See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/373873057

The use of sailfin molly, Poecilia latipinna (Lesueur, 1821) Meal and Spinach, Amaranthus tricolor (Linnaeus, 1759) meal in diet for mud crab, Scylla serrata (Forskal, 1775)

Article *in* International Journal of Fisheries and Aquatic Studies - January 2023 DOI:10.22271/fish.2023.v11.i4b.2836





E-ISSN: 2347-5129 P-ISSN: 2394-0506 (ICV-Poland) Impact Value: 76.37 (GIF) Impact Factor: 0.549 IJFAS 2023; 11(4): 139-144 © 2023 IJFAS www.fisheriesjournal.com Received: 03-05-2023 Accepted: 08-06-2023

Hasnidar

Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Muslim Indonesia, Jl. Urip Sumoharjo Km. 05 Makassar 90231, South Sulawesi, Indonesia

Rosmiati Syaharuddin

Student, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Muslim Indonesia, Jl. Urip Sumoharjo Km. 05 Makassar 90231, South Sulawesi, Indonesia

Andi Tamsil

Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Muslim Indonesia, Jl. Urip Sumoharjo Km. 05 Makassar 90231, South Sulawesi, Indonesia

Syahrul

Department of Marine Sciences, Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Muslim Indonesia, Jl. Urip Sumoharjo Km. 05 Makassar 90231, South Sulawesi, Indonesia

Corresponding Author: Hasnidar

Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Muslim Indonesia, Jl. Urip Sumoharjo Km. 05 Makassar 90231, South Sulawesi, Indonesia

The use of sailfin molly, *Poecilia latipinna* (Lesueur, 1821) Meal and Spinach, *Amaranthus tricolor* (Linnaeus, 1759) meal in diet for mud crab, *Scylla serrata* (Forskal, 1775)

Hasnidar, Rosmiati Syaharuddin, Andi Tamsil, Syahrul

DOI: https://doi.org/10.22271/fish.2023.v11.i4b.2836

Abstract

Sailfin Molly, a pest in ponds, can be utilized as a source of protein because it has good nutritional quality. Spinach is also known to contain ecdysteroid which can accelerate molting in crabs. The study aims to analyze the use of these two ingredients on crab growth. Mangrove crabs weighed 66.43±9.78 g and carapace width 7.40±0.43 cm. The container used was crab box and placed in the pond. Treatments consisted of 4; i.e. the use of sailfin molly meal and spinach, treatments A (40 and 20), B (40 and 25), C (40 and 30) %, D (commercial feed); using a completely randomized design. Test parameters: weight growth (W), number of crabs molting (NM), survival (SR), feed conversion (FCR) and physico-chemical physico-chemical water conditions. Data were analyzed by variance and Duncan's further test. The treatments had a significant effect on W, SR and FCR but no significant effect on NM. Water physico-chemical i.e. salinity was not favorable for crab growth. The use of sailfin molly meal and spinach in feed was as good as commercial feed.

Keywords: Mud crab, sailfin molly meal, spinach meal, growth

1. Introduction

Mud crab, *Scylla serrata* (Forskal, 1775) is a species of crab that is found along the coast of Indonesia, precisely in mangrove areas ^[1]. Crab production apart from the catch in nature also comes from the results of culture activities ^[2]. A very important aspect in mud crab aquaculture is the diet used. Fish feed and artificial feed are two types of feed used in mud crab culture ^(3,4). Feed is a source of energy for mentenence, growth and reproduction, therefore the availability of feed is very important so that culture remains sustainable ⁽⁵⁾. Feed is said to be of high quality if it contains complete protein, lipid, carbohydrates vitamins, minerals, essential amino acids and essential lipid ^[6].

