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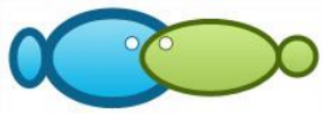
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Feeding preference of white spotted rabbitfish (*Siganus canaliculatus*) on different species of seagrass

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Abstract. The white-spotted rabbit (*Siganus canaliculatus*) is a group of marine herbivorous fish that has the potential to be cultivated. One of the natural food is seagrass. The purpose of this study was to analyze the feeding preferences of *S. canaliculatus* on different species of seagrass. This experimental study used *S. canaliculatus* and four types of seagrass (*Enhalus accoroides*, *Thalassia hemprichii*, *Cymodocea rotundata*, *Cymodocea serrulata*) as test feed. An 80 L aquarium with an aquaculture recirculation system was used as a rearing tank. The study was carried out in 2 experimental stages, namely: 4 species of seagrass were placed in the same rearing container (Experiment I), and 4 types of seagrass were separated, each in a different rearing container (Experiment II). A total of 1 individual fish was put into each container. Feed consumption was recorded every 24 hours, each experiment was carried out for 3 days (72 hours). This study used a completely randomized design (CRD). Experiment I used one treatment with three replications and the data were analyzed descriptively. Experiment II used four treatments with three replications and the data were analyzed using one-way ANOVA and Duncan's test to determine further differences. The results showed that *S. canaliculatus* preferred seagrass feed, respectively, *C. serrulata*, *C. rotundata*, *T. hemprichii*, and *E. acoroides*. Different seagrass feed preferences are thought to be related to 1) proportional proximate composition, 2) protein to energy and higher GE/P ratio, and 3) low content of secondary metabolites (tannins). Thus, *C. rotundata* and *C. serrulata* can be used as the main ingredients for making feed formulations for *S. canaliculatus*.

Key Words: biochemical content, herbivorous fish, natural feed, tannin content.

Introduction. White-spotted rabbitfish *Siganus canaliculatus* (Park, 1797) is a marine teleost widely distributed in the Indo-Pacific region (Allen & Erdmann 2012). It is a herbivorous fish spatially distributed in mangrove swamps, seagrass meadows, and coral reefs (Woodland 2001; Latuconsina et al 2013; Latuconsina et al 2015; Suardi et al 2016; Latuconsina et al 2019). *S. canaliculatus* has a strongly association with seagrass habitats during its life cycle in the juvenile, pre-adult, and adult stages, seagrass being a nursery ground (Latuconsina et al 2013; Latuconsina et al 2020^a).

The distribution of *Siganus canaliculatus* depends on the day and night period, and on moon phases. It always dominates the structure of fish communities in seagrass habitats, especially in the waters of Inner Ambon Bay (Latuconsina et al 2012; Latuconsina & Ambo-Rappe 2013). However, the increasing fishing pressure in the waters of Inner Ambon Bay (Manik 1998; Latuconsina et al 2020^b), and the threat to seagrass habitats due to anthropogenic pressure (Irawan & Nganro, 2016), are feared to reduce their population in the wild and threaten sustainability. One solution that needs to be developed for the sustainable use of *S. canaliculatus* is through cultivation development (Jaikumar 2012).

S. canaliculatus has commercial value as a consumption fish and has the potential to be a cultivated commodity (Lam 1974; Tharwat 2004) in floating net cage systems (Tacon et al 1990; Jaikumar 2012; Visca et al 2017; Paruntu et al 2020). When reared in floating net cages, it can grow to 20 cm (fork length) at 6 months of age (Jaikumar et al

2011), while in the wild, *S. canaliculatus* reaches a body length of 26-28 cm at the end of the first year (Al-Marzouqi 2013; Latuconsina et al 2020b).

One of the natural feeds of *S. canaliculatus* is seagrass leaves and epiphytes attached to seagrass leaves (Lam 1974; El-Sayed 1994; Pradheeba et al 2011; Padang 2011; Latuconsina et al 2013; Ambo-Rappe et al 2013; Muliati et al 2017). Seagrass has a high nutritional value for marine organisms including fish, and plays an important role in the oceanic food chain (Jeyasanta et al 2018). Seagrass leaves are epiphytic shaving sites. Ambo-Rappe et al (2013) noted different feeding preferences of *S. canaliculatus* juveniles for seven species of seagrass (*Halophila ovalis*, *Halodule uninervis*, *Halodule pinifolia*, *Syringodium isoetifolium*, *Cymodocea rotundata*, *Thalassia hemprichii*, *Enhalus accoroides*). Differences in dietary behavior and preference hierarchies indicate that seagrass as a feed source has varied nutritional qualities and secondary metabolite content, which affects the preferences of predators.

