 2007), and therefore, Japanese fishermen benefit from bamboo raft "*tsukee*" to catch them (Sakamoto & Kojima, 1999). Dolphinfish spend >80% of daytime activity and 40% of nighttime activity near the surface (Lin et al., 2020) and inhabit warmer seawater temperatures of 24ºC- 30ºC (Palko et al., 1982). This species occupies water temperatures from 17º to 32ºC but spent 95% of its 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). The highest dolphinfish CPUE occurs at 24◦C and chlorophyll-a concentration of <0.2 mg m^{-3} for 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 columns at night than during the daytime, and nighttime activity increased with increasing lunar illumination (Schlenker et al., 2021).

The IUCN status of dolphinfish is the least concerned (Carlson et al., 2020). Many studies have been done on this species in several oceanic waters, such as India, Mexico, the Mediterranean, Spain, the Atlantic, and United States. This study is aimed at estimating the size at first maturity of Dolphinfish *C. hippurus* caught in the Molucca Sea, North Sulawesi. 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). Knowledge of length at maturity and spawning season is important for the proper management and conservation of fish stocks (Nandikeswari, 2016). Size at first maturity is commonly evaluated for wild populations as a point of biological reference to ensure that a sufficient number of juveniles reaches maturity (Roa et al., 1999) because only fishing the individuals which have reached maturity is one of the basic rules that should be followed to ensure sustainability (Ilkyas et al., 2018). It has been utilized in various exploited animals, such as crustaceans (Skud & Perkins, 1969; Carlucci et al., 2006; Peixoto et al., 2018)), fish populations (White et al., 2011; Tesfahun, 2018), mollusks (Galimany et al., 2015; Pratasik et al., 2015). 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 neighboring countries.

Materials and Methods

Dolphinfish *Coryphaena hippurus* samples were mainly collected from fishermen in the Kalinaun coast, East Likupang district, North Minahasa, North Sulawesi. The fish samples were obtained from May to July 2021, because there was no catch after this period. Fishing activity was conducted near a man-made Fish Aggregating Device (FAD) in the Molucca Sea located in the northeastern part of the village between 125°11'24'' E and 125°13'48'' E and 1°35'24'' and 1°35'24'' N. Local fishermen usually used live bait-handline. Live baits were obtained in the multi-hooks handline fishing before daybreak. Trolling was also carried out around the FAD to obtain more samples. 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 a non-parametric comparative test Chi-Square ($\chi 2$, $\alpha = 0.05$). Gonadal maturation was observed under a dissecting microscope. The maturation cycle is the 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.

The first gonad maturity was estimated 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. The 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:

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

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,…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*

p*i* = 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, …k-*i*

and $p_l = r_l/n$, if $n = n_l = n_{l+1}$ for *i* = 1, 2…..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 a size range of 405 mm - 674 mm FL with a weight range of 670 – 1,640 g, and females were at a length range of 431 mm - 687 mm FL with a weight range of 725 – 2,650 g. Based on Sturges (1926), the length distribution was divided into 6 size class intervals.

Figure 1. Length frequency of *C. hippurus* caught in the Molucca Sea.

Sex ratio, maturity stage, and size at first maturity

Sex ratio information is useful to maximize reproduction. The present study found a 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 mature at a 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 the low market value of this species. Field observations also revealed that the occurrence of *C. hippurus* in this region is seasonal. Besides, although the fish *C. hippurus* are around, they did not bite at all in trolling or live bait fishing. Only a few individuals of *C. hippurus* are caught, usually 1-5 individuals per boat. However, there is still no study on the fishing season of *C. hippurus*, particularly in this area.

A previous study on dolphinfish landing in the Bitung Fisheries Port found 4,160 individuals of *C. hippurus* in the size range of 300 mm FL – 1,210 mm FL with a mean length of 598 ± 13.9 mm FL (Chodrijah & Nugroho, 2016) reflecting small size dominance. The fish samples came from catches of many kinds of fishing gears, such as purse seine, longline, and trolling. The present study found narrower size distribution. The 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 rely on hand-line fishing.