Crab size increase is preceded by the molting process. Molting is the process of changing the old skin to a new skin that is larger in size so that the crab increases in size. Factors that affect molting are internal and external factors. One internal factor is the concentration of molting hormone (ecdysteroid). The addition of molting hormone (20-hydroxyecdyson/20-HE)) at a dose of 1.0 µg/mL increases the number of molting crabs and the duration of molting time is faster ^[7]. The availability of 20-HE molting hormone is limited and the price is expensive, so a substitute material that is available and cheaper is needed. Amaranth plants (*Amaranthus* sp) is a type of plant containing ecdysteroid analogous to growth hormone (ecdisteroid) in insects and some other invertebrates such as crustaceans ^[8], crabs (*Callinectes sapidus*) ^[9]. Ecdysteroids derived from plants are called phytoecdysteroids. Spinach plant extract has been used to stimulate molting in blue swimming crab, *Portunus pelagicus* ^[9], mangrove crab, *Scylla serrata* by injection ^[10]. The addition of phytoecdisteroids through injection is less efficient if done in bulk so that other efforts, i.e spinach extract added to artificial feed ^[11], a combination of injection of spinach extract and feeding through feed ^[12].

Sailfin molly, *Poecilia latipinna* (Lesueur 1821) is a pest fish in ponds. These fish are abundant during the fish or shrimp harvest, and pond farmers simply discard them.

Sailfin molly can be used as a source of feed protein to replace commercial fish meal which is increasingly expensive. According to ^[13], sailfin molly contains 66.40% protein, 12.52 lipid, 0.80% crude fiber, 15.51% ash, 3.70% water; essential amino acids, i.e lysine 4.99%, leucine 4.27%, phenylalanine 2.64%, valine 2.63%, threonine 2.50%, and histidine 1.49%. Essential fatty acids consist of the omega-3 fatty acid group, i.e decosyaxanoic acid (DHA) 0.09%, eicosapentanoic acid (EPA) 0.03% and linolenic acid (HUFA) 0.21%. The omega 6 fatty acid group consists of linoleic acid (LA) 0.25% and arachidonic acid (AA) 0.04%.

Culturing mud crabs using artificial feed requires high quality and cheap local raw materials. In addition, mud crabs are carnivorous animals that require higher feed protein than herbivorous animals ^[6]. The combination of sailfin molly meal and spinach meal in mud crab feed is expected to be very useful information, considering that the two ingredients can complement each other so that molting and crab growth increase. This study aims to analyze the use of sailfin molly meal and spinach meal on the growth of mud crabs, *S. serrata*.

2. Materials and Methods

Mud crabs, S. serrata with an average weight of 66.43±9.78 g

and carapace width of 7.40 ± 0.43 cm, a total of 60 individuals were obtained from crab fishermen around the study site. The research was conducted in the aquaculture area in Minasa Upa Village, Bonto District, Maros Regency, South Sulawesi, Indonesia. Feed preparation was carried out at the Nutrition Laboratory of the Research Center for Brackish Water Aquaculture and Fisheries Extension, Maros.

2.1 Preparation of spinach meal and sailfin molly meal

The spinach used is a type of red spinach, cleaned, dried in the sun for about 3-4 days or using a grill (oven) with a temperature of 150 0 C for 45 minutes, then mashed using a blender for 5 minutes ^[14]. For sailfin molly, first cleaned, dried in the sun, drying time ±12 hours (depending on the intensity of the sun, estimated moisture content <10%). Then the fish is blended into meal, and ready for use ^[13].

2.2 Feed preparation

Feed ingredients, namely spinach meal, sailfin molly meal and other ingredients such as soybean meal, fine bran, tapioca, vitamins and CMC were obtained from a feed ingredients store. All feed ingredients were weighed using o'haus scales accuracy of 0.01 g, the percentage of each ingredient is shown in Table 1.

Fable 1 :	Feed	composition	according	to	treatment

No	Dahar Dahu	Treatment (SMM and ATM)%				
INO	Вапап Ваки	A(40 and 20)	B(40 and 25)	C(40 and 30)	D (CF)	
1	Sailfin Molly meal (SM)	40	40	40		
2	A. tricolor meal (AM)	20	25	30		
3	Soy meal	18	18	18		
4	Baran meal	12	7	6	Comercial Feed (CF)	
5	Tapioca	6,5	6,5	2,5		
6	Vitamins	3	3	3		
7	CMC	0,5	0,5	0,5		
Total ingredients		100	100	100		

All feed ingredients are mixed evenly by mixing with a wooden spoon for 1-2 minutes, then warm water is added so that the ingredients form a paste batter so that it is easier to mold. Mixing of raw materials is carried out for 3-5 minutes after which the material is ready to be molded in a pellet molding machine ^[15]. The resulting pellets are then dried under the sun or in the oven at 50 $^{\circ}$ C for 10 minutes, then the pellet feed is ready to be applied. The proximate test results of

the test feed are presented in Table 2.