Research on the adult size of *S. canaliculatus* feeding preferences on different species of seagrass is still lacking in information. Thus, it is important to determine and select the right natural feed in the efforts to grow *S. canaliculatus*. This information can be used as basis for the preparation of artificial feed formulations.

Material and Method

Time and location of the research. The research stages ranging from acclimatization of test animals (*S. canaliculatus*), feeding, and monitoring of water quality were carried out in July 2016 at the Cultivation Laboratory, Deep-Sea Research Center, Indonesian Institute of Sciences, Ambon. The test for the proximate content of seagrass leaves was carried out at the Laboratory of the Ambon Industrial Research and Standardization Center. Seagrass leaves tannin content test was conducted at the Laboratory of Nutrition and Feed Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang, Indonesia.

Animals and feed tests. Adult *S. canaliculatus* measuring 11-14 cm and weighing 14-33 g were obtained from Inner Ambon Bay and used as test animals. The test animals were acclimatized and given a mixture of fresh seagrass feed *ad libitum*. Then, the animals were fasted for 48 h before being transferred to the experimental container. Four species of fresh seagrass (*Enhalus accoroides*, *Thalassia hemprichii*, *Cymodocea rotundata*, and *C. serrulata*) were used as test feed. Each species used was put together into clumps, planted in plastic pots filled with sand and gravel, and then placed in an aquarium container filled with aerated seawater.

Experiments. The experiment was carried out in 2 stages. Experiment phase I tested the preference level of *S. canaliculatus* for 4 species of seagrass (*E. accoroides*, *T. hemprichii*, *C. rotundata*, and *C. serrulata*) simultaneously (mixed feed) in one rearing container (80 L capacity aquarium equipped with a recirculating aquaculture system containing 1 individual of *S. canaliculatus*). Phase II tested the consumption of *S. canaliculatus* of four species of seagrass as single feed in rearing containers with 1 individual of *S. canaliculatus*. Phases I and II were each carried out for 3 days (72 hours) in three replications, providing feed to the test animals and recording consumption every 24 hours.

The main parameters measured in this experiment included survival and feed intake by *S. canaliculatus*, chemical composition of the seagrass, and tannin content in seagrass. Water quality parameters measured during the trial include temperature, salinity, pH, and dissolved oxygen (Table 1).

Table 1

Parameters and measurement methods

Parameters	Equations / Test Methods / Procedures	References
Feed consumption (FC) (g wet weight ind ⁻¹ day ⁻¹) - measured taking into account autogenic changes	$FC = \left(\frac{U_i \times C_f}{C_i} \right) - U_f$ U_i = initial feed weight; U_f = the rest of the feed, C_i and C_f = control feed weights before and after testing	Dworjanyn et al (2007)
The biochemical composition of seagrass Water content (%) Protein (% dry weight) Lipid (% dry weight)	Thermogravimetric Method Kjeldhal Method Soxhlet procedure (n-hexane solvent)	AOAC (1995)
Crude fiber content (% dry weight)	Obtained by reducing the remaining dry residue during combustion after decomposing the sample with 1.25% H ₂ SO ₄ solution and 1.25% NaOH -	AOAC (1995)
Ash content (% dry weight)	Dry method	AOAC (1995)
BETN (extract ingredients without nitrogen, which is part of carbohydrates after subtracting crude fiber) (% dry weight)	BETN = 100% - (Moisture + Protein + Lipid + Crude Fiber + Ash)	AOAC (1995)
Water quality maintenance medium Temperature (°C) Salinity (‰) pH Dissolved oxygen (mg L ⁻¹)	Digital thermometer Refractometer digital pH-meter digital DO-meter	

Data analysis. This experimental study used a completely randomized design (CRD). Experiment phase I used one treatment factor with three replications. The level of preference was measured by calculating the amount of feed consumption, which was analyzed descriptively (rank order). Meanwhile, the second experiment used four treatment factors with three replications. The amount of feed consumption was tested using one way ANOVA and the Duncan test. Pearson correlation test was used to determine the relationship between seagrass feeding preferences by *S. canaliculatus* with protein content, gross energy, and the ratio of gross energy to protein-energy and tannin content. The statistical analyses were carried out with the help of IBM SPSS Statistics software version 19.

Results and Discussion

Biochemical content of seagrass leaves. The results of the proximate analysis of four species of seagrass showed that *C. serrulata* was the species with the highest protein and carbohydrate content (13.69% and 14.00%, respectively, of dry weight). *E. acoroides* has the lowest protein and carbohydrate content (12.11% and 3.30%, respectively, of dry weight) (Table 2).

