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We have checked the manuscript line by line and made some changes (YELLOW). All references in the text are also consistent with the reference list. So, this is the final form of the article. Thank you

Fisheries and Aquatic Sciences

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

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7 **Abstract.** This study aims to estimate the smallest size of mature individuals that can be exploited. Fish samples of
8 *Coryphaena hippurus* were collected from Kalinaun fishermen's catches in the Molucca Sea. They were sexed, then
9 the fork length (FL) and maturity stage were recorded. Results showed that *C. hippurus* in the Molucca Sea had a sex
10 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-
11 813 mm FL. Size at first maturity was estimated as 529 mm FL for males with a range of 475-588 mm FL and 405
12 mm FL for females. This study provided basic information for future management needs of the dolphinfish, especially
13 in the Molucca Sea.

14 **Keywords (3 to 5):**

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

16

Introduction

17 Fisheries management must be directed to maintain the fish populations remain sufficiently abundant to
18 minimize extinction risk and sustain intact ecosystems (Freshwater et al., 2020). Fish reproduction is an important
19 aspect in maintaining the equilibrium of the fish stock population in the water since stock recovery is highly dependent
20 upon reproductive success that is closely related to environmental changes particularly temperature, photoperiod, and
21 food supply (Bagenal, 1978). Thus, fecundity, sexual maturity, and spawning habits must be understood to explain
22 the variation of the population level to increase the amount of fish harvest and maintain the recovery rate (Das et al.,
23 1989; Brown et al., 2003).

24 Dolphinfish, *Coryphaena hippurus* (Linnaeus, 1758) (Coryphaenidae), known as mahi-mahi, is a commercially
25 important species in tropical and temperate waters worldwide (Benyamin & Kurup, 2012) in line with the tuna catch
26 decline in the Indian Ocean (IOTC 2012). This species has a sufficiently large size, the young one is about 30 cm

27 long and the adults can reach 200 cm long with bodyweight up to 50 kg. The individual weight of fish caught ranges
28 from 7 to 13 kg and rarely reaches 15 kg. The species is caught as bycatch in several types of fishing gears, such as
29 purse seine, longline, and trolling (Chodrijah & Nugroho, 2016).

30 *C. hippurus* is a long-range and fast swimming fish that displace with time and is an opportunistic epipelagic
31 predator and preys on biota associated with a fish aggregating device (FAD) and floating debris, such as fish, squids,
32 and shrimps (Malone et al., 2011; Whitney et al., 2016). *C. hippurus* can stay several days in association with a raft
33 (Taquet et al., 2007). Dolphinfish spend >80% of daytime activity and 40% of nighttime activity near the surface (Lin
34 et al., 2020) and inhabit warmer seawater temperatures of 24°C- 30°C (Palko et al., 1982; Schlenker et al., 2021).
35 When surface sea temperature (SST) rises, dolphinfish use behavioral thermoregulation by moving deeper up to 250
36 m, and the nighttime activity increased with increasing lunar illumination (Schlenker et al., 2021). The IUCN status
37 of dolphinfish is the least concerned (Carlson et al., 2020).

38 This study is aimed at estimating the size at first maturity of Dolphinfish *C. hippurus* caught in the Molucca
39 Sea, North Sulawesi. Size at first maturity is the smallest size of mature legally taken, the size at which 50% of the
40 individuals are sexually mature (Farley et al., 2013). Knowledge of length at maturity and spawning season is
41 important for the proper management and conservation of fish stocks (Nandikeswari, 2016). Size at first maturity is
42 commonly evaluated for wild populations as a point of biological reference to ensure that a sufficient number of
43 juveniles reaches maturity (Roa et al., 1999) because only fishing the individuals which have reached maturity is one
44 of the basic rules that should be followed to ensure sustainability (Ilkyas et al., 2018). It has been utilized in various
45 exploited animals, such as crustaceans (Peixoto et al., 2018)), fish populations (Tesfahun, 2018), and mollusks
46 (Galimany et al., 2015), to protect juveniles, let them grow into adults, and spawn at least once before being caught.
47 Proper estimation of size at first maturity is very useful for fish stock management (Karna et al., 2011). These data
48 provide basic information on fish biology that is crucial for dolphinfish fisheries management in Indonesian waters
49 and other neighboring countries.