2.3 Crab culture activities

The rearing container is a crab box measuring $20 \times 15 \times 15$ cm in length x height x width, placed on a rearing raft made of bamboo, equipped with floats from used bottles and placed in the pond (Fig. 1).



Fig 1: Crab boxes installed in a row and placed in the pond.

Each crab box contains 1 crab, the sides and bottom of the crab box are covered with green netting with a mesz size of 1 inch to prevent feed from falling through the crab box hole.

Crabs were weighed using a digital scale with an accuracy of 0.1 g and the carapace width was measured using a sliding ruler. The sorted crabs were put into the crab box and

acclimatized to the research media and feed used, for 5 days. The feeding dose was 5%/body weight/day, with the frequency of feeding twice a day at 7:00 am and 6:00 pm. Pond water changes were carried out every day following the tides.

Observations of the number of crabs that molted and died were made daily, growth sampling was done every 15 days. Observations of water quality such as temperature, salinity, pH were done every morning, dissolved oxygen and nitrite were done every 15 days. Experimental research using a completely randomized design (CRD) with 4 treatments (Table 1), and each treatment was repeated three replicates, there were 12 treatment units and each unit consisted of 5 crabs.

Donomotor	Treatment (SMM and ATM)%				
Parameter	A(40 and 20)	B(40 and 25)	C(40 and 30)	D(CF)	
% Protein	35.20	35.34	37.76	38.82	
% Carbohydrate	27.41	27.98	26.64	40.69	
% Lipid	3.69	4.30	3.53	4.48	
% Water	8.52	7.51	7.01	7.61	
% Ash	25.18	24.87	25.06	8.40	

2.4 Research parameters

Parameters measured were Weight Gain/WG, Number of Molting/NM, Feed Conversion Ratio/FCR, Survival Rate/SR), and water physico-chemical. The formulation used the ⁽¹⁶⁾ equation as follows:

$$NM = \frac{number of molting crabs}{initial number of crabs} \ge 100$$

$$FCR = \frac{dry \ weight \ of \ feed \ given}{(final \ weight + dead \ crab \ of \ weight) - initial \ weight)}$$

$$SR = \frac{Final number of crabs}{initial number of crabs} x 100$$

2.5 Data Analysis

Data were analyzed using Analysis of Variance (ANOVA), because the treatments influenced the research parameters, Duncant's further test was conducted, using SPSS 18.0 (IBM Corp., Armonk, NY, USA). Water physico-chemical parameters were analyzed descriptively.

3. Results

The research parameters, e.i Weight Growth (WG), number of molting (NM), survival rate (SR), and feed conversion ratio (FCR) of mud crabs treated with a combination of sailfin molly meal and spinach meal and commercial feed are presented in Fig. 2 to 4. Water physica-chemical parameters are: temperature, pH, salinity, dissolved oxygen, ammonia and nitrite (Table 3).



Fig 2: Mud crabs weight growth in each treatment



Fig 3: Number of mud crabs molting in each treatment



Fig 4: Feed Conversion Ratio of mud crabs in each treatment



Fig 5: Survival Rate of mud crabs in each treatment

The results of analysis of variance (ANOVA) showed that the treatment significantly affected the parameters of growth, survival and feed conversion, but the treatment did not significantly affect the number of crabs molting. Duncan's further test results showed that absolute growth (WG) and feed conversion (FCR) of commercial feed treatment (67.33 \pm 19.55 g and 3.1 \pm 0.35) were better but not significantly different from treatment B (61.33 \pm 25.00 g and 3.57 \pm 1.14), and C (58.67 \pm 3.79 g and 3.3 \pm 0.60). Survival (SR), the commercial feed treatment (97.77 \pm 3.87) was higher but not different from treatments A (84.43 \pm 7.67) and B (86.67 \pm 6.65).

Table 3: Parameters water physico-chemical: temperature, pH,

 salinity, dissolved oxygen, ammonia and nitrite, the range during the

 study and the optimum range for mud crab culture.

