Different marine macroalgae feeding preferences of adult white-spotted rabbitfish (*Siganus canaliculatus*)

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Abstract. White-spotted rabbitfish (*Siganus canaliculatus* Park, 1797) is a herbivorous fish that can be cultivated by utilizing seaweed (macroalgae) as its natural food. This study aims to determine the feed preferences of *S. canaliculatus* to three species of marine macroalgae. The research was conducted at The Research Center for Deep-Sea, National Research and Innovation Agency (BRIN), Ambon, Indonesia. Three species of marine macroalgae were used as test feed, namely: *Ulva reticulata* (Chlorophyceae), *Gracilaria lichenoides* (Rhodophyceae), and *Padina crassa* (Phaeophyceae). This research was conducted through two experimental stages. In the first stage of the experiment, each test animal was placed in three aquariums containing 3 different species of macroalgae, and in the second stage of the experiment, nine test animals were placed in nine aquariums, where each aquarium contained 1 different species of macroalgae (1 treatment with 3 tests). A completely randomized design was adopted in this study. The first experiment used one treatment three times and analyzed it descriptively. The second experiment used three treatments with three replications and was analyzed using one-way ANOVA, followed by Duncan's test. The results showed that *S. canaliculatus* preference to the type of macroalgae was based on consumption from the highest to the lowest, namely, *P. crassa, G. lichenoides*, and *U. reticulata*. Concurrent feeding of macroalgae resulted in a higher consumption level than single feeding. The high feed preference of *S. canaliculatus* based on the consumption value of marine macroalgae species was associated with high proximate content, especially protein and lipids, and tended to be inversely related to high carbohydrate content. Therefore, the selection of macroalgae species as natural feeds or as raw materials in artificial feed formulations must consider the proportion of proximate content to support optimal feed consumption by *S. canaliculatus*.
Key Words: feed consumption, *Gracilaria lichenoides*, *Padina crassa*, proximate content, *Ulva reticulata*.

Introduction. Marine macroalgae are increasingly getting attention as an alternative protein source for fish farming, especially in developing countries in the tropics, because of their high protein content (Hasan & Chakrabarti 2009). Marine macroalgae can be used as additives in feed to effectively use nutrients in cultured fish (Mustafa et al 1995). The potential of marine macroalgae as a food source has been exploited commercially and intensively cultivated with polyculture techniques (Munifah 2008). *Ulva reticulata*, *Gracilaria lichenoides*, and *Sargassum polycystum* are used as stimulants in artificial feeds to improve the quality of sea urchins (Purbiantoro 2014). *Gracilaria verrucosa*, *Eucheuma spinosum*, *Ulva* spp., and *Gracilaria arcuata* in abalone (*Haliotis asinina*) cultivation (Nurfajrie et al 2014). *Gracilaria vermiculophylla*, *Porphyra dioica*, and *Ulva* spp. as feed ingredients for tilapia (*Oreochromis niloticus*) (Silva et al 2014).

White-spotted rabbitfish (*Siganus canaliculatus* Park, 1797) is an herbivorous fish commonly found in seagrass habitats (Munira et al 2010; Latuconsina et al 2013; Suardi et al 2016; Latuconsina et al 2020a). Their diet consists of seagrass leaves, macroalgae, copepods, amphipods, hydrozoa, Ostracoda, gastropod and bivalves' larvae attached to seagrass leaves (El-Sayed 1994; Latuconsina et al 2013; Latuconsina et al 2021; Kwak et al 2015). *S. canaliculatus* has the potential to be cultivated en masse using floating net cages (Jaikumar 2012; Visca et al 2017; Paruntu et al 2020; Salampessy & Irawati 2021) because it is not cannibalistic, grows fast, and feeds on macroalgae such as *Kappaphycus alvarezii*, *Ulva reticulata*, *Hypnea* spp., and *Enteromorpha* (Jaikumar et al 2011). The digestive tract of rabbitfish can adapt well to different foods and adjust physiologically to the nutritional status of the feed given (Xie et al 2018).

