Mar. Sci. Tech. Bull. (2021) 10(2): 154-162
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.820627

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Effects of two different macroalgae (*Ulva lactuca* and *Jania rubens*) species on growth and survival of juvenile red swamp crayfish (*Procambarus clarkii*) as feed additive

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ARTICLE INFO

Article History:
Received: 03.11.2020
Received in revised form: 24.12.2020
Accepted: 29.12.2020
Available online: 22.01.2021

Keywords:
Red swamp crayfish
Procambarus clarkii
Seaweed
Ulva lactuca
Jania rubens
Growth performance
Survival

ABSTRACT

The effects of dietary supplementation of two different macroalgae *Ulva lactuca* and *Jania rubens* on the growth performance, survival and feed conversion ratio of juvenile red swamp crayfish juvenile (*Procambarus clarkii*) were investigated. Red swamp crayfish with an average total length of 56.2±6.67 mm and an average weight of 3.77±0.2 g were placed at tanks (10 crayfish at each tank) and offered diets 8 weeks. Different levels of macroalgae were added to commercial sea bass feed, and no seaweed was used as a control group. It was observed that crayfish fed with 10% feed had higher growth performance (in terms of length and weight) than those fed with 15% diet and control group ($P<0.05$). The lowest feed conversion rate was observed in juvenile crayfish fed with 15% feed ($P<0.05$). The highest survival rate was 50.0% at group fed with 15% feed, followed by 46.66% (control group) and 43.33% (10% diet groups), respectively. This study showed that there was no statistical difference in survival rate among treatment groups ($P>0.05$). However, the frequency of molting was mostly observed in the group fed with 10% diet. Therefore, the results showed that seaweed (*Ulva lactuca* and *Jania rubens*) could be used as a supplement for red swamp crayfish diet (*Procambarus clarkii*) at 10% to improve growth performance with no adverse effects on feed efficiency or survival rate.

Please cite this paper as follows:

Mazlum, Y., Yazıcı, M., Sayın, S., Habiboğlu, O., Uğur, S. (2021). Effects of two different macroalgae (*Ulva lactuca* and *Jania rubens*) species on growth and survival of juvenile red swamp crayfish (*Procambarus clarkii*) as feed additive. *Marine Science and Technology Bulletin*, 10(2): 154-162.

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Introduction

The red swamp crayfish *Procambarus clarkii* is an important commercial aquaculture freshwater crayfish with an annual globally production of almost 700 tons (FAO, 2017). *P. clarkii* is successfully grown in most tropical regions of the world due to its rapid growth at low oxygen levels and high temperatures (23-31°C), high egg productivity and the ability to grow and reproduce rapidly (Mazlum & Eversole, 2004, 2005). Crayfish are invertebrates and have polytrophic feeding habits. Crayfish are classified as herbivorous, detritivorous, omnivorous and sometimes mandatory carnivores (Correia, 2002; Nystrom, 2002; Mazlum & Yilmaz, 2012; Mazlum & Şirin, 2020). They are capable of living in many habitats in terms of physiological, morphological and behavioral characteristics. Crayfish are abundant and predominantly found among all invertebrates. This organism play an important role in the freshwater food chain by feeding on the residues and detritus of thousands of animals, from living and rotten plants, cereals, algae and vertebrates to smaller vertebrates such as small fish species (Lodge et al., 2012; Twardochleb et al., 2013).

Recently, seaweed (known as macroalgae) have been drawing attention as alternative protein sources for aquaculture as nutritional content of aquatic organisms due to their relatively high protein values (Sinurat & Fadjriah, 2019) , essential amino acid content, vitamins and trace metals (Morais et al., 2020; Peñalver et al., 2020) and also low cost availability in tropical countries (Nakagawa & Montgomery, 2007). Annual seaweed production was reported to be 31.2 million tons in 2016 (Buschmann, 2017; Ferdouse, 2018), and the seaweed industry worldwide was over 6 million USD (FAO, 2018). However, it is still difficult to use the applicable and sustainable methodology in seaweed cultivation and to be economical and productive in various parts of the world. (Golberg et al., 2020; García-Poza et al., 2020).