Parameters	Value Range During Research	Optimum Range
Temperature (°C)	10 °C to 30 °C	29 °C ^[17] ; 20 to 30 °C ^[18]
pH	6.0 to 8.0	7.75 to 8.50 ^[19]
Salinity (ppt)	5 to15 ppt	25 ppt ^[20] ; 20 ppt ^[21]
Dissolved Oxyagen (ppm)	3 to 5 ppm	±5.51 ppm ^[22] ; >5 ppm ^[23]
Ammonia (mg/l)	0.10 to 0.30 mg/l	< 0.5 [17]
Nitrite (NO ₂)	0.00 to 0.002 mg/l	<0.05 [17]

4. Discussion

The results showed that quantitatively the commercial feed treatment was better than the feed formulation of this study, but the results of statistical analysis showed that the commercial feed was not significantly different from treatments B and C. This shows that the feed with a combination of SMM and ATM, e.i 40% and 25-30%, is as good as the commercial feed. The observation in the field shows that the commercial feed has a higher water stability than the feed made in this study. The water stability referred to here is the resistance of the feed when the feed is held by the crab's claws, which is not easily crumbled so that a lot of it enters the crab's mouth. In contrast, feed treatments A, B and C, are easily crumbled when held by the crab claws so that a lot of feed is wasted.

The resistance of the feed when held by the crab claws is one of the obstacles in the process and formulation of small-scale crab feed, because the process of mixing the ingredients is done manually. Crabs have a unique way of taking their food, which is using the claws to hold and deliver the food into the mouth ^[24, 25]. Therefore, a compact feed texture that does not crumble easily is required. Feed pellets for crustaceans such as crabs should have high water stability, nutrients that are not easily leached and a larger size because crabs are benthic organisms and slow eaters ^[26]. It is therefore recommended that crustacean pellets should maintain a dry matter retention of at least 90% even after 1 hour in water the nutrients are not

lost [27].

The use of spinach meal did not affect the molting and growth of crabs in this study. The use of spinach meal in doses of 20-30% is thought to not be able to produce enough ecdysteroids to play a role in increasing body protein. The most important metabolic action of steroids is the activation of protein metabolism^[28]. Protein synthesis is the most basic growth process, without massive protein production, growth will not occur [29]. The 20-30% dose of spinach meal in feed is equivalent to 1 g of spinach extract in 1 kg of feed ^[23]. This dose is thought to be unable to stimulate crab molting. In addition, artificial feed is washed out while in water so that feed nutrients will be reduced [30]. The future of crab feed includes finding binders that will help bind feed components together, minimize empty space so that the integrity of the pellets is maintained, resulting in more compact and durable pellets ^[31].

The high FCR value in this study, which was 3.3 to 8.3, indicates that the feed provided was inefficient. Crabs rely on their sense of smell, taste and sight to find food ^[31]. Animals that rely on the olfactory organ in finding food, the food attractant must be strong so that the crab is stimulated to consume the food given. According to ^[30], attract ability and palatability are two factors that determine feed efficiency in crustaceans. Palatability is the animal's acceptance of food and attract ability is the attractiveness of food. Good attract ability and palatability of feed causes increased feed consumption resulting in increased growth.

Water quality conditions during the study were in a tolerable range, except salinity was at a non-optimal value to support crab molting and growth. Salinity is one of the most important factors affecting the physiological status of aquatic animals. Changes in salinity are directly related to osmoregulatory capacity. Osmoregulation is an attempt by aquatic animals to control the balance of water and ions between the body and its environment through the mechanism of regulating osmosis pressure ^[20, 23]. The further the difference in osmotic pressure between the body and the environment, the more energy is needed to perform osmoregulation as an adaptation effort, up to the limit of tolerance it has ^[32, 33].

Growth requires a condition of a range of water quality that is small compared to its survival. This situation causes the reallocation of metabolic energy for growth to energy for environmental adaptation ^[10]. Mud crabs are eurihaline aquatic organisms, but the optimum salinity for mangrove crabs is around 25 ppt ^[20]. In optimum salinity conditions, the level of osmotic work reaches a minimum level so that energy expenditure for osmoregulation is low and energy for growth increases.