Feed is an essential factor in fish farming activities. Feed preferences, cost, and availability must be considered in selecting feeds other than nutritional content. Seaweed (marine macroalgae) is a source of minerals and nutrients and is the leading food for various marine herbivorous fish species (Tolentino-Pablico et al 2008), one of which is *Siganus canaliculatus* (Siganidae). A study of feed preferences revealed that *S. canaliculatus* prefers Chlorophyceae (*Enteromorpha intestinalis*) and Rhodophyceae (*Gracilaria confervoides*) (von Westernhagen 1974). You et al (2014a) found that three macroalgae species, namely *Ulva prolifera* (Chlorophyceae), *Gracilaria lemaneiformis* (Rhodophyceae), and *Chaetomorpha linum* (Chlorophyceae), favoured by *S. canaliculatus*. This difference in macroalgae feeds preference is related to differences in proximate content.

In Ambon Bay waters, 21 species of marine macroalgae were found, which were very abundant, including 10 species from the Rhodophyceae class, six species from the Chlorophyceae, and five species from the Phaeophyceae (Litaay 2014), but macroalgae have not been used as natural feed to support the development of herbivorous fish culture such as *Siganus canaliculatus*. *S. canaliculatus* was found abundantly in the waters of Inner Ambon Bay and is strongly associated with various types of seagrass habitats (Latuconsina et al 2013; Latuconsina et al 2020a; Latuconsina et al 2022) but with a higher level of exploitation (Latuconsina et al 2020b), will threaten its existence in the wild. This fact is a consideration for developing *S. canaliculatus* cultivation in the future. As an initial stage, it is necessary to test the natural feed preferences of macroalgae to obtain ideal feed candidates who are expected to be the essential ingredients of feed formulations for the development of mass cultivation of *S. canaliculatus*.

Material and Method.

Time and location of research. This research was conducted from December 2016 to February 2017. The stages of this research started from acclimatization of test animals (*Siganus canaliculatus*), feeding, and monitoring of water quality carried out at the Aquaculture Laboratory of The Research Center for Deep-Sea, National Research and Innovation Agency (BRIN), Ambon, Indonesia

Animal and feed test. A total of 9 specimens of *Siganus canaliculatus* adults measuring 12-14 cm and weighing 15-35 g were obtained from Inner Ambon Bay, Maluku-Indonesia and used as test animals, and the tested feeds were three species of macroalgae, namely, *Ulva reticulata* (Chlorophyceae), *Gracilaria lichenoides* (Rhodophyceae), and *Padina crassa* (Phaeophyceae). The test animals were placed in an 80L capacity aquarium equipped with a Recirculating Aquaculture System (RAS).

Experiment. The three marine macroalgae species were administered simultaneously (mixed) in experiment I and separately (single) in experiment II. Each stage of the experiment was carried out for three days. Each trial used a completely randomized design adopted in this study, with three replications for each treatment. During the observation, the measurement of water quality parameters (temperature, salinity, pH, and dissolved oxygen) parameters were measured. Macroalgae proximate analysis was conducted at the Research and Industrial Standardization Laboratory of Ambon, Maluku - Indonesia.

Data analysis. Measurement of water content in each marine macroalgae species was carried out before being given as test feed using the equation:

WC=(GW-DW)/GW x 100% .... (1)

where:
WC = water content, GW = gross weight, DW = dry weight.

Before calculating the dry weight of the feed, first, marine macroalgae were dried using an oven at 96°C for 24 hours. The feed preference of the test animal (*S. canaliculatus*) was obtained from the data on the difference in weight of each macroalgae species eaten by the test animal. Feed consumption is calculated using the equation (Dworjanyn et al 2007):

Feed consumption (g) = (Ui x Cf /Ci) - Uf .... (2)

where: Ui= initial feed weight, Uf= final feed weight (leftover feed), Ci = control feed weight before testing, Cf = control feed weight after testing.