Ulva lactuca (Chlorophyta) and *Jania rubens* (Rhodophyta) are the dominant seaweed plants in Iskenderun Bay. The biochemical composition, nutritional ingredient and mineral composition of these two species were previously evaluated by Turan et al. (2015). *U. lactuca* is also known as "sea lettuce", which has high nutritional value due to its high polysaccharides, proteins, vitamins and trace minerals (Ashour et al., 2020). *Jania rubens* has significant nutrient compounds such as protein, lipids and carbohydrates (Morais et al., 2020). The nutritional value of dietary supplements of seaweed in aquaculture is mostly estimated in terms of growth performance, feed intake and survival rate. It has been revealed by the researchers seaweed have a good vitamin and mineral profile and are particularly rich in ascorbic acid (Ortiz et al., 2006; García-Casal et al., 2007).

The use of seaweed as a feed supplement has shown favorable positive effects on human and animal health and nutrition. These findings by scientific researchers have revealed that the application of seaweed or extract as a feed additive has many benefits. Adding seaweed to diets has been shown to significantly alter the gut flora, improve immunity, and improve growth performance, feed utilization and disease resistance for many aquatic animals and reduced nitrogen release to the environment (Ashour et al., 2020). Therefore, studies conducted with the addition of macroalgae to feed in recent years have focused on both shellfish and fish species. These studies mainly on pacific shrimp (Rodríguez-González et al., 2014; Cárdenas et al., 2015), trout (Soler-Vila et al., 2009; Güroy et al., 2013), European seabass (Valente et al., 2006), African catfish (Abdel-Warith et al., 2016; Al-Asgah et al., 2016), sea bream (Emre et al., 2013), red tilapia *Oreochromis sp.* (El-Tawil, 2010), Nile tilapia (Güroy et al., 2007; Marinho et al., 2013; Valente et al., 2016), Senegalese sole (Moutinho et al., 2018) and Atlantic salmon (Kamunde et al., 2019). They reported that adding macroalgae to feed at low rates did not adversely affect growth. Although some studies have investigated the use of seaweed as a partial feed substitute for the shrimp diet, there is no study on (*Ulva* + *Jania*) being used as a feed additive in the crayfish diet. Therefore, this study is considered as a preliminary study as the determination of alternative feed sources for crayfish is one of the key factors for successful crayfish culture. Thus, the specific purpose of this study was to determine the effect of two different seaweed (*Ulva* + *Jania*) dietary supplements on growth performance, survival and feed conversion rate of juvenile red swamp crayfish.

Material and Methods

The study was conducted at Aquaculture Research and Application Center (ISTE-DUM) breeding laboratory of Iskenderun Technical University, Hatay, Turkey during a period of 60 days.

Experimental Crayfish

Brood crayfish, *Procambarus clarkii,* were obtained from an aquarium pet shop in İskenderun, Turkey. Once the adult crayfish transported to the Aquaculture Research and Application Center (ISTE-DUM), they were kept in troughs and fed with a commercial feed until hatching time. Adult crayfish were fed with a commercial trout diet (50% crude protein and 12% cure lipid) during the acclimation period. A total of 90 juvenile crayfish with an initial mean total length (56.2±6.67 mm) and weight of 3.77±0.2 g were used in this study. Ten juvenile crayfish were randomly stocked in 9 tanks (100 L water capacity) in triplicate and supported by continuous aeration. Juvenile crayfish were fed twice a day, five days a week. Uneaten food was siphoned out from the aquariums 1 h after feeding. PVC pipe pieces with a diameter of 2-3 cm were placed into all aquariums as shelters. Experimental crayfish were weighed at the beginning of the experiment and then at 4-week intervals for 8 weeks. Water temperature was maintained at 25.2±1.23°C, dissolved oxygen at 4.05±0.48 mg/L (YSI oxygen meter) and pH at 7.49±0.14 (YSI). During the experiment, a 12-hour light and 12-hour dark photoperiod was applied. Crayfish were fed twice a day, on the morning and evening. Growth performance was monitored monthly. Morphometric characteristics of crayfish were measured as total length (mm) and weighed (g) with an electronic balance. Total length measurements were performed at from the rostrum tip to the telson by using a vernier caliper.