5. Conclusions

The use of sailfin molly meal and spinach meal is as good as commercial feed on the growth, survival, and feed conversion ratio of crabs. These ingredients can be considered as a cheap and quality alternative to local ingredients. However, the diet must be enhanced with the addition of binders and attractants. Water physico-chemical specifically salinity, does not support optimal survival and growth of mud crabs.

6. Acknowledgments

To Mr. Burhan thank you for the pond facilities as a research location, Mr. Kamaruddin for his help in making feed, the team of crab research students, Nursaadah Kasim and Hamdani Akbar for their cooperation during the research.

7. References

- 1. Putri A, Bengen DG, Zamani NP, Salma U, Kusuma NP, Diningsih NT, *et al.* Mangrove habitat structure of mud crabs (*Scylla serrata* and *S. olivacea*) in the Bee Jay Bakau Resort Probolinggo, Indonesia. Ilmu Kelaut Indones J Mar Sci. 2022;27(2):124–132.
- Hasnidar H, Tamsil A. Effect of amphipods (Grandierella megnae) density on the growth and survival rate of mangrove crab (*Scylla tranquebarica*). Asian J Fish Aguatic Res. 2023;23(5):26–31.
- 3. Aslamyah S, Fujaya Y. The frequency of feeding artificial based waste to produce soft shell mud crabs. Torani. 2014;24(1):44–52.
- 4. Permadi S, Juwana S. Determination of daily requirement of trash fish feed to fatten the mangrove crab, *Scylla paramamosain* in bottom net cages. Oseanologi dan Limnol di Indones. 2016;1(1):75–83.
- Hanif A, Herlina S. Feeding with different percentages of raw fish on the growth of mangrove crab (*Scylla* spp). J Ilmu Hewani Trop. 2021;10(1):1–5.
- Craig S, Kuhn DD. Understanding fish nutrition, feeds, and feeding. In: Virginia Cooperative Extension. Virginia State University; c2017. p. 1–6.
- Tamsil A, Hasnidar. The effect of molting hormone (20hydroxyecdyson) on molting of mud crab (*Scylla olivacea* Herbst, 1976). Ecol Environ Conserv [Internet]. 2018;24(2):960–967. Available from: https://api.elsevier.com/content/abstract/scopus_id/85053 280976
- 8. Gorelick-feldman JI. Phytoecdysteroid Understanding their anabolic activity. The State University of New Jersey; c2009.
- Fujaya Y, Trijuno DD, Nikhlani A, Cahyono I, Hasnidar H. The use of mulberry (*Morus alba*) extract in the mass production of blue swimming crab (*Portunus pelagicus* L.) larvae to overcome the mortality Rate Due to Molting Syndrome. Aquat Sci Technol. 2014;2(1):1–14.
- Hasnidar H, Tamsil A, Wamnebo MI. The effects of the amaranth extract (*Amaranthus* spp.) on the molting of orange mud crab (*Scylla olivacea*). AACL Bioflux [Internet]. 2021;14(2):1036–1045. Available from: https://api.elsevier.com/content/abstract/scopus_id/85106 205561
- Aslamyah S, Fujaya Y. Effectiveness of artificial diet enriched by spinach extract on molting stimulation to produce soft shell crab. J Akuakultur Indones. 2011;10(1):8–16.
- 12. Usman Z. Molting time, chemical composition and body energy of mud crab (*Scylla olivacea*) given vitomolt through a combination of injection and artificial feeding. Agrominansia. 2017;2(2):195–210.
- Hasnidar, Tamsil A. Chemical characteristics of sailfin molly meal, *Poecilia latipinna* (Lesueur 1821). J Pengolah Has Perikan. 2020;23(2):392–401.
- Salim C, Artina V, Ayu AS. Processing spinach meal as a substitute for glutinous rice meal in making klepon. J Pariwisata. 2019;6(1):56–70.
- Mubaraq A, Hamzah RNA, Mulyasari SP, Nurhabiba S, Rusdi I. Fish Feed Making Guide. Jumadi O, Mulyaningrum SRH, Juanda M, editors. Makassar: Jurusan Biologi FMIPA UNM; 2022. 101 pp.
- Boonyaratpalin M. Methodologies for vitamin requirement studies. In: De Silva S., editor. Fish Nutrition Research in Asia. Manila, Philippines: Asian

Fisheries Society, Philippines, and International Development Research Centre of Canada; 1989. p. 58–67.