Table 2
Biochemical content of four species of seagrass leaves as test feed for *Siganus canaliculatus*

Seagrass	Water content	Biochemical composition (% dry weight)				
		Ash content	Protein	Lipid	Carbohydrate Crude fiber	
<i>Enhalus acoroides</i>	79.69	27.23	12.11	2.61	3.30	54.75
<i>Thalassia hemprichii</i>	77.61	22.47	13.44	1.92	7.95	54.22
<i>Cymodocea rotundata</i>	76.50	27.83	12.94	1.57	14.00	43.66
<i>Cymodocea serrulata</i>	77.72	22.76	13.69	3.28	6.96	53.32

E. acoroides contained the highest water content, 76.69% and the lowest was in *C. rotundata*, 76.5% (Table 2). Water content is the amount of water contained in foodstuffs and is a very important characteristic of foodstuffs because water can affect the appearance, texture, taste, and durability of foodstuffs (Winarno 2008). *C. serrulata* had

the highest protein content, 13.69%, and the lowest was in *E. acoroides*, 12.11%. The highest lipid content was found in *C. serrulata* at 3.28%, and the lowest in *C. rotundata*, 1.57% of dry weight (Table 2). Although the data on lipid and water content in this study did not show a clear relationship, Setyati et al (2005) found a negative correlation between high water content and a drastic decrease in lipid content in *E. acoroides*. The carbohydrate content in *C. rotundata* was 14.00%, and the lowest was in *E. acoroides* at 3.30% of dry weight (Table 2). Carbohydrates have an important role in determining the characteristics of food ingredients, such as taste, color, texture, and others (Winarno 2008).

Jeyasanta et al (2018) found higher levels of protein (19.1%), lipid (4.8%), and carbohydrates (19.8%) in *C. serrulata* compared to other seagrass species in the Gulf of Mannar, India. Protein content, lipid, and carbohydrates in seagrasses are very dependent on the nutrients present in their environment.

The highest crude fiber content obtained was in *E. acoroides* at 54.75%, while the lowest was in *C. rotundata* at 43.66% (Table 2). According to Kordi (2009), the high crude fiber content in fish feed will affect digestibility and absorption in the digestive tract. The highest ash content was found in *C. rotundata*, 27.83%, and the lowest in *T. hemprichii*, 22.47%.

Tannin content in seagrass leaves. The content of tannins in seagrass leaves varied between seagrass species. *E. acoroides* have a high tannin content of 0.68% (dry weight), *T. hemprichii* 0.49%, *C. serrulata* 0.45%, and the lowest tannin content was in *C. rotundata*, 0.42%.

Baby et al (2017) found the highest tannin content in *E. acoroides* compared to *C. serrulata* and *T. hemprichii* in Palk Bay and Gulf of Mannar, India. The high content of tannins is thought to affect the feed preferences of *S. canaliculatus*, which require a high carbohydrate content, because the tannin content tends to be inversely related to carbohydrates. According to Bele et al (2010), tannins are water-soluble polyphenols found in many plant foods and are known to be responsible for the reduction in feed intake, growth rate, feed efficiency, clean metabolizable energy, and protein digestibility in animals. Hence, tannin-rich foods are considered to have a low nutritional value. Tannin is a secondary metabolite compound produced by seagrass vegetation. According to Kavitha et al (2020), *E. acoroides* has a high concentration of tannins, phenols, and flavonoids, and is low in alkaloids, steroids, saponins and does not contain sugars or amino acids. *T. hemprichii* contains high levels of tannins and flavonoids and is low in phenols, alkaloids, sugars, saponins, and amino acids. *C. rotundata* contains high levels of tannins and saponins, and is low in phenols, alkaloids, sugars, steroids, flavonoids, and does not contain amino acids. Meanwhile, *C. serrulata* contains high amounts of phenols, alkaloids, and saponins, and low amounts of sugar, tannins, steroids, amino acids, and flavonoids.

Phase I results. The values of the water quality parameters are presented in Table 3.

Table 3

Values of water quality parameters in phase I

No	Water quality parameters	Observation time (days)		
		1	2	3
1	Temperature (°C)	26.07	26.57	26.57
2	Salinity (‰)	35.67	35	35
3	pH	7.96	7.94	7.95
4	Dissolved oxygen (mg L ⁻¹)	16.83	17.4	16.5

Feeding preference of *S. canaliculatus* towards four species of seagrass as mixed feed. *S. canaliculatus* preferred, in order, *C. serrulata*, *C. rotundata*, *T. hemprichii*, and *E. accoroides* (Figure 1). *C. serrulata* had the highest average consumption, followed by *C. rotundata*, *T. hemprichii*, and *E. accoroides*.