Experimental Diets

The macroalgae *Ulva lactuca* and *Jania rubens* were collected from June to July 2019 along the Konacık coastline in Iskenderun Bay. The macroalgae species were collected freshly and the samples were brought to Iskenderun Technical University, Faculty of Marine Sciences and Technology, Algal Biotechnology Laboratory in cooling containers. Macroalgae were identified according to their species, then they were cleaned from pollutants such as sand, epiphyte, and stored at −20°C until analysis after washing and drying processes were performed (Ye et al., 2009). The ash, lipid and protein amounts of macroalgae were determined by following the methods of Vollenweider (1974) and Bligh & Dyer (1959). Nutrient components (Nitrogen (N), Carbon (C), Hydrogen (H)) of algae samples were determined according to method described by Dumas (1831). Methods suggested by Santoso et al. (2006) were followed in the determination of macro (Na, Ca and Mg) and micro mineral contents of *J. rubens* (Rhodophyta) and *U. lactuca* (Chlorophyta). The macroalgae required for the experiment dried in an oven at 50°C for 48 hours, and seaweed powder was prepared. A 55% CP was used in the present study (crude protein for control diet was formulated with two different seaweed).

Diets Formulation and Preparation

The percentage of the biochemical (protein, lipid, ash), nutrient components (Nitrogen (N), Carbon (C), Hydrogen (H) and mineral contents of two different macroalgae species (*J. rubens, U. lactuca*) (g/100g dry weight) are given in Table 1.

Table 1. The percentage of the biochemical (protein, lipid, ash), nutrient components (Nitrogen (N), Carbon (C), Hydrogen (H)) and mineral contents of two different macroalgae species (*J. rubens, U. lactuca*) (g/100g dry weight).

Species	***Ulva lactuca***	***Jania rubens***
Chemical Composition		
Crude protein	16.89±0.12	5.991±0.773
Lipid	1.08±0.33	0.39±0.103
Ash	26.47±0.20	78.740±0.066
Nutritional Ingredients (%)		
Nitrogen	1.004 ± 1.061	1.716 ± 1.030
Carbon	16.382 ± 0.541	17.415 ± 0.022
Hydrogen	1.702 ± 0.129	1.339 ± 0.007
Micro mineral ingredients		
Fe	97.12 ± 0.71	51.09 ± 0.02
Zn	1.08 ± 0.03	0.09 ± 0.26
Cu	0.53 ± 0.01	0.69 ± 0.12
Macro Mineral Ingredients		
Na	1098±0.07	806±0.22
Ca	16345±0.19	359±0.01
Mg	1934±3.01	973±1.09

The experimental commercial diets were pelleted and placed into the mixer chamber of Alphie (Hexagon Product Development Pvt. Ltd. India) with two different seaweed 3-D mixing feature and 25 min (1000 μ), 20 min (1200μ), 15 min. (1500 μ) at 80 rpm with stirring. Feed sizes were adjusted according to crayfish measurements in 30-day periods. Prepared feeds were stored at +4°C until used in plastic containers. A new diet was formed by adding 10% and 15% seaweed (*U. lactuca and J. rubens*) to commercial sea bass feed containing 55% crude protein and 9.8% cure lipid (Sirin and Mazlum, 2016; Yazıcı et al., 2020).

Growth Performance

Crayfish in the different experimental groups were weighted at the end of the 8th week feeding trail to estimate the growth parameter. Growth parameter was analyzed in terms of final individual crayfish body weight (BW g), weight gain (WG g), specific growth rate (SGR, %day^{-1}), feed conversion ratio (FCR). The formulas were used as follows:

$$\text{WG} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{SGR} = \frac{\ln \text{final body weight} - \ln \text{initial body weight}}{\text{Rearing duration (60 days)}} \times 100$$

$$\text{FCR} = \frac{\text{Dry feed intake (g)}}{\text{wet weight gain (g)}}$$

$$\text{Survival rate} = \frac{\text{Total number of crayfish harvested}}{\text{Total number of crayfish stocked}} \times 100$$

$$\text{Total Molting Frequency} = \frac{\text{Number of molt crayfish}}{\text{Total number of crayfish}} \times 100$$

Statistical Analysis

The data were analyzed by using the Statistical Package for the Social Sciences software (SPSS, 2012, Version 17.0, SPSS, Chicago, IL, USA). The results were subjected to Levene's test to determine homogeneity of variance and no transformation was required. One-way ANOVA was used to determine the effects of the diets on the various responses including final wet weight, molting frequency, survival, SGR, and FCR for all treatments. Multiple range test (Duncan's) was used to compare means of treatments. Results were considered to be significant at the (P<0.05) level. Mean values were presented with ± standard deviation (SD) in tables.