- 17. Hastuti YP, Affandi R, Millaty R, Tridesianti S, Nurussalam W. The best temperature assessment to enhance growth and survival of mud crab, *Scylla serrata* in resirculating system. J Ilmu dan Teknol Kelaut Trop. 2019;11(2):311–22.
- 18. Alberts-Hubatsch H, Lee SY, Meynecke JO, Diele K, Nordhaus I, Wolff M, *et al.* Life-history, movement, and habitat use of *Scylla serrata* (Decapoda, Portunidae): current knowledge and future challenges. Hydrobiologia. 2016;763(1):5–21.
- 19. Hastuti YP, Nadeak H, Affandi R, Faturrohman K. Penentuan pH optimum untuk pertumbuhan kepiting bakau, *Scylla serrata* dalam wadah terkontrol. J Akuakultur Indones. 2016;15(2):171.
- Hastuti YP, Affandi R, Safrina MD, Faturrohman K, Nurussalam W. Optimum salinity for growth of mangrove crab *Scylla serrata* seed in recirculation systems. J Akuakultur Indones. 2015;14(1):50.
- 21. Suyono. Success rate of mangrove crab (*Scylla serrata*) molting with different salinity treatments in controlled containers. IOP Conf Ser Earth Environ Sci. 2021. P.755(1).
- Faturrohman K, Nirmala K, Djokosetiyanto D, Hastuti YP. The concentration of optimum dissolved oxygen levels for growth of mangrove crab *Scylla serrata* seed in recirculation system. J Akuakultur Indones. 2017;16(1):107–15.
- 23. Hasnidar. Dinamika Molting Kepiting bakau. c2018. 1–154 p.
- 24. Ruscoe IM, Jones CM, Jones PL, Caley P. The effects of various binders and moisture content on pellet stability of research diets for freshwater crayfish. Aquac Nutr. 2005;11(2):87–93.
- 25. Volpe MG, Varricchio E, Coccia E, Santagata G, di Stasio M, Malinconico M, *et al.* Manufacturing pellets with different binders: Effect on water stability and feeding response in juvenile *Cherax albidus*. Aquaculture [Internet]. 2012;324–325:104–10. Available from: http://dx.doi.org/10.1016/j.aquaculture.2011.10.029
- 26. Obaldo LG, Divakaran S, Tacon AG. Method for determining the physical stability of shrimp feeds in water. Aquac Res. 2002;33(5):369–77.
- 27. Sudaryono A. Pellet water stability studies on lupin meal based shrimp (*Penaeus monodon*) Aquaculture Feeds: Comparison of lupin meal with other dietary protein sources. J Coast Dev. 2001;4(3):129–40.
- 28. Hoar W, Randall D, Brett JR. Fish Physiology. VIII. Hoar W., Randall D., Brett J., editors. Academic Press. New York: Academic Press; 1979;8:1-805.
- 29. Mente E. Protein nutrition in crustaceans. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour. 2006;1(043):1– 7.
- 30. Suresh AV, Kumaraguru Vasagam KP, Nates S. Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry byproduct meal. Aquaculture. 2011;319(1–2):132–40.
- 31. Aaqillah-amr MA, Hidir A, Azra MN, Noordiyana MN. Interactions between food, feeding and diets in crustaceans: A review. 2021.

- Aragão C, Costas B, Vargas-Chacoff L, Ruiz-Jarabo I, Dinis MT, Mancera JM, *et al.* Changes in plasma amino acid levels in a euryhaline fish exposed to different environmental salinities. Amino Acids. 2010;38(1):311– 7.
- Denisse Re A, Díaz F, Ponce-Rivas E, Giffard I, Muñoz-Marquez ME, Sigala-Andrade HM, *et al.* Combined effect of temperature and salinity on the Thermotolerance and osmotic pressure of juvenile white shrimp *Litopenaeus vannamei* (Boone). J Therm Biol. 2012;37(6):413–418.