A completely randomized design was adopted in this experimental study. In the first stage of the experiment, one treatment with three replications was analysed descriptively (ranking order). The second stage of the experiment used four treatments with three replications and used one-way variable analysis (ANOVA) with Duncan's test for further testing at a 95% confidence level.

Results and Discussion. The range and mean values of the water quality parameters measured during maintenance in the experimental aquarium containers are as shown in Table 1.

Table 1

Value of water quality parameters during maintenance in aquarium containers

Water quality parameters	*Unit*	*Range*	*Average ± SD*
Temperature	(°C)	25.16 – 28.71	26.55 ± 0.30
Salinity	(‰)	23.21 – 34.12	33.31 ± 0.34
pH		7.25 – 8.15	7.95 ± 0.02
Dissolved oxygen	(mg/l)	4.55 – 8.20	6.84 ± 0.70

The average water temperature value in the aquarium containers is still in the optimal range to support the metabolic process of *S. canaliculatus* (Table 1). The optimal temperature range for the life of *S. canaliculatus* is 25°C-34°C (Lam 1974). Water temperature affects the metabolic activity of fish related to dissolved oxygen concentration and oxygen consumption. The metabolic rate of fish will increase with increasing water temperature and, at the same time, increase the need for dissolved oxygen consumption (Effendi 2003; Kordi & Tancung 2007). According to Floeter et al (2005), herbivorous fish experience physiological constraints in feeding activities and the process of food digestion related to temperature. Thus, according to Carr et al (2018), the grazing rate of herbivorous fish increases with increasing water temperature, and vice versa. *S. canaliculatus* can live in a wide salinity range of 17-37‰ but is sensitive to high pH (>9) and low dissolved oxygen content <2 mg/l (Lam 1974), thus the average value of salinity, pH, and the dissolved oxygen content during the trial in the aquarium containers is still feasible to support the metabolic process of *S. canaliculatus*.

Macroalgae proximate content. The results of the analysis of the proximate content of the three marine macroalgae species used as test feed are as shown in Table 2.

Table 2

Proximate content of marine macroalgae fed to *Siganus canaliculatus*

Species of marine macroalgae	*Water content (%)*	*Proximate composition in dry weight (%)*				
		Ash level	*Protein*	*Lipid*	*Carbohydrate*	*Coarse fiber*
Ulva reticulata	19.24	36.18	8.73	0.82	2.93	51.34
Gracilaria lichenoides	8.44	50.33	7.97	0.5	13.81	27.39
Padina crassa	7.29	47.2	9.08	1.87	2.61	39.24

The results of the analysis of the proximate content of three species of marine macroalgae (Table 2) showed that the *P. crassa* species had higher protein and lipid content than the other two species of macroalgae. While *U. reticulata* contains the highest water content of 19.24%, the lowest value is recorded for *P. crassa* of 7.29%. According to Winarno (2008), water content is the amount of water contained in foodstuffs that can affect the appearance, texture, and taste of foodstuffs; high water content makes it easy for bacteria, molds, and yeasts to breed so that changes will occur in foodstuffs that can accelerate spoilage.

The highest protein content value was found in *P. crassa* at 9.08%, and the lowest was *G. lichenoides* at 7.97% (Table 2). According to Hasan and Chakrabarti (2009), only about 10-15 percent of food protein needs can be met by algae in the test diet without compromising growth and food utilization by fish. According to Winarno (2008), protein is a macromolecule formed from amino acids bound to peptides and contains elements carbon (C), hydrogen (H), oxygen (O), and nitrogen (N) and functions for the body as enzymes, movement regulators, and body defences.