Results

The initial total length (ITL) and initial body weight (IBW) of the treatment group were the same at the beginning of the experiment (P>0.05). The results of this study indicated that final total length (FTL), final body weight (FBW), weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) of red swamp crayfish (*Procambarus clarkii*) were affected (P<0.05) by using different levels of seaweeds (*U. lactuca*) in the diet (Table 2).

Juvenile crayfish fed on control and experimental groups (10% and 15%) attained 62.57, 66.15 and 60.06 mm final length. Final total length was found significantly different among the treatment groups (Table 2). The highest FBW, WG and SGR%/day values were obtained with the crayfish kept at 10% level, were found to be 7.72 g, 3.95 g (Table 2) and 1.19 g% day (Figure 1), respectively. Followed by crayfish maintained control and %15 level did not significant differences (P<0.05) (Table 2). However, the least values of FBW, WG and SGR%/day were recorded with crayfish maintained at 15% level with the value of 6.25 g, 2.46 g and 0.83 g%/day, respectively (Table 2).

FCR was found to be 2.25, 1.98 and 3.07, respectively. FCR did not enhanced with increasing the level of addition rate of seaweed in the diet. The substantial improvements (P<0.05) in feed conversion ratio were achieved when seaweed level in the diet 10%. The best SGR was detected with the crayfish maintained at 10% seaweed level (1.19). It was considerably different from SGR of the crayfish maintained at 15% seaweed level and control group. Lastly, the lowest (P<0.05) SGR value (0.83) was detected with crayfish maintained at 15% seaweed level in the diet. At the end of the experiment, the survival rate was the same among treatments, and there was no significant difference (P>0.05). The highest survival rate (50.0%) was obtained using 15% diet, followed by control diet (46.66%), and then by 10% diet (43.33%). However, molting frequency was mostly observed in the group fed with 10% diet.

Table 2. Supplementation of macroalgae on growth performance of red swamp crayfish (*Procambarus clarkii*) fed at different dietary levels.

	Treatments		
Parameter	**Control**	**10%**	**15%**
ITL(mm)	56.2±6.67^{a}	56.2±6.67^{a}	56.2±6.67^{a}
FTL (mm)	62.57±5.21^{a}	66.15±6.02^{b}	60.06±7.43^{a}
IBW (g)	3.76±0.09^{a}	3.77±0.01^{a}	3.79±0.15^{a}
FBW (g)	7.13±1.86^{a}	7.72±2.92^{b}	6.25±2.26^{a}
WG (g)	3.37±0.08^{a}	3.95±0.06^{b}	2.46±0.02^{c}
SGR (%/day)	1.06±0.02^{a}	1.19±0.010^{b}	0.83±0.006^{c}
FCR	2.25±0.02^{a}	1.98±0.01^{b}	3.07±0.09^{c}
SR (%)	46.66^{a}	43.33^{a}	50.0^{a}
MF (%)	53.33^{a}	66.66^{a}	56.66^{a}

Note: Means values expressed as mean± standard deviation (SD). Means in each row followed by different letters are significantly different (P<0.05). Initial total length (ITL mm), Final total length (FTL mm), Initial body weight (IBW), Final body weight (FBW gr), Weight gain (WG gr), specific growth rate (SGR %/day), feed conversion ratio (FCR), Survival rate (SR %) and Molting frequency (MF %)

Discussion

Seaweed is alternative protein sources for aquaculture as nutritional content of aquatic organism due to their relatively high protein values, essential amino acid content, vitamins and trace metal and production rates in tropical countries (Nakagawa & Montgomery, 2007). It has been reported that seaweed chemical structure can be affected several parameters such as by temperature, light, salinity or food source during cultivation (Sinurat & Kusumawati, 2017). The results of this study showed that different level of seaweed supplementation improved the growth performance and feed efficiency of red swamp crayfish with no adverse effects in the diet (Table 2). Similar results were reported by Qiu (2017) could addition up to 2% fish meal in shrimp feed without causing negative effects on the growth of shrimp. He noted that adding up to 2% to shrimp feed did not have a negative effect on shrimp growth.