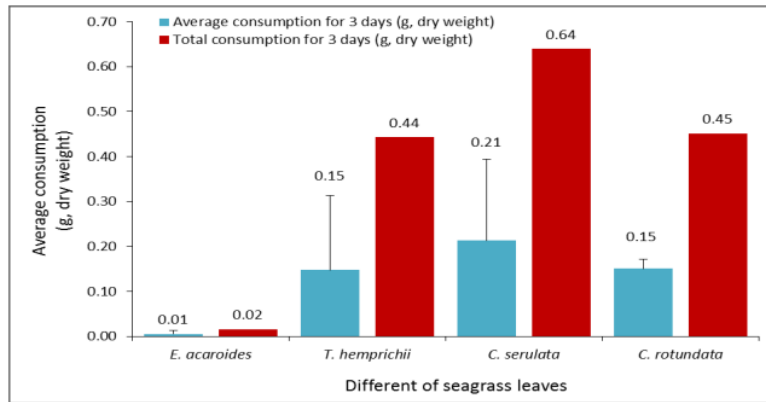


Figure 1. Feeding preferences of *Siganus canaliculatus* on different seagrass in phase I.

Ambo-Rappe et al (2013) observed the preference of *S. canaliculatus* for *Halophila ovalis* and *C. rotundata*, because they are associated with a superior carbohydrate content. According to Masyamsir (2001), herbivorous fish need up to 50% carbohydrates in their feed, being able to produce amylase enzymes along their digestive tract.

The low consumption of *E. acoroides* by *S. canaliculatus* is thought to be related to the high chemical content of secondary metabolites such as tannin. This was also observed by Ambo-Rappe et al (2013) for *S. canaliculatus* juveniles, who preferred less *E. acoroides*. According to Muchtadi (1989), tannins are polyphenolic compounds that form more complex, insoluble compounds with proteins; also, tannins can inhibit the activity of several digestive enzymes such as trypsin, chymotrypsin, amylase, and lipase.

Relationship between feeding preference and protein and energy content. The four species of seagrass used in the preference testing have different protein content and energy values (Table 4). Pearson's correlation test results illustrate a strong relationship between protein content and gross energy with the consumption of seagrass leaves ($r=0.748$ and $r=0.665$, respectively). This means that a higher protein and energy level in the seagrass will increase the consumption within a certain limit. The test results were reinforced by a two-sided significance test which concluded that there was a relationship between protein and gross energy with the level of seagrass leaves consumption by *S. canaliculatus* ($p<0.005$ and $p<0.025$, respectively).

Table 4
Protein content, protein energy, gross energy, and the ratio of gross energy to protein-energy (GE/P) in seagrass used in experiments of phase I

No	Seagrass	Protein (% dry weight)	Protein energy (kcal kg ⁻¹)	Gross energy (kcal kg ⁻¹)	GE/P
1	<i>Enhalus acoroides</i>	12.11	678.29	1058.84	1.56
2	<i>Thalassia hemprichii</i>	13.44	752.84	1259.31	1.67
3	<i>Cymodocea rotundata</i>	12.94	766.61	1359.83	1.77
4	<i>Cymodocea serrulata</i>	13.69	724.43	1446.43	2

Note: gross energy is estimated using a conversion factor: protein - 5.4 kcal g⁻¹; lipid - 9.3 kcal g⁻¹; carbohydrate - 4.1 kcal g⁻¹

The relationship between the ratio of gross energy to protein-energy (GE/P) and the amount of seagrass consumption has a weak correlation ($r=0.461$), strengthened by a two-sided significance test, which concluded that there is no relationship between GE/P and consumption of seagrass by *S. canaliculatus* ($p<0.025$). The results of this analysis indicate that the preference hierarchy of *S. canaliculatus* is probably influenced by protein content and the gross energy of the feed.

7 Optimization of the protein to energy (P/E) ratio in fish diets is widely accepted as a means of protein storage, thereby promoting growth at lower costs in fish farming (Lupatsch et al 2001; Ali & Jauncey 2005; Ali et al 2008). At low P/E ratios, the use of dietary protein for growth and maintenance of the body is maximized, whereas, at a higher P/E ratio, more protein is used for energy or stored (Ali et al 2008).