The highest lipid content was in *P. crassa* at 1.87%, and *G. lichenoides* had the lowest at 0.50% (Table 2). In contrast, the highest carbohydrate content was in *G. lichenoides* at 13.81%, and the lowest was in *P. crassa* at 2.61%. According to Hasan and Chakrabarti (2009), the poor performance of fish feed containing higher algae inclusions may be due to the high carbohydrate content. The dominance of the complex and structural carbohydrates can cause low digestibility. According to Muchtadi (1989), carbohydrates in food can be classified into two types, namely digestible carbohydrates that function as an energy source for the body and indigestible carbohydrates, which function to prevent various diseases. According to Winarno (2008), carbohydrates play an essential role in determining the characteristics of food ingredients, such as taste, color, texture, and others.

The highest crude fiber content was found in the macroalgae *U. reticulata* at 51.34% and the lowest in *G. lichenoides* at 27.39% (Table 2). According to Kordi (2009), the high crude fiber content in fish feed will affect the digestibility and absorption in the digestive tract of fish. The highest ash content was found in the macroalgae *G. lichenoides* at 50.33% and the lowest in *U. reticulata* at 36.18%. According to Winarno (2008), ash content is a mixture of inorganic or mineral components in a food ingredient where food consists of 96% organic matter and water, while the rest is mineral elements.

Feed preference of Siganus canaliculatus. The results of the feed preference test of *Siganus canaliculatus* on three species of marine macroalgae through experiments I and II are shown in Figures 1, 2, 3, and 4.

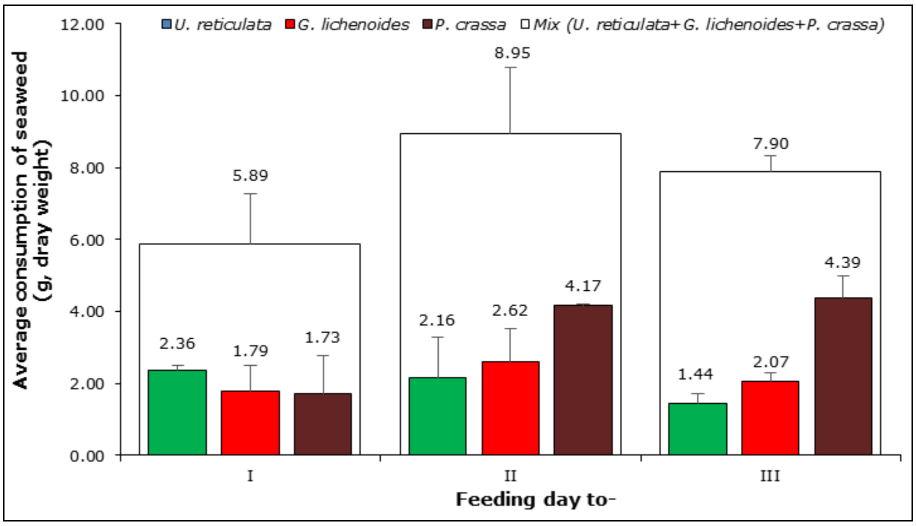

Figure 1. Graph of experiment I (feed given together). Daily consumption value.

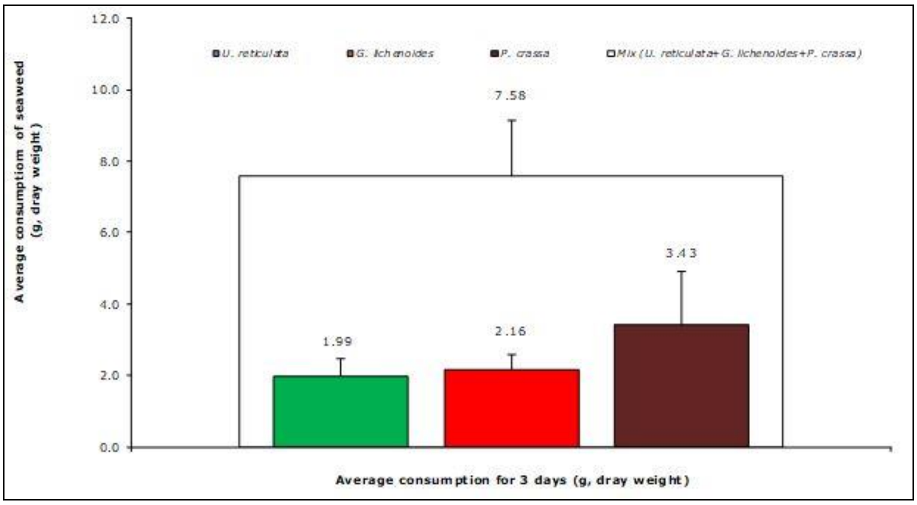

Figure 2. Graph of experiment I (feed given together). Average consumption value.