50

51 **Materials and Methods**

52 Dolphinfish *Coryphaena hippurus* samples were mainly collected from fishermen in the Kalinaun coast, East
53 Likupang district, North Minahasa, North Sulawesi. The fish samples were obtained from May to July 2021, because
54 there was no catch after this period. Fishing activity was conducted near a man-made Fish Aggregating Device (FAD)
55 in the Molucca Sea located in the northeastern part of the village between 125°11'24" E and 125°13'48" E and

56 1°35'24" and 1°35'24" N. Local fishermen usually used live bait-handline. Live baits were obtained in the multi-hooks
 57 handline fishing before daybreak. Trolling was also carried out around the FAD to obtain more samples. The fish were
 58 sexed on the beach. The fork length and weight were also recorded, then the gonads were removed and brought to the
 59 Laboratory of the Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado, for further
 60 observation. The estimation of sex ratio used a non-parametric comparative test Chi-Square (χ^2 , $\alpha = 0.05$). Gonadal
 61 maturation was observed under a dissecting microscope. The fish maturity stage was identified following Effendie
 62 (2002) (Table 1).

63
 64 Table 1. Gonad maturity characteristics.

65 The first gonad maturity was estimated by setting the size class intervals, from the smallest to the largest one. Length
 66 distribution analysis followed Sturges (1926) as follows:

67
$$k = 1 + 3.3 \log n$$

68 where k is number of classes and n is number of data. The class interval was estimated as

69
$$C = \frac{X_n - X_1}{k}$$

70 where C is class interval, X_n is the largest data value, X_1 is the smallest data value, and
 71 k is number of classes.

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

73
$$m = x_k + \frac{x}{2} - (x \sum p_i)$$

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

75
$$x = \log \text{ size increment} = x_{l+1} - x_l, l = 1, 2, \dots, k-1$$

76 and x_0 = log last size in which no fish are fully mature

77
$$r_i = \text{number of fully mature fish in size group } i$$

78
$$p_i = \text{proportion of fully mature fish in size group } i$$

79
$$p_i = r_i/n_i, \text{ if } n_i \neq n_{i+1} \text{ for } i = 1, 2, \dots, k-i$$

80 and
$$p_i = r_i/n, \text{ if } n = n_i = n_{i+1} \text{ for } i = 1, 2, \dots, k-i$$

81 Size at first maturity was obtained with $\text{antilog}(m) = M$.

82
$$\text{antilog} [m \pm 1.96 \sqrt{x^2 \sum_i \left\{ \frac{(p_i - q_i)}{n_i - 1} \right\}}]$$

83

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 classes.

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 due to the low local market value of this species. Field observations also revealed that the occurrence of *C. hippurus* in this region is seasonal. Besides, although the fish 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, and it could result from less number of samples obtained due to high dependence on local artisanal fishermen who rely on hand-line fishing.

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

127 This sex deviation is similar to that reported in the western and central Mediterranean (Potoschi et al., 1999;
128 Benseddik et al., 2019) reflecting sex segregation in *C. hippurus* until reaching the mature stage. Mature individuals
129 seem to gather in the same area for spawning and feeding around the rafts so that more females were caught than
130 males. This result also agrees with Perle et al. (2020) and Oxenford (1999) that sex segregation occurs in *C. hippurus*
131 or males are more susceptible to fisheries than females, even though our finding found more females than males. A
132 higher proportion of females from FADs captures could result from greater availability of females, higher natural
133 mortality in males, or differential growth of both sexes (Benseddik et al., 2019). Moreover, males and females show
134 different maturity stages with size class (Table 2). Both sexes show bigger individual sizes than 400 mm FL with
135 more females at mature stages (III and IV). It indicates that males need a bigger size to reach gonad maturity or females
136 reach gonad maturity earlier than males. These data are consistent with Beardsley (1967) that female dolphinfish begin
137 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
138 mature at 550 mm FL, whereas males are mature at a slightly larger size (427 mm FL). Nevertheless, in the Eastern
139 Tunisian Coast, Central Mediterranean, Benseddik et al. (2019) found that the first maturity size of *C. hippurus* occurs
140 at 553 mm FL for females and 605 mm FL for males. In the present study, females above 400 mm FL reached maturity
141 stages III and IV. This difference could result from different environmental conditions in localities. It means that 50%