Furthermore, this size range is far below the maximum individual size previously reported (Chodrijah & Nugroho, 2016) reflecting that the mean individual size of *C. hippurus* has been declining. The recovery rate of a population is related to the 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 a high mortality rate. However, so many factors influence 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 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 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 another factor causing changes in fish populations, which 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. The rate of recovery cannot 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 also agrees 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 females than males. Reports on the sex ratio of the dolphinfish population from North Carolina, Gulf Stream, Florida Current, Puerto Rico, Virgin Islands, Gulf of Mexico, and

142 Barbados have been consistent with the present finding with a higher proportion of females than males (Oxenford,
143 1999). According to Benseddik et al. (2019), a higher proportion of females from FADs captures could result from
144 greater availability of females, higher natural mortality in males, or differential growth of both sexes. Moreover, males
145 and females show different maturity stages with size class (Table 2). Both sexes show bigger individual sizes than
146 400 mm FL with more females at mature stages (III and IV). It indicates that males need a bigger size to reach gonad
147 maturity or females reach gonad maturity earlier than males. These data are consistent with Beardsley (1967) that
148 female dolphinfish begin to mature (reach stage II) at about 350 mm FL (about 6-7 months old), 50% are mature at
149 450 mm FL, and 100% are mature at 550 mm FL, whereas males are mature at a slightly larger size (427 mm FL). In
150 the present study, females above 400 mm FL had reached maturity stages III and IV. The present study suggested that
151 mature individuals seem to gather in the same area for spawning and feeding around the rafts. Therefore, more females
152 were caught than males around the FAD.
153 Although the present first maturity size estimate is smaller than the previous report (Benseddik et al., 2019),
154 553 mm FL for females and 605 mm FL for males, both studies have suggested that females mature earlier than males.
155 This difference could result from different environmental conditions in localities. It means that 50% of mature
156 individuals that occurs at this size, particularly in the Molucca Sea population, could be set as the minimum legal size
157 of this species to meet the sustainability criteria and avoid economic loss due to fishing immature individuals. The use
158 of minimum legal size in fisheries is intended to protect juveniles, let them grow into adults, and spawn at least once
159 before being caught. This minimum legal size could be considered a management tool to maintain the spawning stock
160 and control the fish size caught. The size range of *C. hippurus* caught in the Molucca Sea reflects adult mature
161 individuals and has mostly passed the size at first maturity. Nevertheless, since fishing is a major factor in reducing
162 size and age at first maturity (McIntyre & Hutchings, 2003) and a decline in age and size at maturity may negatively
163 affect the fish recovery (Hutchings, 2002), it needs to be controlled. The individual size decline of *C. hippurus* far
164 below the maximum size could have indicated a reduced population size and should not be ignored. Earlier maturity
165 can be associated with reduced longevity, increased post-reproductive mortality, and smaller sizes at reproductive age.
166 Populations composed of small individuals will reduce reproductive potential (Scott et al., 1999), increase variance in
167 offspring survival (Hutchings & Myers, 1993), and eventually negatively affect population growth.
168 Mesh size control and escapement could be an alternative to maintain or increase the individual size range or
169 even increase the longevity, and the reproductive potentiality of dolphinfish. Larger fish have higher fecundity and
170 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 sizes. 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 a sufficient number of smaller fish to pass through the mesh. Also, fishing gear separation should be established for commercial small pelagic and large pelagic fisheries 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. A long-term study on the 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 advantage of the resources should also participate in sustainable resource utilization programs by maintaining the exploitation level and the ecosystem equilibrium.

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Respond to Reviews

1. We would firstly thank your evaluation. We highly realize the limitation of this finding due to the small sample size despite having good consistency with previous studies. So we agree to put this paper as a short paper or short communication.

2. We have edited it to be more concise and short. For this, we took out the map and replaced it with the geographic position of the raft localities in the Molucca Sea where the samples were obtained.