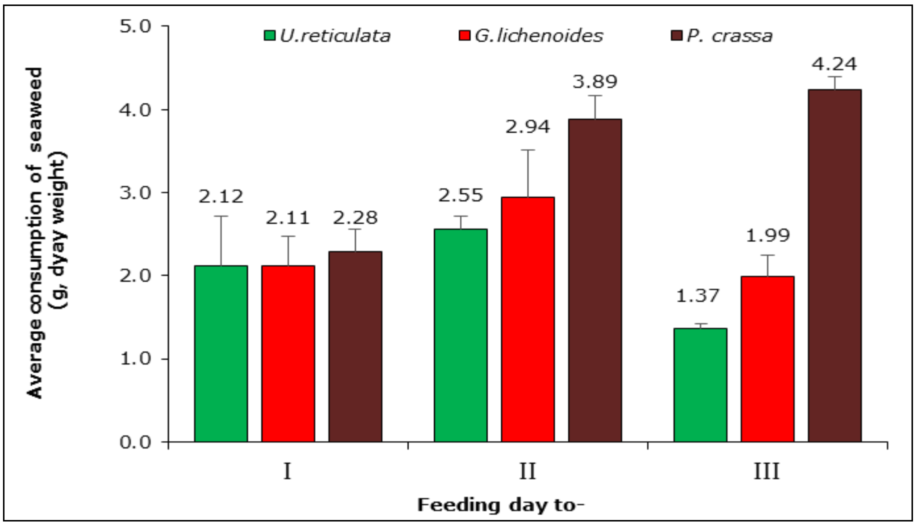

Figure 3. Graph of experiment II (feed is given separately, single). Daily consumption value.

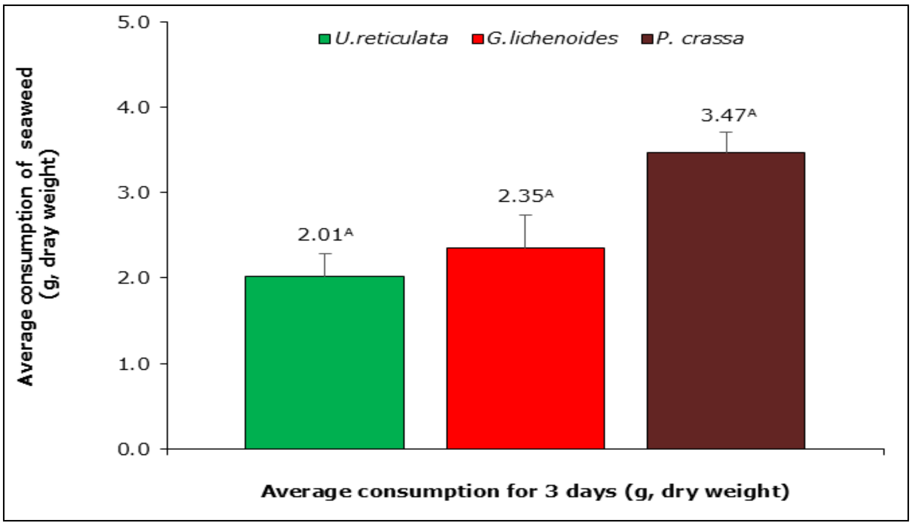

Figure 4. Graph of experiment II (feed is given separately, single). Average consumption value.