142 of mature individuals that occurs at this size, particularly in the Molucca Sea population, could be set as the minimum
143 legal size of this species to meet the sustainability criteria and avoid economic loss due to fishing immature individuals.
144 The size range of *C. hippurus* caught in the Molucca Sea reflects mature individuals and has mostly passed the size at
145 first maturity. Nevertheless, since fishing is a major factor in reducing size and age at first maturity (McIntyre &
146 Hutchings, 2003) and a decline in age and size at maturity may negatively affect the fish recovery (Hutchings, 2002),
147 it needs to be controlled. The individual size decline of *C. hippurus* far below the maximum size could have indicated
148 a reduced population size and should not be ignored. Earlier maturity can be associated with reduced longevity,
149 increased post-reproductive mortality, and smaller sizes at reproductive age. Populations composed of small
150 individuals will reduce reproductive potential (Scott et al., 1999), increase variance in offspring survival (Hutchings
151 & Myers, 1993), and eventually negatively affect population growth.

152 Mesh size control and escapement could be an alternative to maintain or increase the individual size range or
153 even increase the longevity, and the reproductive potentiality of dolphinfish. Larger fish have higher fecundity and
154 can produce more eggs. So far, commercial purse seiners (< 30 GT) for small pelagic fish have fished any fish schools
155 encountered in the open sea using small mesh sizes. As a result, small yellowfin tuna, skipjack, and dolphinfish are
156 also caught (field obs.). Fishing gear separation should be established for commercial small pelagic and large pelagic
157 fisheries to maintain stock availability and prevent individual size decline. This effort limitation could help reduce the
158 risk of population collapse and become one of the remedies to population recovery. Fish population recovery,
159 therefore, requires institutional structures that either entice fishers to leave the business, through expensive buyout
160 schemes of fishing boats and licenses or force them to reduce fishing activity (Hutching & Reynolds, 2004).

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

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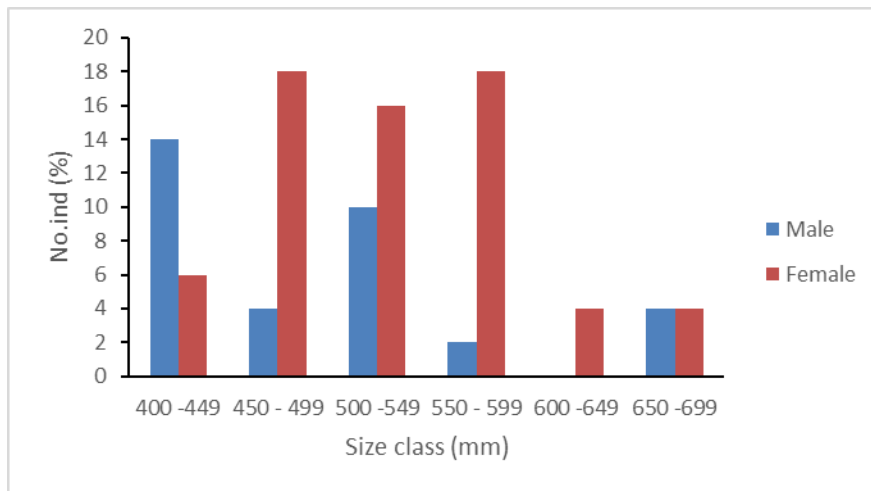
Table 1. Gonad maturity characteristics

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*



Respond to Review

We have checked the manuscript line by line and made some changes (YELLOW). All references in the text are also consistent with the reference list. So, this is the final form of the article. Thank you