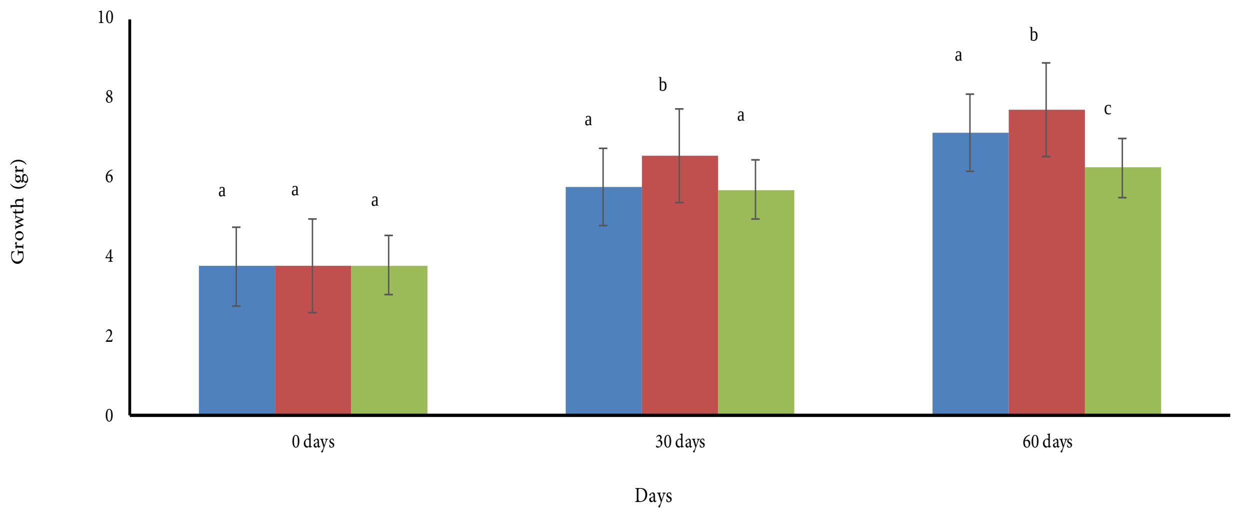

Figure 1. Mean and standard deviation of red swamp crayfish growth for macroalgae added to feed in an 8-week study.

Also, in a number of studies, involvement levels of less than 5% of seaweed meals include Pacific white shrimp (Rodríguez-González et al. 2014; Cárdenas et al., 2015), rainbow trout (Soler-Vila et al., 2009; Güroy et al., 2013), European perch (Valente et al., 2006), African catfish *Clarias gariepinus* (Abdel-Warith et al., 2016; Al-Asgah et al., 2016), gilthead seabream *Sparus aurata* (Emre et al., 2013) red tilapia *Oreochromis sp.* (El-Tawil, 2010) and Nile tilapia (Güroy et al., 2007; Marinho et al., 2013; Valente et al., 2016) were reported not to adversely affect growth.