There are differences in the level of preference of *S. canaliculatus* to different species of marine macroalgae, either given together (mixed) or separately (Figures 1, 2, 3, 4). The highest average preference based on the value of marine macroalgae feeds consumption was *P. crassa* (Phaeophyceae), and the lowest was *U. reticulata* (Chlorophyceae). The highest preference for *P. crassa* species showed a high level of palatability of *S. canaliculatus* against this macroalgae species. If it is associated with proximate content, it is suspected that the high feed preference of *S. canaliculatus* for each marine macroalgae species is closely related to the high protein and lipid content and inversely proportional to the carbohydrate content of each marine macroalgae species.

The results of experiments I and II showed the preference of *S. canaliculatus* to marine macroalgae species based on the amount of consumption, sorted as follows: *P. crassa*, *G. lichenoides*, and *U. reticulata*. The combination of experimental data I and II showed that feeding marine macroalgae simultaneously (mixed) resulted in a higher level of feed consumption than if given separately (single) (significantly different, Duncan's test, $p<0.05$). This fact indicates that *S. canaliculatus* will choose marine macroalgae to feed by taste and nutritional content for its metabolic needs.

The low consumption value of *S. canaliculatus* against the macroalgae from species *U. reticulata* is thought to be related to the high water content, thereby accelerating the decay and decreasing the appetite of *S. canaliculatus*. You et al (2014a) found that three species of marine macroalgae, namely *Ulva prolifera* (Chlorophyceae), *Gracilaria lemaneiformis* (Rhodophyceae), and *Chaetomorpha linum* (Chlorophyceae), were favoured by *S. canaliculatus*, and the least preferred was *Ulva pertusa* (Chlorophyceae) and *Porphyra haitanensis*. In contrast, the least favoured was *Sargassum fusiforme* (Phaeophyceae) and *Corallina sessilis* (Rhodophyceae).

The marine macroalgae favoured by *S. canaliculatus* are richer in protein and soluble sugars so that they are higher in energy than the least preferred. In addition, these fish prefer fibrous and flat algae over calcified ones. Thus, the nutrient richness and morphological characteristics determine the preference of *S. canaliculatus*. According to von Westernhagen (1974), a factor that seems to be important in terms of feed preference for *S. canaliculatus* fish is the texture of the algal thallus. The most preferred marine macroalgae are crispy, and the thallus is relatively thin, so the siganids can easily bite them.

According to Tolentino-Pablico et al (2008), many marine herbivorous fish associated with coral reefs, such as the family Siganidae (*Siganus* spp.), selectively feed on filamentous and fleshy macroalgae such as brown algae (Phaeophyceae), which are preferred over calcareous and crusty ones. In addition, the high consumption of marine macroalgae by herbivorous fish is due to the low levels of tannins and phenolics they contain, as found in brown algae (Steinberg 1986). Generally, marine macroalgae that are calcified and contain a high number of secondary metabolites are not favoured by herbivore animals (Paul & Hay 1986).

The results of this study prove that marine macroalgae can be used as natural food to support the growth of *S. canaliculatus*. So that it can be used as an alternative natural feed or can be developed as a base for cheap artificial feed formulations to support the cultivation of *S. canaliculatus*, the addition of marine macroalgae into the feed formulation according to You et al (2014b), it can improve growth performance and increase antioxidant capacity. It is practical and efficient in feed formulations for cultivating herbivorous fish such as *S. canaliculatus*.

Conclusions. The feed preferences of *Siganus canaliculatus* based on the consumption value of marine macroalgae species are associated with high proximate content, especially protein and lipids, and tend to be inversely related to high carbohydrate content. The high water content also accelerates spoilage which can reduce the freshness of macroalgae, thus being a low preference of *S. canaliculatus*. Therefore, selecting macroalgae species as natural feeds or as ingredients into artificial feed formulations, the proportion of proximate content such as high protein and lipid and low carbohydrates and water content must be considered to support optimal feed consumption by *Siganus canaliculatus*.

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Conflict of interest. The authors declare that there is no conflict of interest.

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