Relationship between feeding preference and tannin content. The varying content of tannin in seagrass leaves is thought to affect the preference for feed of *S. canaliculatus*. The four seagrass species had different tannin levels (Table 4). *E. acoroides* had the highest tannin content, while *C. rotundata* the lowest. The Pearson correlation test results illustrated a strong negative relationship between the amount of tannin content in seagrass leaves and the level of seagrass consumption by *S. canaliculatus* ($r=-0.777$). It means that a lower the tannin content increases the level of consumption ($p<0.005$). The preference hierarchy of *S. canaliculatus* is influenced by the amount of tannin in the seagrass.

Phase II results

Water quality in the maintenance media. During the experiment, water quality parameters such as temperature, salinity, pH, and dissolved oxygen were in good condition (Table 5).

Values of water quality parameters in phase II

Table 5

No	Water quality parameters	Observation time (days)		
		1	2	3
1	Temperature (°C)	26.6	27.02	26.48
2	Salinity (‰)	35.5	35	35.67
3	pH	7.94	7.93	7.98
4	Dissolved oxygen (mg L ⁻¹)	17.83	15.83	16.66

The average value of water quality parameters measured in the maintenance tank (aquarium) during the experiment was optimal for supporting the life of *S. canaliculatus* (Table 5). The optimal temperature range for *S. canaliculatus* is between 25-34°C. It can tolerate changes in salinity up to 5‰ and is very sensitive to pH values waters above 9. The dissolved oxygen content should be higher than 2 mg L⁻¹ (Lam 1974).

Preference of S. canaliculatus towards four types of seagrass as single feed. No seagrass species were rejected as feed by *S. canaliculatus*. *C. serrulata* was had the best average feed consumption, followed by *C. rotundata*, *E. accoroides*, and *T. hemprichii* (Figure 2).

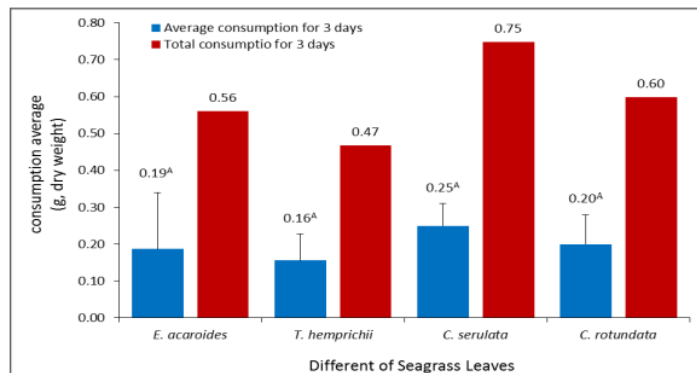


Figure 2. Feeding preferences of *Siganus canaliculatus* on different seagrass in phase II.

The overall results of phase II show that apart from preference, the availability of feed is also an important factor affecting the consumption level of each seagrass by *S. canaliculatus*.

The feed preference of *S. canaliculatus* based on the total amount and on the average consumption of seagrass ordered from highest to lowest is *C. serrulata*, *C. rotundata*, *E. acoroides* and *T. hemprichii*. The high preference for *C. serrulata* could be related to the proportionate content of protein, lipid, carbohydrates, and crude fiber. Mariani & Alcoverro (1999) found that herbivorous fish preferred seagrass feeds with low fiber and carbon content from in short-lived seagrass species such as *Cymodocea rotundata* and *Syringodium isoetifolium*, compared to long-lived seagrass species with higher fiber and carbon content such as *E. acoroides* and *Thalassodendron ciliatum*. According to Unsworth et al (2007), the feeding selectivity of herbivorous fish in seagrass is based on the proximate availability of seagrass.

Feed preference of *S. canaliculatus* for seagrass was higher in seagrass species with smaller leaves such as *C. serrulata* and *C. rotundata*, compared to seagrass species with large leaves such as *E. acoroides* and *T. hemprichii*. Ambo-Rappe et al (2013) found that juveniles *S. canaliculatus* preferred small seagrass (*H. ovalis* and *C. rotundata*) as their feed, the preference being related to the size of the mouth opening of the fish. According to Effendie (2002), fish will choose food according to the size of their mouth. Preferences of *S. canaliculatus* for different species of seagrass leaves are also thought to be due to differences in the content of different secondary metabolites (Kavitha et al 2020).

Conclusions. Feed preference of *S. canaliculatus* for different seagrass species is closely related to the proportional proximate composition, higher protein to energy ratio, and low content of secondary metabolites (tannins). Thus, *C. rotundata* and *C. serrulata* can be used as the ingredients in formulating feed for *S. canaliculatus*.

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Conflict of Interest. The authors declare no conflict of interest.

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