However, in many studies, the results of low, medium, and high inclusion have shown a discrepancy between the levels of seaweed meals. Qiu (2017) reported that high level of *Ulva* meal as an additional in meal resulted in depressions in shrimp growth performance. In other studies, *Ulva rigida* species added to Nile tilapia feed were reported to have the best performance at the level of 5% algae inclusion and decreased by 10-20% (Diler et al., 2007; Güroy et al., 2007). Rodríguez-González et al. (2014) reported that adding *U. lactuca* meal to the diet meaningfully reduced the weight of *Litopenaeus vannamei* by 10% and 15%, whereas shrimps fed diets containing similar levels of seaweed (*Gracilaria parvispora*) showed no change in weight gain. In a study by Felix and Brindo (2014), they showed that 10, 20, and 30% inclusion of raw *U. lactuca* flour in the diet causes a decrease in growth performance in the giant freshwater shrimp *Macrobrachium rosenbergii*. Ergun et al. (2009) stated that the inclusion of 5% Ulva flour (*U. rigida*) in diets improved *Oreochromis niloticus* growth performance, feed efficiency, and nutrient and body composition. They concluded that Ulva meal can be added to feeds as a dietary component. In addition, Nakagawa et al. (1993) showed that adding 2.5-5.0% of the Ulva meal to the diet gives good results. Therefore, in our study, crayfish fed a diet supplemented with 10% seaweed (*Ulva* + *Jania*) showed a slight increase in growth performance, whereas these parameters decreased for crayfish fed with a 15% diet. At the end of this study, it shows that the growth performance of red swamp crayfish was affected by the addition of various levels of seaweed in the diet. The highest values of final live weight, weight gain and SGR %/day were obtained by keeping crayfish at 10% level. Increasing the level of Ulva added to the food above 15% did not have a significant effect on growth compared to the control group. These results are consistent with the results of previous studies in terms of growth performance.

Azaza et al. (2008) concluded in their study that green algae (*U. rigida*) can be included in *Oreochromis niloticus* diets up to 20% no any adverse effects on growth parameters. Güroy et al. (2007) observed that two seaweed meals effects (*U. rigida* and *Cystoseira barbata*) on feed intake, growth, and food use at three levels (5%, 10% and 15%) of young Nile tilapia (*Oreochromis niloticus*). They concluded that the adding of 5% ulva meal to the fish diet did not have a adverse effect on growth performance, feed use and body composition; however, they stated that using levels between 10% and 20% may result in negative consequences.

Valente et al. (2006) found that the young sea bass *Dicentrarchus labrax* had maximum fish growth performance in 10 weeks when 10% seaweed (*Ulva sp.*) was added to their diet. Elmorshedy (2010) showed that the starting body weight of 0.094 g in mullet *Liza ramada* significantly increased with the body weight, weight gain and specific growth rate up to 28% seaweed level (*Ulva sp.*) In addition, Diler et al. (2007) recommended that the addition of 5 to 15% dietary Ulva pulp instead of wheat flour in carp diets better growth and could be suitable for carp. Also, Guroy et al. (2007) indicated that fish mass gain was high of *Oreochromis niloticus* fed diets added with numerous levels of Ulva flour were achieved for in fish fed 5 to 10% Ulva diets. They think that the differences in the results of certain proportions of seaweed can be variable depending on dietary habits, age, and species of algae and fish.

Our result showed that 10% seaweed significantly increased the growth performance of red swamp crayfish (P<0.05). Mustafa & Nakagawa (1995) stated that adding algae as a feed supplement significantly improves growth, digestive efficiency, disease tolerance, chemical composition and quality meat. In addition, Nakagawa & Montgomery (2007) stated that fish diet prepared with the exception of fish fed a diet containing 25% *Ulva* has a positive effect on FCR. However, previous study results such as Güroy et al. (2007) and Diler et al. (2007) showed that adding 20% *Ulva* meal to the diet effect negatively on FCR value.

Conclusion

This study supports previous outcomes displaying that seaweed can be used as possible additive in crayfish feed. Inclusion of seaweed up to 10% has shown that the red swamp crayfish allows for an increase in growth performance and feed utilization. However, the extent to which higher feed additions and continued long-term testing affect growth and feed use should be studied in more detail. The results of this study concluded that the use of seaweeds can provide dietary alternatives supplement of juvenile crayfish as they increase growth performance and feed utilization. With the results obtained, it was concluded that seaweed can be supplemented with an optimum 10% diet of red swamp crayfish (*Procambarus clarkii*) no adverse effects to increase growth parameter.

Compliance with Ethical Standards

Authors' Contributions

YG: experimental design, feed preparation, data collection, writing and analysis, original draft review and editing. MY: data collection, feed preparation, original draft review and editing. SS: supply macroalgae, analysis of macroalgae, species identification, review and editing. OH: analysis of algae SU: analysis of algae.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. According to scientific research procedures in Turkey, if you are investigating arthropods such as shrimp and crayfish you do not have to get ethical permission.

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